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CHEMICAL STIMULI AND FEEDING BEHAVIOR IN OCTOPUS, *OCTOPUS VULGARIS*

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ABSTRACT: The effects of chemical stimuli in the feeding behavior of *Octopus vulgaris* were studied using bait pellets, to elucidate the effect of bait quality on feeding behavior, to observe the chemoreceptive function of the arms and lip, and to see if they play a role in bait quality selection. The pellets were made from a binder (starch and cellulose) and 14 types of solutions: amino acids (L-Ala, L-Pro, L-Met, L-Ser, Gly or Bet), sugars (Glucose, Galactose or Sucrose), Quinine-HCl, fish extract (blue sprat, *Spratelloides gracilis*), and cephalopod inks [same species, same genus (*Octopus aegina*) or different order (*Sepioteuthis lessoniana*)]. Subjects differed in behaviors depending on test bait's contents. The behavioral differences occurred when individuals touched the bait with arm(s) or lip. The ink from a different order induced a significantly higher rate of rejection, compared with the control bait when touched by the arm(s). Comparisons of ingested ratios for each type of bait indicated that fish extract resulted in significantly higher ingestion, and the group containing Met, Gly, galactose and ink from same genus in lower ingestion rates. It was found that chemoreception in the arms and lip is responsible for the feeding behavior in terms of bait quality selection. It is also suggested that the properties of chemoreceptors on the arms and lip might be different.

INTRODUCTION

Octopus vulgaris is an important fisheries resource in Japan, with annual landings around 100,000 t and a landed value of more than $350 million. The capture methods used include skin diving, shelter pots, baited traps and artificial or natural lures. Fishermen claim that live baits are more effective than fresh ones, and much more than artificial lures. The effectiveness of bait seems to result from the combination of both the visual and chemical stimulus they send.

It has been thought that all cephalopods are probably sensitive to chemicals as well as tactile stimuli (Hanlon and Messenger, 1996). Histological research has revealed the presence of chemosensory structures in both suckers and lips of *Octopus* (Graziadei, 1962, 1964, 1965). It is known that ciliated chemoreceptor-like cells are densely distributed along the rim of the sucker (Graziadei, 1964). Behavioral studies also have confirmed the presence of chemoreception in the sucker in *Octopus* (Wells, 1963, Lee, 1992). But on the whole research on chemoreception in cephalopods is scarce compared with that of vision, and there is little information about which stimulatory substances are responsible for the attractiveness of their food. Lee (1994) found that the amino acid Proline and some nucleotides (ATP, AMP) were attractive in both *O. maya*, while the amino acids Bet and Tau were arrestants.

The purpose of our study was to elucidate the effect of bait quality on feeding behavior, to observe the chemoreceptive function of the arms and lip, and to see if they play a role in bait quality selection. The significance of this research is the perspective of improving current baiting technology, such as developing baits that are selective for octopus or that will exclude them from the catch of other fisheries, which would be extremely useful for a sustainable management of fisheries. Also this research can be applied to improve the palatability of formulated diets for maintaining octopus in captivity.

MATERIALS AND METHODS

Experimental animals and test tank

Experiments were carried out at the Education and Research Center for Marine Resources and

Environment of the Faculty of Fisheries, Kagoshima University, southern Japan. The two octopuses *Octopus vulgaris* (195 and 210 g) used in these experiments were purchased at the local fish market, and individually housed in aquaria (60 x 45 x 35 cm). An open circulation system ran seawater through the tanks. Each aquarium contained a clay pot for shelter. Black plastic sheets were taped on each wall to prevent an effect of the observer's presence on the subjects. For two weeks prior to experimentation the octopus were fed 4 g of blue sprat *Spratelloides gracilis* three times a day (9:00, 13:00, and 17:00). Water temperatures during the experiments ranged from 21° to 27° C.

Test chemicals and bait preparation

Six amino acids (L-Ala, Gly, L-Pro, L-Ser, Bet and Met), three sugars (glucose, sacharose, galactose), a bitter substance (quinine-HCl), three types of ink [conspecific (*O. vulgaris*), same genus (*O. aegina*), and different order (*Sepioteuthis lessoniana*)], and fish extract (blue sprat) were used as chemical stimuli. Each amino acid, sugar, and Q-HCl was made up to a 1 M seawater solution. Inks were extracted from the dissected ink sacs collected from live animals. Fresh blue sprats were crushed and only the liquid portion that passed through a filter paper was used. The above solutions were used as the stimulating substances, and 4 ml of each was mixed with a binder, a mixture of 5.7 g starch and 0.3 g cellulose. As the control bait, mixtures of binder and seawater were prepared. The binders were shaped into cylindrical pellets, 5 mm in diameter and 7 mm in length, by pressing the mixture through a syringe. Test baits were prepared one day before the experiments, and all the baits were air dried before use.

