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Alarm substance Detection and Fright Reaction in Giant Danio (*Danio malabaricus*)

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A series of experiments were conducted to examine on what stimulus giant danio (*Danio malabaricus*) were mainly responsible for detecting alarm substance.

When 0.15 ml alarm substance (10 ppm) was introduced into the tank, fish showed no notable change in swimming pattern. However, the introduction of 1.5 ml alarm substance (100 ppm) could induce fright reaction about in 6 minutes. Further, when 15 ml alarm substance (1,000 ppm) was introduced into the tank, fish showed the following fright reaction in a few seconds; suppressing to feed diet, no swimming, strong chasing, and visual alertness.

In detecting alarm substance, fish were mainly depending on the chemical stimulus (nares) rather than the vision stimulus (eyes) because fish detected alarm substance by the chemical cue showed the significantly stronger fright reaction than by the visual cue. The time for fish to show the initial fright reaction after detecting alarm substance by the chemical cue was shorter than by the visual cue. Also after alarm substance was introduced into the tank, olfaction-deprived fish showed significantly weaker fright reaction and less frequency of chasing than the normal fish which detected alarm substance by both olfaction and gustation stimulus.

These results indicated that chemical stimulus, especially olfaction might be the primary sensory modality used in the detection of the alarm substance for giant danio.

Key words : Giant danio (*Danio malabaricus*), Alarm substance, Fright reaction, Olfaction, Gustation.

Introduction

Fish have the highly sensitive chemoreception systems involving in species and individual recognition, feeding, reproductive behavior, predator-prey interactions and orientation (Barnett, 1977). Alarm substance usually exists in a large epidermal cell, the club cell in the skin of fish. The club cell can be distinguished from the mucous cells which secret the mucus at the skin surface through a pore. In juvenile Fancy Carp (*Cyprinus carpio*), alarm substance cells were found in the over side over the scale, concentrated in a central area toward the posterior border of the scale of skin (Strussmann et al., 1994).

The Ostariophysi and Gonorynchiformes have alarm substance in common which is absent in all other groups of teleostean fishes (Pfeiffer, 1977); fright reaction elicited by alarm substance produced by special epidermal alarm substance cells. When active alarm substance induced the fright reaction, fish were usually terrified. The fright reaction was not species-specific, fish reacted to alarm substance emitted by others, but the intensity of the response was related to the phylogenetic proximity of the species. Ear-

lier, Pfeiffer (1967) reported that alarm substance from Genorynchiformes was also effective in Ostariophysi. Verheijen (1963) reported the presence of an alarm reaction to intraspecific skin extracts in nine species of cyprinid fish.

The fright reaction may be treated as a visual signal by other conspecifics, leading to rapid transmission of the signal through school of fish. The fright reaction is generally accepted to be mediated by olfaction. Although the fright reaction may be initiated by chemical stimulus, it can be transmitted through school of fish by vision.

It could be hypothesized that olfaction might be a primary cue in detecting alarm substance for giant danio (*Danio malabaricus*). The objective of this study is to figure out on which stimulus (chemical stimulus or visual stimulus) giant danio are usually depending in detecting alarm substance. If giant danio are mainly depending on chemical stimulus in detecting alarm substance, the next question was "Do fish mostly depend on olfaction (smell) or gustation (taste) cue in detecting alarm substance?"

Materials and Methods

Preparation of Experimental Fish

Giant danio were purchased from a local pet shop. Fish were allowed to acclimate in the tanks for 4 to 5 days before experiments. While 50 ml of water was poured through a glass funnel, the experimental fish were fed by the commercial dry diets. All experiments were performed during the night (between 19:00 and 22:00) to minimize unwanted disturbances. After each experiment was done, fish were caught and mixed with other fish. Before holding fish into the tanks for the next experiment, all tanks were clearly cleaned. The average size of fish used in this study was 5.35±0.29 cm and 1.36 ±0.22 g for total length and wet weight, respectively.

