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T. Teyke

Food-attraction conditioning in the snail, *Helix pomatia*

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Abstract Adult pulmonate snails (*Helix pomatia*) were released equidistant between two types of food, carrot and potato, respectively. Naive snails moved in different directions and did not locate either food above chance, although both foods were readily eaten upon direct contact. After a single carrot feeding episode, 75% of the carrot-fed snails moved directly towards the carrot and ate it. Conversely, potato-fed snails located the potato in 67% of the cases. Snails that were fed apple or lettuce behaved like naive animals, with the majority of animals (75% in both cases) locating neither the carrot nor the potato.

The ability of snails to locate this particular food after a single feeding episode was maintained for at least 11 days, provided that the snails were not exposed to other foods in the interim. If the animals were fed a different food (but still tested for food-finding ability to the initially conditioned food) their orientation preference decreased gradually over a period of 5 days.

Although the snails' orientation is based upon olfactory cues, exposure to food odor alone is not sufficient to enable food-finding; additional feeding related stimuli are necessary.

These findings indicate that *Helix* do not possess a predisposition for the foods tested, and further suggest that processes underlying food-finding and food selection are strongly influenced by learning experiences. The conditioning phenomenon underlying food-finding behavior has been called *Food-Attraction Conditioning*, and appears to be a crucial link between the ecologies of learning and foraging behaviors. The accessibility of the snail's nervous system should permit neuronal analysis of the mechanisms underlying such a unique and complex learning phenomenon.

T. Teyke
Institut für Zoologie (III) Biophysik, Johannes Gutenberg-Universität, D-55099 Mainz, Germany

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Introduction

Molluscs are established animal models used in the study of the neuronal basis of behavior. Particular attention has been devoted to the identification of various forms of learning and memory and of the mechanisms underlying these phenomena (see e.g., Willows 1973; Kandel 1976; MacPhail 1993). The majority of learning phenomena in molluscs have been studied using rather simple aversive conditioning paradigms (see e.g., Gelperin 1975; Kandel 1976; Alkon 1985), which has been criticized for their lack of behavioral relevance (see e.g., Gallistel 1990; MacPhail 1993). Molluscs are, however, capable of more complex learning tasks, such as the nocturnal homing behavior of *Limax* (Newell 1966; Gelperin 1974), and *Helix's* homing to hibernation sites (Edelstam and Palmer 1950; Lind 1989, 1990). As of today, neither phenomenon has been investigated in detail.

Here, I describe a novel learning phenomenon in *Helix*, which develops as a corollary to feeding and profoundly influences the behavior of the animals. Given the easily attainable conditioning and the accessibility of the nervous system further studies of the cellular mechanisms underlying the learning phenomenon should be possible.

Materials and methods

Adult snails (*Helix pomatia*) with shell diameter > 30 mm were collected locally in wastelands encircled by roads; thus, prior exposure to the cultivated foods tested was unlikely. Each experimental group consisted of 12 animals, which were assigned randomly to the various experimental groups.

Individual snails were released equidistant (20 cm) between two feeding sites containing palatable foods (see Frömming 1938, 1950):

potato tubers (*Solanum tuberosum*); and carrot root (*Daucus carota*); 1 cm^3 piece, each. Through a series of control experiments it was ascertained that the animals, upon direct contact with the foods, readily eat them. An open field arena (1.6 × 1.2 m) was situated in a small, enclosed room with no detectable air currents (draft checker). Air temperature was maintained at 20°C and relative humidity was kept above 60%. The starting positions of the snails and orientation of the feeding sites were varied randomly and standardized in the figures for display of data (carrot at 90° from snail; potato at 270°). The position of each animal was determined at fixed time intervals using a computerized video recording system, and the path was subsequently reconstituted. Most of the experiments were performed and scored blind. Animals were scored when they had reached the carrot (c), or the potato (p), respectively; null(–) was scored when the animals located neither food after a maximum search time of 60 min (which is more than 3 times the average food-finding time of 18 min); or alternatively, when the animals reached the border of the area. Food-finding scores of the animals following the experimental treatments were compared to those of naive animals using χ^2-tests.

