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A test of time-place learning in a cichlid fish

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Abstract

This paper presents a test of time-place learning in fish. Convict cichlids, *Cichlasoma nigrofasciatum*, were offered food several times a day for 10–30 consecutive days. A signal was given 1 min before each food presentation. If the food was always delivered in the same corner of the aquarium, the fish spent 66% of their time in that corner after the signal was given. But if the food was given in different corners throughout the day, each corner being associated with a specific daily time, the fish failed to show preference for the target corner, even after 30 days. Instead they learned which corners yielded food at any time of the day and visited these corners successively after the feeding signal was given. Failure to associate time and place may have been caused by a low cost of travel between corners, a limited number of rewards each day, and/or interference from learning the signal-food association.

Key words: *Cichlasoma nigrofasciatum*; Convict cichlid; Foraging; Time-place learning

Introduction

For some animals, food availability may vary spatio-temporally throughout the day. A prey item may be available only in the morning at one place, and only in the afternoon at a different place. If this pattern remains consistent from day to day, an efficient forager should learn to visit specific places at specific times of the day (a behaviour called time-place learning). To date, the only published evidence of time-place learning has come from studies by Wahl (1932) and Gould (1987) on honey bees (*Apis mellifera*), and by

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Biebach et al. (1989) on garden warblers (*Sylvia borin*). In the latter study, warblers in the laboratory could visit four different feeders but each feeder offered food at a different time of day. After 11 days, the birds had learned to go to the correct feeder at the correct time.

To my knowledge, time-place learning has never been tested in fish. Yet there is no compelling reason to believe that fish could not show learning of this type. With a few exceptions, the learning ability of fish does not qualitatively differ from that of birds and mammals (Gleitman and Rozin, 1971). Fish also have circadian clocks (e.g. Kavaliers 1978, 1980, 1981) which seem to be an important part of the mechanism of time-place learning (Biebach et al., 1991). Finally, the ecological benefits of time-place learning (increased foraging efficiency) apply equally to fish as to other animals.

In this study, I tested time-place learning in the convict cichlid (*Cichlasoma nigrofasciatum*). Convict cichlids are small freshwater fish native to Central America. They live in streams and small lakes, where they usually feed on invertebrates and algae (Konings, 1989). In the laboratory, this species is capable of learning to differentiate between food patches of unequal value and predictability (Grand and Grant, 1993). It also shows evidence of circadian rhythmicity (Tobler and Borbély, 1985; Reeb and Colgan, 1991).

Materials and Methods

Experiments I, II and III

Only juvenile cichlids (total length 2.9–4.5 cm) were used. Juveniles usually have robust appetites and their behaviour is unaffected by reproductive interactions. Each experiment involved four solitary individuals and four groups of five individuals. Groups were used in addition to solitary individuals because learning is sometimes enhanced in group situations (Welty, 1934; O'Connell, 1960).

Eight aquaria (32 × 62 × 31 cm) were equipped with a water heater that maintained temperature at 26–28°C, and with a 'corner' filter installed midway along one of the long sides of each tank. In all four corners a vertical tube led from the surface to a 5 × 5 cm white Plexiglas sheet lying on the bottom. Small granules of Kyowa dry food were dropped directly into the tubes at feeding time, but a cover prevented fish from seeing in which corner the food was being dropped. Only after the granules had sunk to the bottom could fish see that food had arrived in a particular corner. Each aquarium was surrounded by partitions which prevented fish from seeing activity outside their own tank. However, these partitions were distant enough from the tanks to permit illumination by overhead fluorescent lights. At one end of each aquarium there was a one-way mirror through which observations could be made (lights did not shine on the observer behind the one-way mirror).

Lights came on at 0700 h and went off at 1900 h. All fish were fed four times a day, at 0830, 1130, 1430, and 1730 h (these times represent the mid-points of each of the 3-h periods that make up a 12-h day). At each feeding session the procedure was the same: first the observer noted which corner of the tank each fish was closest to; then the air supply to the filter was turned off as a signal to the fish that they were about to be fed; at 5-s intervals for the next 60 s, the nearest corner to the fish (or, in the case of groups, the majority of fish) was noted; food (4–5 mg/fish) was then dropped into the appropriate tube; and 2 min later the air pump was restarted.

In experiment I, all feeding sessions were in the same corner for a given aquarium (for each tank, this corner was initially chosen at random). This experiment did not test time-place learning *per se*; rather it tested whether fish could distinguish among corners, a necessary condition for time-place learning. This experiment lasted 10 days.