Behavior observation protocol

Experimental set-up for observing the feeding behavior towards the test baits is shown in Fig. 1. Observations were carried out everyday at 11:00 and 17:00. On experimental days, octopuses were previously fed 2 g of blue sprat meat 2 hours before the experiment to avoid results being confounded by hunger drive and to keep the feeding motivation the same in each trial. At the end of each experimental day, a whole blue sprat was given to each subject to satisfy their nutritional need. The number of tests in one trial was also limited to observe the response behavior with an equal condition of the subjects. In one trial, only one test bait was examined for one individual, therefore a total of four test baits were examined using two individuals a day. The bait was tied with thin nylon monofilament line and moved near the subjects to obtain a visual response and accelerate their attention towards it. The response behavior was video recorded from the top of the aquarium using a camera fixed on a tripod.

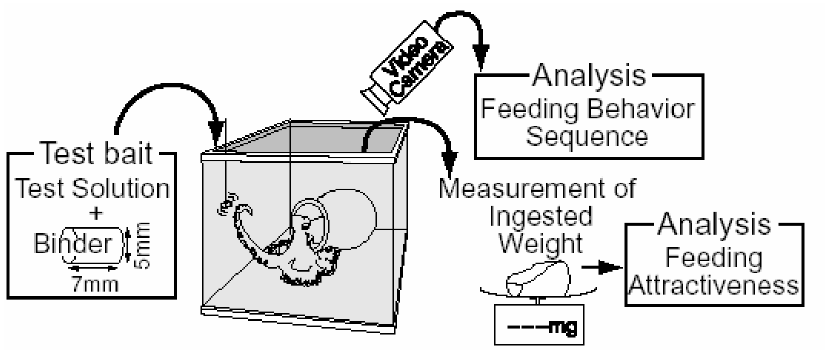

Figure 1. Experimental protocol to analyze the feeding behavior sequence and feeding attractiveness towards various types of chemicals.

Analyses

Based on the images recorded on the video, the behavioral sequences towards each chemical substance after the subject touched the bait with its arm(s) were determined. The time required for each sequence was also measured. Fisher's exact probability test was used to compare the number of behavioral sequences that appeared for each chemical with those of the control bait (binder only). To analyze the effects of the chemical substances on ingestion, ingested weights of the bait (ingested percentages relative to given weights) were used as the index of feeding attractiveness. Weight of each bait was measured by an electronic balance (10 mg resolution) before giving it to an octopus, and the repelled bait was weighed after drying for 2 hours in a drier. Weights of each bait before and after the experiment never showed the same values because of a small dilution of the binder. Therefore, data for lost weight percentages were collected from the experiments in which the octopus did not bite the bait, and we calculated the average (12.4 ± 10.3%, n = 84) as the control loss. The control loss was subtracted from all the ingested weight percentages. Mann-Whitney's *U* test was used to compare the ingested weight percentages, and Spearman's rank order correlation coefficients were calculated to analyze the relationships between ingested weight percentages and the terms required for the ingestion for each chemical.

RESULTS

Behavior sequences towards the test baits

All the behaviors observed in this experiment were indeed triggered by the visual stimulation of the moving bait, and that stimulus led the octopus to touch the bait within 10 s. Since visual stimulation was no longer available after subjects held the baits and transferred them to the mouth, it was clear that bait quality evaluation by the subjects was done based on the chemicals present in them. It is also important to notice that the effects of distant chemoreception were not involved in this experiment since the subjects responded to the baits immediately after exposure to them it in the tank. Subjects stayed in the shelters throughout the experiment. By moving the test bait in front of the subjects, they would extend and touch the bait with the arm(s) using 1^{st} and/or 2^{nd} arm(s) in all trials. As shown in Fig. 2, the behavior after touching it could be categorized into the following, i) hold by the arm(s), ii) transfer to the mouth, iii) reject at touch by arm(s), iv) partial or complete ingestion, and v) reject at touch with the mouth. It was clearly indicated that the behavioral sequence differed by the type of chemicals contained in the bait.

Differences in behavioral sequences according to chemicals

The frequencies of behavior sequence until transfer to the mouth in Fig. 2 are indicated in Table 1. Control baits induced positive behavior progress in 63% (12 of 19 trials) of the presentations. Significantly higher rates of positive behavior towards the baits when compared with the control were only found in the fish extract pellets (Fisher's exact probability test, $p < 0.05$), though other stimulatory substances induced higher transfer rates, such as Bet (88.3% transfers), sucrose (81%), and Met (79%). On the other hand, it was found that baits containing inks from cephalopods induced lower transfer rates to the mouth. Highly significant differences (Fisher's exact probability test, $p < 0.01$) were found only for baits containing ink from a different order, while those from the same genus and conspecifics had transfer rates as low as 30 and 50%.