Preparation of Alarm Substance

Alarm substance was daily prepared by the method of Suboski et al., (1990). Briefly, several giant danio average weighing 1.35 g were used get 100 ml of alarm substance. Fish were sacrificed and then lightly cut 20 times on each side of fish. Then fish, scales, and released fluids were placed with 100 ml of aerated dechlorinated water together in the 2 ℓ flask. And the mixed solution in the flask was stirred for 10 minutes. The solution was strained through filter paper and used immediately. Three different types of experiments were performed.

Experiment I

The optimal concentration of alarm substance for giant danio to show the fright reaction was determined. With the transparent 15 ℓ tanks (35 ×21×26 cm), three concentrations of alarm substance (0.15, 1.5 and 15 ml) were prepared to get final concentrations of 10, 100 and 1,000 ppm in water. Paper divided into four vertical and two horizontal lines was attached to the back of the tanks, so that the movement of fish could be well observed based on these lines. While alarm substance was introduced into the tanks, behaviour of fish was recorded. Also the time for giant danio to show the initial fright reaction was measured after alarm substance was introduced.

Experiment II

To investigate which stimulus (chemical stimulus or visual stimulus) giant danio are responsible for detection of alarm substance in giant danio. Group of fish were composed of

three fish for easy observation. In this experiment, three different treatments were designed. While alarm substance was introduced into the tank, the commercial diets were supplied.

One treatment was that fish could detect alarm substance by both chemical and vision stimulus. To set up this treatment, a plastic panel with many big holes was inserted into the middle part of the transparent 30 ℓ glass tank (51×26×30 cm), so that the tank was divided into the two sections. After alarm substance was introduced into one section, it could be dispersed to the other section through big holes. Also fish in one section could see other fish in the other section through holes. Therefore, fish could detect alarm substance by both chemical and vision stimulus.

Another treatment was that fish could detect alarm substance by chemical stimulus, but not vision stimulus. To set up this treatment, the plastic panel with many small holes after wrapped with black nylon was inserted into the middle part of the transparent 30 ℓ glass tank as same as above treatment. After alarm substance was introduced into one section, it could be dispersed to the other section through holes, but fish in one section could not see other fish in the other section. Therefore, fish could detect alarm substance by only chemical stimulus.

The other treatment was that fish could detect alarm substance by vision stimulus, but not chemical stimulus. To set up this treatment, the 15 ℓ glass tank was closely placed to the 30 ℓ tank. So fish in 15 ℓ tank could detect alarm substance by vision stimulus while fish in the 30 ℓ tank showed fright reaction after alarm substance was introduced. The behaviour of fish after detecting alarm substance were recorded.

All observations followed behavior and response in two out of three fish. After behaviour of fish in the tank was observed for 10 minutes, alarm substance was introduced with diet. And then fish were observed for the next 20 minutes. The times for fish to show the initial fright reaction were compared.

Experiment Ⅲ

Group of olfaction-deprived fish and normal fish were placed into the 30 ℓ tanks. In this experiment, two treatments were designed. While alarm substance was introduced into the tank, the commercial diets were supplied.

One treatment was that group of three fish could detect alarm substance by both olfaction and gustation. And the other treatment was that group of three olfaction-deprived fish plugged nares by applying super glue could detect alarm substance by only gustation, but not olfaction. Before applying super glue to the nares of fish, group of fish were anesthetized in MS 222 for 2 minutes. Olfaction-deprived fish were given 2 to 3 days to recover from the stress. Olfaction-deprived fish seemed to be healthy because fish fed diet well before the experiment was performed. The experiment was observed for 40 minutes (10 minutes of the pre-experiment and 30 minutes of the experiment). After alarm substance was introduced into the tank, the frequency of the fright reaction (strong chasing referred that a fish swam fast and another fish followed it. Sometimes, a fish was nipping the tail part of others) were compared.

Duncan's multiple range test was employed for statistical analysis (Duncan, 1955). The difference was considered to be significant at the level of 5 %.