Results

Food-finding requires prior experience

Naive *Helix* (as a rule, naive refers to animals which were collected the day prior to testing and had not been fed in the laboratory) were released in an open field arena between two food pellets (potato and carrot). As shown in Fig. 1A, the snails moved in different directions (for clarity, tracks of only 12 animals are shown), and the majority located neither food. In total, only 3/24 snails located and consumed one of the foods. This rate is similar to expectations based upon the likelihood of a snail with an average tentacle span establishing tactile contact with food by moving from the center in any random direction (which is approx. 1/12 or 8%). Following control tests with naive snails, half of the experimental group were fed potato (0.5 g), while the other half received carrot. When tested the following day, according to the same protocol, the majority of the animals moved directly towards the food sites (Fig. 1B), and 21/24 snails located and consumed the food. The snails' orientation was strongly biased towards the food heretofore eaten: 9/12 animals that were fed carrot located carrot, and, conversely, 8/12 animals that received potato moved directly towards the potato (see also Table 1).

In contrast, snails that were fed lettuce or apple (but tested for carrot/potato food-finding ability) behaved like naive animals. The majority of animals (75% in both cases) located neither food (Table 1). The performance of the apple-fed animals differed when they were tested in an apple/carrot orientation test rather than carrot/potato. In these tests, 9/12 apple-fed animals (75%) located the apple.

These results indicate that *Helix* do not possess a predisposition for the selection of foods tested, however exposure to a food enables the snails to subsequently locate this, and only this, particular food. The learning phenomenon underlying food-finding was thus termed *food-attraction conditioning.*

Acquisition and retention of food-attraction conditioning

In a separate set of experiments, acquisition and retention of conditioned food-attraction was investigated under different experimental conditions. Before feeding, none of the naive animals of the four experimental groups located the food (see Fig. 2A and B, naive). Then, 6 snails from each group were fed carrot, while the other 6 received potato (except for controls, which remained unfed). Conforming to expectations (based on the results of the experiments described above), the snails developed a strong preference for the food after a single feeding episode (Fig. 2A, B) : the next day (day 1), 8 to 10 snails out of each group located the conditioned food (except controls). Following the initial food conditioning, each experimental group was treated differently and the animals were tested daily for their

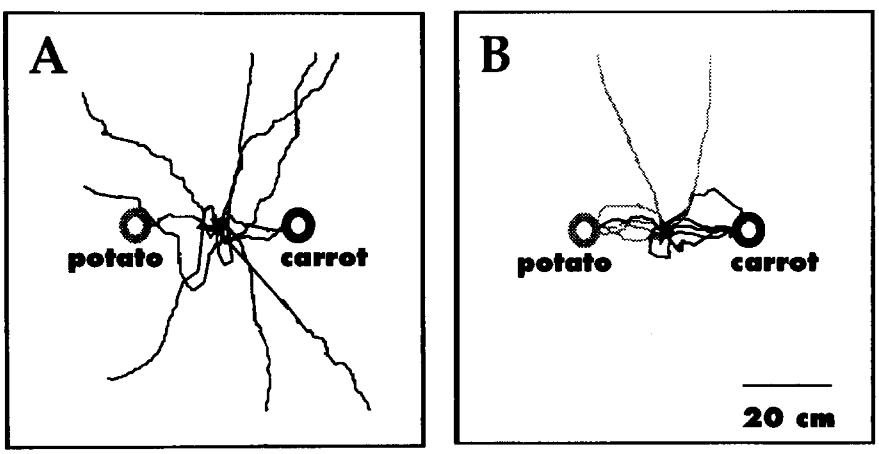

Fig. 1A, B A single feeding episode alters orientation and food-finding behavior. Composite of locomotor paths of 12 snails released midway between two feeding places potato and carrot in an open field situation. **A** Naive animals. **B** Same group of animals fed over-night and tested the next day: 6 snails were fed with carrot (*dark tracks*) and the remaining 6 with potato (*light tracks*)

Table 1 Consumption of food determines food-finding ability in *Helix*. Food located in carrot/potato orientation test (% animals); For statistical purposes, performance of animals after feeding was compared to that of naive animals; *n.s.*: not significant

	carrot	potato	(−)	*P*
Naive (animals combined) before feeding [*n* = 60]	8	7	85	-
Food consumed				
carrot [12]	75	25	0	<0.005
potato [12]	8	67	25	<0.005
apple [12]	17	8	75	n.s.
lettuce [12]	17	8	75	n.s.
Odor alone				
carrot [12]	8	25	67	n.s.

ability to locate the conditioned food. The snails were able to locate the conditioned food at high levels for at least 11 days when in the interim they did not receive any food, i.e., there was no evidence that the animals had forgotten the food (Fig. 2A, filled squares). Unfed control animals did not locate the food above the expected chance level (Fig. 2A; open circles); although there appeared to be a slight increase in the food-finding rate after 2 to 3 days. This increase may be attributed to the fact that in some cases, an animal may have obtained a small piece of food, and thus conditioned itself.