Comparisons of feeding attractiveness in each chemical

The means of percentages for ingested weight (%) and terms (ingestion times) (s) required for the ingestion in each chemical are shown in Table 2. The mean ingested weight of the control bait was 23.7%. Significant higher ingestion values were observed only in baits containing the fish extract (75.7%, Mann-Whitney's *U* test, $p < 0.001$). Baits containing Bet, L-Ala, and glucose had higher ingestion weights than the control, 39.9%, 33.3%, 26.1 %, respectively; but were not significantly different. In contrast, baits containing substances that elicited significantly lower ingested weights than the control were baits containing galactose ($p < 0.01$), Gly ($p < 0.01$), Met ($p < 0.01$) and ink

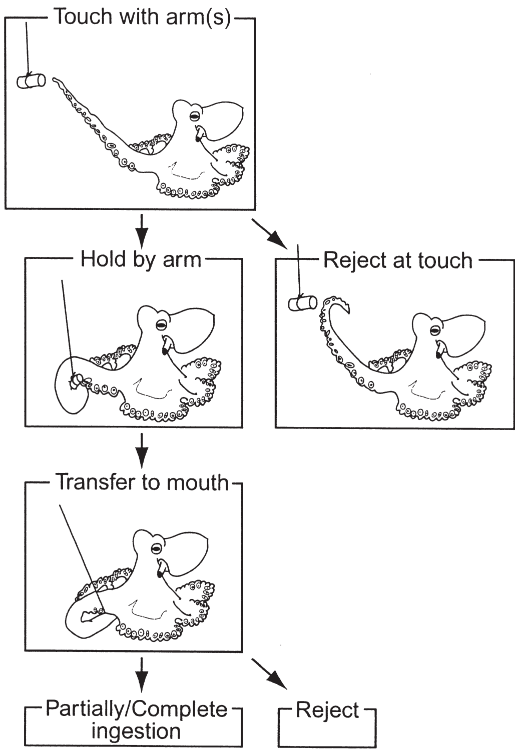

Figure 2. Behavioral sequences towards test baits.

Table 1. Comparisons of the appearance frequencies of the behavior sequence until bait transfer to mouth in reponse to various types of chemicals. Frequencies in each chemical were compared with that of control using Fisher's exact probability test and *p* values are shown in the Table.

Type of bait	# of trial	# of transfer to mouth		Comparison with control
		#	%	*p*
Control (binder)	19	12	63.2	
Fish extract	26	23	88.5	0.05*
Bet	12	10	83.3	0.22
Sucrose	16	13	81.3	0.21
Met	14	11	78.6	0.29
L-Gly	11	8	72.7	0.45
L-Pro	24	17	70.8	0.42
Q-HCL	19	13	68.4	0.50
L-Ser	11	7	63.6	0.65
L-Ala	31	19	61.3	0.57
Glucose	12	7	58.3	0.74
Galactose	11	6	54.5	0.80
Ink of conspecific (*O. vulgaris*)	16	8	50.0	0.33
Ink of same genus (*O. aegina*)	10	3	30.0	0.10
Ink of different order (*S. lessoniana*)	10	1	10.0	0.01**

Symbols respectively indicate the statistical significance levels, ** $p<0.01$; * $p<0.05$

Table 2. Comparisons of the ingested weights (%) and the terms (s) required for ingestion in response to various types of chemicals. The weights and terms were respectively compared with that of control using Mann-Whitney's *U* test and the *p* values are shown in the Table.

Type of chemical	# of sample	Ingested weight		Comparison with control	Ingest term		Comparison with control
		mean %	range %	*p*	mean s	range s	*p*
Control (binder)	12	23.7	0– 56		65	6– 222	
Fish extract	23	75.7	0–100	0.00***	221	47– 717	0.00***
Bet	10	39.9	0–100	0.45	424	7–1023	0.03*
Sucrose	13	11.9	0– 35	0.07	64	3– 198	0.98
Met	11	3.6	0– 13	0.00**	68	2– 184	1.00
L-Gly	9	2.8	0– 17	0.00**	56	6– 226	0.13
L-Pro	17	25.3	0–100	0.72	169	3– 742	0.30
Q-HCL	13	21.8	0– 62	0.64	63	2– 312	0.33
L-Ser	7	19.4	0–100	0.08	122	3– 349	0.80
L-Ala	19	33.3	0–100	0.67	100	3– 349	0.52
Glucose	7	26.1	0–100	0.73	57	4– 116	0.93
Galactose	6	2.3	0– 13	0.00**	29	4– 60	0.24
Ink of conspecific (*O. vulgaris*)	8	31.0	0–100	0.85	83	5– 384	0.56
Ink of same genus (*O. aegina*)	3	3.6	0- 11	0.04*	24	6– 55	0.17

Symbols respectively indicate the statistical significance levels, ***$p<0.001$; $p<0.01$; *$p<0.05$.

from the same genus ($p < 0.05$). However, terms required for ingestions varied in each trial for the same chemical. Significant positive correlations between ingested weight percentages and the terms required for ingestion were observed only in Q-HCl ($r = 0.87$, $p < 0.005$), conspecific ink ($r = 0.86$, $p < 0.05$), L-Ser ($r = 0.86$, $p < 0.05$) and Bet ($r = 0.66$, $p < 0.05$).