Results

Experiment I

Giant danio showed the different types of the fright reaction depending on the concentration of alarm substance introduced. When 0.15 ml alarm substance (10 ppm) was introduced into the tank, fish did not show any change of swimming pattern. But when 1.5 ml alarm substance (100 ppm) was introduced into the tank, fish showed the fright reaction about in 6 minutes. After detecting alarm substance, fish stayed on the bottom of the tank and showed motionless and a long period of visual alertness as the fright reaction. When 15 ml alarm substance (1,000 ppm) was introduced into the tank, fish showed the fright reaction in a few seconds. Fish did not move and gathered closely. Also fish strongly avoided the place of the tank where alarm substance was introduced. And at 1,000 ppm of alarm substance, fish showed the typical fright reactions, such as suppressing to feed on diet, no swimming, strong chasing, and visual alertness. Because fish showed the fright reaction well at 1,000 ppm of alarm substance, this concentration was used for all following experiments.

Experiment II

Before giant danio detected alarm substance, fish in all treatments fed diet well and swam normally. After detecting alarm substance, fish in both treatments (chemical and vision stimulus or chemical stimulus) showed a lot of frequency of chasing and were suppressed to feed diet. Although diet remained a lot in both treatments, fish almost never feed any diet for 1 hour. However, fish which detected alarm substance by vision stimulus showed a few frequency of chasing during the first 30 minutes after alarm substance was introduced, but fish fed diet well. And fish did not show any chasing reaction and swam normally during the next 30 minutes.

In three of four times, fish which detected alarm substance by chemical stimulus were very suppressed to feed diet and showed the increased visual alertness and motionless and gathering closely. Most fish showed the fright reaction within a few minutes (Table 1) and almost never fed any diet during 20 minutes observation. There was no notable difference in the time for fish to show the initial fright reaction between both chemical and vision stimulus treatment and chemical stimulus treatment. But the times for fish to show the initial fright reaction in both treatments were significantly shorter than that in vision stimulus treatment.

Unlike above, once fish showed the very strong chasing reaction after detecting alarm substance. Chasing frequency per minute was

Table 1. The time for giant danio to show the initial fright reaction depending on 3 different stimulus

Treatments	Time for fish to show the initial fright reaction (minute)			Mean ± variance
Chemical and vision stimulus	1	5.3	4	$3.4^{a} \pm 4.9$
Chemical stimulus	3.3	0.25	3	$2.2^{a} \pm 2.8$
Vision stimulus	20	17	16	$17.7^{b} \pm 4.3$

Mean in column with same superscript letters are not different at $P<0.05$.

counted every 5 minutes for 1 hour (Fig. 1). Chasing frequency in both chemical and vision stimulus treatment and chemical stimulus treatment were higher than that in vision stimulus treatment. Except in 35 minutes after alarm substance was introduced, there was trend toward in decrease of chasing frequency.

Experiment III

Shortly after alarm substance was introduced into the tank, fish which detected alarm substance by both olfaction and gustation showed fast swimming and strong chasing. And fish did not take any diet and stayed on the bottom of the tank. Fish seemed to be very suppressed to feed diet. Although a lot of diets had remained on the surface of water, normal fish did not feed any diet and showed strong chasing reaction as the fright reaction (Fig. 2). However, olfaction-deprived fish showed a few of chasing reaction, but fed diet after alarm substance was introduced. There was significant difference in chasing frequency between olfaction-deprived fish and normal fish.

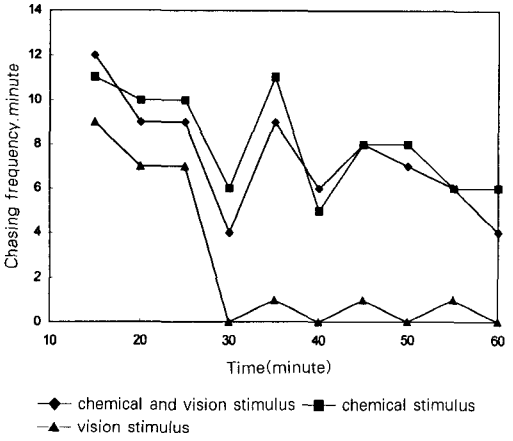

Fig. 1. Changes of chasing frequency for giant danio after detecting alarm substance by chemical and vision, chemical, or vision stimulus.