Animals in the other groups were first conditioned for one food and thereafter fed a different food. When snails received the alternate food as a test for reversal learning (carrot-conditioned animals were fed potato, and vice versa) their food-finding rate declined over 4 or 5 days to control level (Fig. 2B; filled triangles). Similarly, in animals fed lettuce to test for extinction of memory, their preference for the initial food also declined over 4–5 days (Fig. 2B; open inverse triangles). These results indicate that – although the snails' food-finding ability can change rapidly following exposure to novel foods, previous experience with food exerts long-term effects upon the snails' behavior. In other words: food-attraction conditioning is accomplished by a single feeding episode, and contains a long-term memory component.

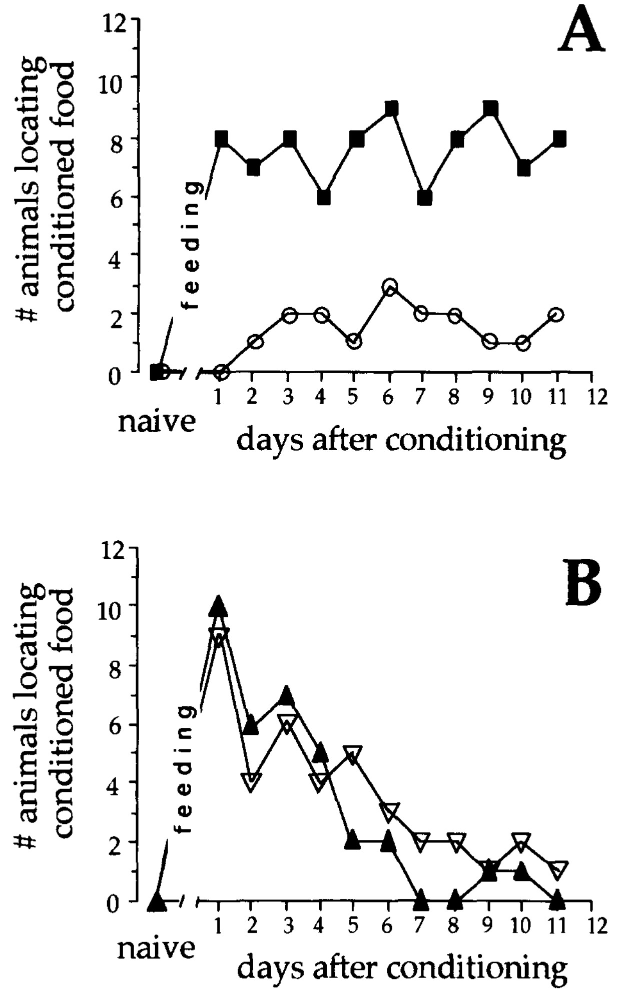

Fig. 2A, B Retention of food-attraction conditioning. The orientation preference for the initially conditioned food was tested over successive days in the carrot/potato test. Each group consisted of 12 animals, which were first tested for spontaneous preferences (*naive*). Thereafter, 6 snails of each group (except control group) were fed carrot and 6 potato (*feeding*). **A** Retention of conditioned food-attraction after the snails were fed only a single meal (*solid squares*) as compared to responses of control animals, which remained unfed (*open circles*). **B** Effects of feeding additional meals on the preference for the initially conditioned food. To test for reversal learning, animals were subsequently fed with the alternate food; carrot fed animals received potato, and vice versa (*filled triangles*). *Inverse open triangles*: all animals received lettuce daily, as a test for extinction of learning