In experiment II, the morning (0830 and 1130 h) feeding sessions were both in the same corner (initially chosen at random for each tank, but thereafter remaining the same from day to day) and the afternoon (1430 and 1730 h) sessions were both in the diagonally opposed corner. In experiment III, each of the four feeding sessions was associated with a different corner (initially randomized for each tank), but the associations remained the same from day to day. Experiments II and III lasted 18 days each.

The percentage of time spent in each corner was inferred from the scan sampling conducted between signal and food drop. (Scan sampling yields frequency data which can be used to estimate percentage of time spent in various activities or places; see Lehner 1979.) Throughout the paper, 'target corner' refers to the corner in which food was going to be dropped. To determine whether time-place learning had taken place, I defined the following criterion: after the feeding signal, a fish had to spend more time in the target corner than in any of the other corners, at all daily feeding times and for two consecutive days. The probability of this happening by chance during a 18-day experiment is 0.00026 if we assume a 25% chance of preferring a given corner, or 0.06 if we assume a 50% chance of preferring a given corner (see results of experiment II).

Experiment IV

This experiment was similar to Experiment II except that: (1) larger aquaria ($33 \times 124 \times 52$ cm) were used; (2) only two feeding tubes were installed, one at each end, and rocks placed in front of the bottom plates prevented the fish from seeing the food from a distance; (3) there were only two feeding times, at 1000 and 1600 h; (4) only four solitary juveniles (no groups) were tested; and (5) the experiment lasted 30 days. Food (9–10 mg/fish) was delivered at one end of the tank in the morning (this end was initially chosen at random, but thereafter it remained the same from day to day) and at the other end in the afternoon. At feeding time, the filter was turned off, the first tube approached by the fish to within 15 cm was noted, the amount of time spent in the target half-tank during the following minute was measured with a stopwatch, and finally the food was dropped into the tube. This experiment was designed to test whether a simpler feeding schedule, a larger distance separating the feeding stations (30–40 times the total length of the fish), and a longer duration of the experiment would facilitate expression of time-place learning. However, because of the more limited number of choices a fish could make and the resulting greater probability of making a correct choice just by chance, the criterion for time-place learning was modified to four, rather than two, consecutive days of correct choices. The probability of this happening by chance in a 30-day experiment is 0.08.

Results

In all experiments, no qualitative or quantitative difference could be detected between the behaviour of solitary and grouped fish. Outside of the feeding sessions, groups tended to break up and spread themselves evenly across the aquaria; they did not tend to be near

TABLE 1

Location of grouped convict cichlids ($n = 5$ in each group) within their tank before the signal was given at each of four daily feeding times

Time	Experiment	Corner				χ^2 (P)
		target	near neighbour	far neighbour	diagonal	
0830	I	5	5	5	5	0.0 (NS)
	II	3	6	6	5	1.2 (NS)
	III	6	2	3	9	6.0 (NS)
1130	I	4	6	4	6	0.8 (NS)
	II	6	5	7	2	2.8 (NS)
	III	6	4	2	8	4.0 (NS)
1430	I	5	5	3	7	1.6 (NS)
	II	3	13	3	1	17.6 (< 0.001)
	III	4	6	7	3	2.0 (NS)
1730	I	5	7	5	3	1.6 (NS)
	II	3	9	5	3	4.8 (NS)
	III	5	5	4	6	0.4 (NS)

'Target' refers to the corner where food was to arrive, 'near neighbour' to the closest corner to the target, 'far neighbour' to the second closest corner, and 'diagonal' to the corner diagonally opposed to the target. Data show the total number of fish near each corner, added over the last four days of the experiment, for three representative groups (one each from experiments I, II and III). The χ^2 -goodness-of-fit test was performed on the totals against a uniform distribution, assuming independence among days. NS = non-significant.

the target corner before feeding time (Table 1). However, as soon as the feeding signal was given, the fish quickly assembled in a coherent school that swam vigorously from corner to corner. Solitary fish also appeared to be located at random relative to the target corner before the feeding signal (Table 2), but they started swimming from corner to corner once the signal was given. Hereafter, results from individuals and groups are presented together.

In Experiment I (same-corner feeding), fish quickly learned which corner yielded food. The average percentage of time spent near the target corner rose from about 25% (chance level) on the first day to 59% on the third day (Fig. 1). From the fifth day on, values remained stable around 66%. The remaining 34% was spent almost evenly between the two corners nearest to the target corner. These results indicate that the fish could learn to discriminate between corners and that their preference for the target corner could lead to a fairly high percentage of time spent near it.