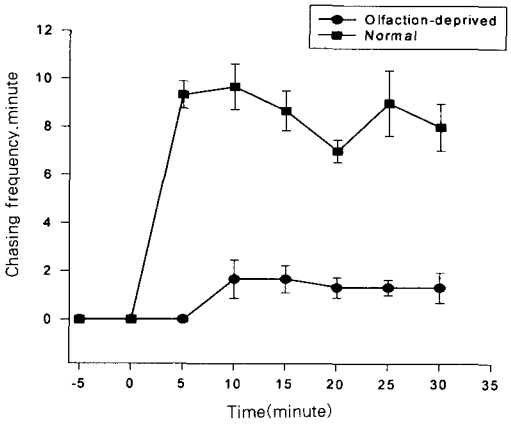

Fig. 2. Changes of chasing frequency for the normal and olfaction-deprived giant danio at 5 minute interval after detecting alarm substance (mean±standard error).

Discussion

When alarm substance was introduced into the tank, giant danio avoided the area where alarm substance was infused. This result was consistent with Hemmings (1966)'s study. After fish detected alarm substance, fish showed the following fright reactions in this study; suppressing to feed diet, no swimming (motionless), strong chasing, visual alertness or fast swimming,

The time for giant danio to show the initial fright reaction was different depending on by which stimulus fish detected alarm substance (Table 1). There was no significant difference in the time for fish to show the initial fright reaction between both chemical and vision stimulus treatments and chemical stimulus treatment. Also more chasing frequency was observed in both treatment (Fig. 1). Fish detected alarm substance by vision stimulus showed the weak fright reaction (a few times of chasing) during 30 minutes. In some extents, fish also

showed the fright reaction by vision stimulus. But soon after detecting alarm substance by vision stimulus, fish showed the stable condition (swam normally) because fish could not detect any alarm substance by chemical stimulus. This indicated that giant danio mainly detected alarm substance by chemical stimulus. But we can not conclude that fish could not detect alarm substance by vision stimulus yet because fish showed the fright reaction in some extents.

It was difficult to plug nares of fish by applying super glue. Also sometimes applied super glue was off in 2 or 3 days after plugged because of movement of fish. Olfaction-deprived fish showed some alertness, but fed diet well as before the experiment started. Olfaction-deprived fish showed the weak fright reaction after detecting alarm substance by gustation (Fig. 2). But the normal fish which had the function of gustation and olfaction showed the very strong fright reaction (strong chasing) after detecting alarm substance. This result indicates that giant danio mainly detect alarm substance by olfactory stimulus.

Male fathead minnows lost the cells which contain alarm substance during the breeding season (Smith, 1973) and female-donor extract was less potent than male-donor alarm substance in zebra fish (Gandolfi et al., 1968). The difference of the for giant danio to show the initial fright reaction in this experiment may be due to these reasons. Reed (1969) reported that fish showed the different types of the fright reaction depending on the different habitats. After perceiving the alarm substance, the top-water fish was generally motionless, the mid-water fish was rapid swimming and darting, and the bottom-water fish showed motionless. Also the initial fright reaction of fish after detecting alarm substance were reported in several ways; increased respiration rate (Pfeiffer, 1962), increased visual alertness (Pfeiffer and Riegelbauer, 1978), increased rate of cover seeking, cohesion and polarization of fish (Heczko and Seghers, 1981). The influence of alarm substance on feeding in zebra danio was also reported (Jakobsen and Johnser, 1989). In their studies, zebra danio fish normally preferred high density of prey (*Daphnia magna*), but when exposed to alarm substance, they preferred lower and less confusing prey densities.

In considering these results, it may be concluded that giant danio are mainly depending on the chemical cue, especially olfaction stimulus, in detecting alarm substance. Further study is needed to improve the technique for blocking gustation of fish in order to figure out how extent fish can detect alarm substance by gustatory stimulus.

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