Conditioned food-attraction requires more than olfactory inputs

Because food-finding per se in *Helix* (von Buddenbrock 1916; Schulz 1938; Farkas and Shorey 1976) as in other terrestrial pulmonates (Gelperin 1974; Chase and Croll 1981), is based on olfactory cues, it is conceivable that the conditioning phenomenon underlying food-finding is mediated by the exposure to olfactory stimuli during feeding. There are several lines of evidence which suggest that olfactory input alone is insufficient to enable food-finding in *Helix*. First, exposing snails for 12 hours to the food-odor while preventing ingestion (a perforated box containing carrot chips was placed in the snails' container) did not increase the rate at which they found the food above that of naive animals. Only 1/12 snails that were exposed to carrot odor located the carrot (Table 1; persisting sensory adaptation was unlikely because the odor source was removed several hours before testing). Second, conditioned food-attraction cannot be attributed to an increase in sensitivity to food odor. Naive animals are unable to locate food at close proximity (10 cm) at rates higher than those expected by chance. Moreover, naive snails were

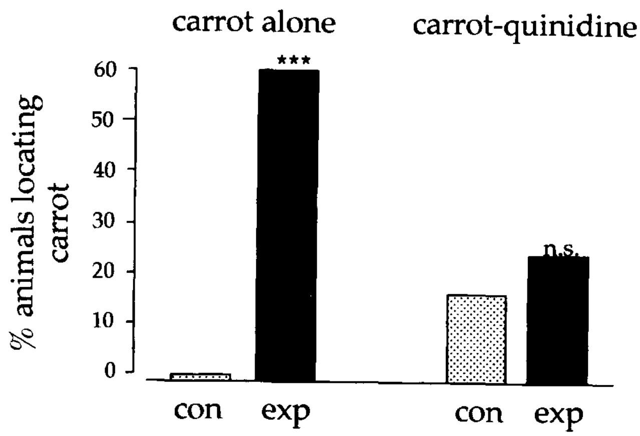

Fig. 3 Pairing food with a noxious stimulus prevents conditioning. Two groups of naive snails were tested for their ability to locate carrot (*con*). Thereafter, animals of one group (*exp*) were fed carrot 3 times for 2 min each, every feeding episode followed by placing the snails for 2 min on moist filter paper (*carrot alone*). The second group was also fed 3 × 2 min carrot, but then placed on filter paper soaked with quinidine solution (*carrot-quinidine*), which is a highly aversive stimulus for the snails (***: $P < 0.005$; *n.s.*: not significant)

occasionally observed passing potential foods at a distance of no more than a few millimeters without turning towards it, thus indicating that the detection of food is not a matter of perception but of recognition.

These results suggest that olfactory stimulation alone cannot account for the change in the food-finding ability of *Helix*. Additional gustatory/ingestive inputs, which have not been identified, are necessary for conditioned food-attraction.

Aversive conditioning paradigm prevents acquisition of food-attraction

In this set of experiments, I adapted an aversive conditioning paradigm, using quinidine as a noxious, aversive stimulus, which was proven to be effective in altering preferences for food odors in *Limax* (Sahley et al. 1981). Prior to the experiments, naive snails were tested for their ability to locate carrot in the orientation test (Fig. 3, con; the relatively high food-finding rate of the second group of animals is within the variance for naive animals). Thereafter, the animals of one group were fed a piece of carrot 3 times for 2 min each. Immediately after each feeding episode, the snails were placed on moist filter paper for 2 min. The second group was also fed carrot 3 times for 2 min. However, they were placed on filter paper soaked with a 1% quinidine solution. During exposure to quinidine the snails showed typical aversive behaviors, such as lifting their foot off the substrate. Exposure was terminated by rinsing the animal with water. Both groups were tested the following day in the carrot/potato orientation test. As shown in Fig. 3, 60% of the carrot-fed animals (carrot alone) located the carrot (exp). This is a significant increase from the food-finding rate of naive animals ($P < 0.005$). In contrast, only 25% of the animals of the experimental group which received carrot paired with the aversive stimulus, quinidine, located the carrot (exp). This rate is not significantly different from that of naive animals ($P > 0.25$), thus indicating that food-attraction conditioning does not occur when the food stimulus is paired with an aversive stimulus. These results show further that short exposure (3 times 2 min) to food is sufficient to condition the animals to that particular food.