In Experiment II (two-corner feeding), none of the fish or groups of fish met the criterion for time-place learning. The fish nevertheless seemed to learn which two corners yielded food, and in the minute following the feeding signal they swam back and forth between these two corners with few visits to the other corners. They showed a tendency to first go to the appropriate target corner in the morning, but not in the afternoon: pooling the last four days of the experiment and all eight tanks, fish in the morning went 22 times to the morning target corner first, 15 times to the afternoon target corner, and 27 times to any of the other two corners; in the afternoon, they went 22 times to the morning target corner

TABLE 2

Location of solitary convict cichlids within their tanks before the signal was given at each of four daily feeding times

Time	Experiment	Corner				χ^2 (P)
		target	near neighbour	far neighbour	diagonal	
0830	I	4	5	6	1	3.5 (NS)
	II	5	6	3	2	2.5 (NS)
	III	4	5	3	4	0.5 (NS)
1130	I	6	1	6	3	4.5 (NS)
	II	5	8	2	1	7.5 (0.05)
	III	2	1	6	7	6.5 (NS)
1430	I	7	0	6	3	7.5 (0.05)
	II	3	1	4	8	6.5 (NS)
	III	3	7	4	2	3.5 (NS)
1730	I	4	3	5	4	0.5 (NS)
	II	3	0	5	8	8.5 (< 0.05)
	III	5	4	3	4	0.5 (NS)

Headings and abbreviations are as in Table 1. Data show the total number of times a fish was found near each corner, added over the last four days of each experiment and pooling all four fish in each experiment. The χ^2 -goodness-of-fit test was performed against a uniform distribution, assuming independence among days.

first, 16 times to the afternoon target corner, and 26 times to any of the other two corners. The total percentage of time spent in the morning and afternoon target corners reached 80% after 8 days, and remained stable thereafter (Fig. 2). In the morning (0830 and 1130

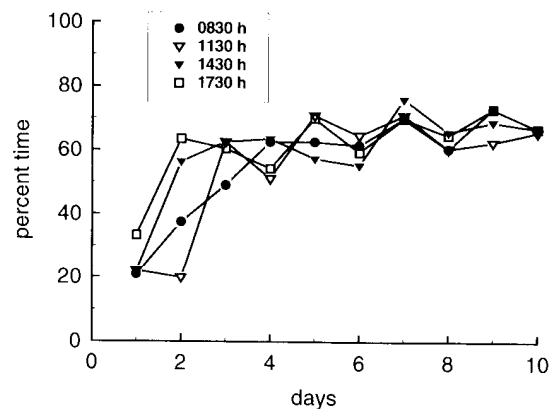


Fig. 1. Percentage of time spent by convict cichlids in the target corner of their aquarium during the min following a feeding signal, at four different times of day and on consecutive days, when food is always delivered in the same corner. Data points are the means of four solitary fish and four groups of five fish.

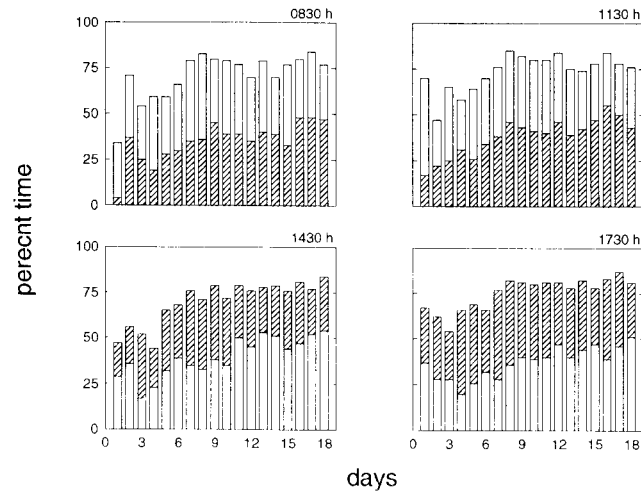


Fig. 2. Percentage of time spent by convict cichlids in the morning target corner (bottom part of each stack) and in the afternoon target corner (top part of each stack) of their aquarium during the min following a feeding signal, at four different times of day (panels) and on consecutive days, when food is always delivered in the same corner in the morning and in the diagonally opposed corner in the afternoon. Hatched bars indicate the target corner for that particular time of day. Data are the means of four solitary fish and four groups of five fish.

h) the fish on average spent more time in the morning target corner than in the afternoon target corner (average for all eight tanks over the last 4 days and over both 0830 and 1130 h: morning target corner = 46%, afternoon target corner = 33%). However, in the afternoon (1430 and 1730 h) the fish continued to prefer the corner where they had been fed in the morning; accordingly they spent on average less time in the afternoon target corner (33%) than in the morning target corner (48%).