DISCUSSION

The present study showed that *O. vulgaris* is capable of qualitative discrimination of chemicals by its arms and lip. The bait selection by the arms was not widely affected by the chemicals. Only two types of chemicals, fish extract and ink from *S. lessoniana*, significantly differed in the behavioral sequence positively and negatively. Amino acids, the bitter substance and sugars did not cause differences in the feeding behavior. On the other hand, bait selection by the mouth was more specific depending on the chemicals present in the baits. Only fish extract was more highly ingested than the others, while the amino acids (Met and Gly), sugar (galactose) and ink from *O. aegina* were ingested less. Comparing the ingested weights with the behavior sequences after touching, Met and Gly induced a high rate of positive behavior progress (79% and 73%, respectively) while the ink from *O. aegina* showed a low rate (30%). We could not find any remarkable correlation between the frequencies of positive behavior progress and the ingested weights in each chemical. These inconsistencies for the bait selection at touching with arms and lip imply that the properties of the chemoreceptors in both portions might be different.

The chemoreceptor in the sucker has been well studied morphologically in *O. vulgaris* and *Sepia officinalis* (Graziadei, 1962, 1964 a b). A tapered sensory cell with microvilli and ciliary apparatus is presumed to be the chemoreceptor. These cells densely line the columnar epithelium layer and end at the free surface of the epithelium. Although there is no direct evidence to prove that tapered sensory cells indeed respond to chemical stimulus, behavioral researches on *Octopus* including the present study have indicated the presence of chemoreception in the sucker (Wells, 1963: Lee, 1992). It is important to specify the chemoreceptive cells and their capabilities for the further studies of chemoreception. Chemoreception and its relation to feeding have progressed for the purpose of developing artificial baits and diets. Lee (1994) compared the behavioral responses of *Octopus maya* and *Sepioteuthis lessoniana* to amino acids (Pro, Gly, Bet, Tau) and nucleotides (ATP, AMP). The results showed the species specific characteristics in behavior as follows. Pro and nucleotides were found to be attractants in both *O. maya* and *S. lessonniana*, and Bet was an arrestant also to both species. On the other hand, Tau was arrestant only in *O. maya* while *S. lessonniana* showed no response to it. Additionally, comparing those results with our present results in *O. vulgaris* and our previous study in *Sepia esculenta* (Anraku *et al.*, 1998), the behavioral effect of Pro and Bet agreed in *O. maya*, *S. lessonniana* and *S. esculenta,* but differed in *O. vulgaris*, and Tau was an attractant in *S. esculenta*. Thus, it has been shown that there is a species specific feature of sensory capability and/or response behavior to the chemical stimuli.

It was also noted that subjects discriminated the inks of other species from that of a conspecific and that they arrested the feeding behavior. Moreover, the ink from a different order affected an individual more than that from the same genus. The belch of ink (so called inking) is a peculiar behavior in the coleoid cephalopod. The meaning of such behavior is generally presumed to act as 'pseudomorph' or 'smoke screen' (Hanlon and Messenger, 1996), and both are basically hypothesized on the assumption that cephalopods emit ink when they are attacked by predators. On the other hand, it is interestingly suggested that some chemical contents of the ink can act as an alarm substance towards a conspecific. The squid *Loligo opalescens* was induced to escape by stimulating its olfactory organ with ink from a member of this same species (Gilly and Lucero, 1992). Although the visual effects of ink towards predators are unknown, it is possible to suggest that the chemical stimulants in the ink mediate some biologically important information among the group of the same species. Squid jigging fisherman actually hesitate to use inked gear because they have empirically found out about such biological meaning. Furthermore, the present study revealed the specific effects of each type of ink from a conspecific and different species towards *O. vulgaris*. The relevance of the uses of ink should be various among the coleoid cephalopods.

In conclusion, the present study could indicate the role of chemoreception in the feeding behavior of *O. vulgaris*. However, the specificity of bait selection was limited only to several types of chemicals. We expected a higher palatability than that observed, because of the very dense distribution of the chemoreceptive cells (about 10,000 / sucker) found in a sucker of *O. vulgaris* (Graziadei, 1964b). The chemicals used here were all prepared as a single substance and the use of crab extract, a common octopus prey, was not performed. It is necessary to examine the feeding behavior in relation to mixtures of individual stimulants.

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