Discussion

The central finding of this paper is that foraging and food-finding behavior in *Helix* is strongly influenced by the animal's prior experience with food. As documented by the failure of naive snails to locate highly palatable foods even at close proximity, they do not possess a predisposition for any of the foods utilized in this study. Preliminary studies have expanded the list of foods tested, and shown further that the snails' performance is independent of their hunger level. Successful food-finding requires prior exposure to the food so that the animals have the opportunity to acquire certain characteristics of this particular food through a rapid learning process. The quasi one-trial learning phenomenon described here – *food-attraction conditioning* – strongly influences the snails' foraging behavior by determining the selection of foods, thus expanding previous reports on plasticity of odor preferences in snails (Croll and Chase 1977, 1980). The term 'food-attraction conditioning' was chosen partly to allude to the discrepancy that arises from the fact that the selection of food is generally thought to be shaped by negative experiences, most dramatically by 'food-aversion conditioning'. Similar to food-aversion conditioning, food-attraction is a learning phenomenon which does not conform to the established framework of classical and instrumental conditioning, most often employed to explain invertebrate learning (Willows 1973; Alkon 1985; Gelperin 1975; Kandel 1976; but see Gallistel 1990; MacPhail 1993). Given the accessibility of the nervous system in *Helix*, an analysis of the neuronal mechanisms underlying food-attraction conditioning appears to be possible.

Food-attraction conditioning is a direct consequence of feeding, and as such, is readily modified by subsequent feeding experiences. For all foods tested to date, a single feeding episode was sufficient to enable more than 60% of the animals to locate that particular food. The snails continued to locate this food for more than 10 days at sustained high rates provided that they did not receive any other food in the interim, thus indicating that long-term memory is involved in food-attraction conditioning. This long-term component may also explain why the snails' preference for the initially conditioned food decreased only slowly when the animals were fed with a different food but still offered the initial food as a choice in the orientation tests.

The learning mechanisms underlying food-attraction conditioning do not appear to be selective for palatable foods. In preliminary experiments, snails were fed a piece of paper towel soaked with coffee. When tested the next day, the majority of snails oriented towards the coffee odor. This suggests that a variety of odorous substances, including those not associated with natural foods, are permissible for the conditioning to occur. Conditioned food-attraction cannot be attributed to an increase in sensitivity to the odor of the food due to exposure which would occur during feeding. Exposure to the odor alone while preventing ingestion of food did not increase food-finding success (see also Croll and Chase (1980) for alteration of odor preferences), indicating that the learning phenomenon is not dependent upon the olfactory system alone, but requires other feeding related stimuli, which are yet to be determined.

The current findings cast a new light on the established theory of food selection and foraging behavior. As exemplified by a small selection of palatable foods, I have shown that naive snails are unable to locate potential foods unless they had experienced that particular food before. Furthermore, preliminary observations have indicated that naive *Helix* do not locate substances, which reportedly are spontaneously attractive in other terrestrial gastropods, such as 2-octanol, amyl acetate, and EMOP (Chase 1982; Hopfield and Gelperin 1989). These differences might be attributed to the different experimental protocols employed. In this study, I have focused on a natural behavior of the snails by assessing food selection (foraging success) under quasi natural, open arena conditions, which should be a very discriminative assay to study food choices.

The current findings also contrast to the common notion that food selection is based upon a predisposition for food in general, encompassing innate preferences (often paraphrased as 'nutritional wisdom', etc.), further shaped by experience based processes (see, for example, Garcia et al. 1977; Gould 1986). Such processes (not all apply to every animal or situation) are: (i) associative conditioning paradigms [of which food-aversion conditioning is an extreme case] (Garcia et al. 1974; Milgram et al. 1976); (ii) prolonged exposure to food [ingestive conditioning] (Thorpe and Jones 1937; Croll and Chase 1977, 1980; see also Pickett and Stephenson 1980); and, (iii) brief exposure to food during a critical period in juvenile animals [food imprinting] (Burghardt and Hess 1966; Capretta 1977; Hudson 1993).

As shown in this paper, in the absence of a predisposition for the cultivated foods tested, *adult* snails acquire the ability to locate a particular food by a rapid, robust learning phenomenon. This learning, food-attraction conditioning, has acquisition and retention characteristics that resemble those found in food imprinting, and its effects oppose those of food-aversion learning. While the current studies have been conducted under laboratory conditions (although in an open field arena), one can predict that conditioned food-attraction has wide-ranging effects on the natural foraging behavior of the snails (Kamil and Roitblat 1985). After a meal of a specific food, the snails are expected to actively search for more of the same. Because most foods (plants) appear in patches rather than randomly distributed, such a *win stay* strategy might endow the snails with a selective advantage for successful exploitation of food resources.

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