In Experiment III (four-corner feeding), none of the fish or groups of fish met the criterion for time-place learning. In fact, none of the fish showed even one complete day of correct preferences. After the feeding signal, fish swam quickly around the aquarium, going from one corner to another in no apparent order. The fish showed no consistent tendency to go to the target corner first (Table 3), and they spent similar amounts of time in all corners (Table 4).

In Experiment IV (two-distanced-corner feeding), none of the four fish met the criterion for time-place learning. After the feeding signal was given the fish swam first to the middle of the tank near the filter, and then back and forth from one end of the tank to the other. They did not show a consistent tendency to first go to the target corner, nor did they spend consistently more time in the target half-tank during the minute that followed the feeding signal (Table 5).

Discussion

The convict cichlids used in this study did not show solid evidence of time-place learning. Instead, they learned which corners yielded food at any time of the day, and quickly inspected these corners in turn once the feeding signal was given, irrespective of the daily time. One can argue that more than 18–30 days may be needed for the fish to

TABLE 3

Number of times each corner was visited first by convict cichlids after the feeding signals in Experiment III

Time	Corner				
	target	near neighbour	far neighbour	diagonal	previous target
0830	9	8	7	8	4
1130	6	12	7	7	7
1430	12	8	3	10	8
1730	12	8	6	6	10

'Previous target' refers to the corner where food had arrived during the last preceding session. Other headings are as in Table 1. Data show the totals added over the last four days of the experiment for all eight experimental tanks.

TABLE 4

Percentage of time spent near each corner by convict cichlids during the four daily feeding sessions of Experiment III

Time	Corner				
	target	near neighbour	far neighbour	diagonal	previous target
0830	29.4	20.4	26.1	24.0	30.5
1130	18.3	29.0	22.4	30.3	24.5
1430	27.1	24.8	21.9	26.2	26.0
1730	31.6	25.4	24.3	18.7	29.5

Headings are as in Table 3. Values are the means of all eight experimental tanks over the last four days of the experiment.

TABLE 5

Percentage of times when convict cichlids correctly approached the target corner first and percentage of time spent near the target corner in Experiment IV

	% correct choice		% time	
	am	pm	am	pm
first 10 days	58	38	58	43
middle 10 days	48	52	53	48
last 10 days	48	46	58	40

Values are the means from four solitary fish over each of the three 10-day periods of the experiments. 'am' and 'pm' refer to the morning and afternoon feeding session respectively.

learn spatio-temporal tasks. However, the results did not suggest this possibility. From the eighth day on, the percentage of time spent in each corner remained relatively stable; at the very least, there was no indication that it increased simultaneously at all target corners.

Other conditions in the present experiments may have impeded time-place learning. First, the ease with which fish could sample the different corners in succession may have made it unnecessary to associate a specific time with a specific corner, even in the distanced-corner experiment. There was little cost to not being in the right place at the right time, as a fish could quickly correct a mistake by swimming to the corner where food had arrived. The only possible cost was in the group situation, where a correctly positioned conspecific could eat most of the food before the other fish arrived. This potential cost was mitigated by the fact that groups tended to remain together during their movements from corner to corner.

Second, the use of only four discrete feeding times each day may have unduly limited the number of rewards for the fish and the chances to associate time and place. Finally, the use of a feeding signal led to the formation of a signal-and-food association which may have interfered with the time-place learning task. The fish may have come to rely more on a signal-food association than on a time-place association to obtain food. To circumvent these problems, it would be appropriate to feed the fish more or less continuously for long daily periods, without feeding signals, and successively at places that are widely separated. Such an experiment is in progress.

Choice of the study species may also be important. Convict cichlids appear to be omnivorous: in nature they feed on both animal and vegetable matter (Konings, 1989). Yet, because mobile animal prey are more likely to show daily spatio-temporal routines than more sedentary prey, one could expect time-place learning to evolve mostly in strictly carnivorous species feeding on wandering prey. Omnivorous species may simply switch prey rather than switch place when one type of food becomes less abundant geographically. Future studies of time-place learning could use fish species that are piscivorous or insectivorous and fairly specialized in their choice of prey species, as well as species that have to travel long distances during their foraging activities.

In the present experiments, convict cichlids spent similar amounts of time in the corners that yielded food at any time of the day. This suggests that the fish adjusted their foraging behaviour to the daily, rather than time-specific, profitability of each corner. A test of this idea would require the use of feeding stations with various degrees of daily profitabilities. For example, one could feed fish twice in the morning in corner A, once in early afternoon in corner B, and once in late afternoon in corner C, and see whether the fish allocate their time between the corners in a 2 : 1 : 1 ratio at all feeding times.

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