LAKEHEAD UNIVERSITY

FOOD and MIRROR IMAGE as REINFORCERS in DISCRIMINATION REVERSAL LEARNING with SIAMESE FIGHTING FISH (*Betta splendens*)

by

ALEXANDER RNIC [1]

A THESIS
SUBMITTED to the FACULTY of ARTS
in PARTIAL FULFILLMENT of the REQUIREMENTS
for the DEGREE of MASTER of ARTS

DEPARTMENT of PSYCHOLOGY
THUNDER BAY, ONTARIO, CANADA
1974

ABSTRACT

The present study attempted to examine the effects of food and mirror image reinforcement on discrimination reversal learning in Siamese Fighting Fish (*Betta splendens*). The operant task used for both reinforcement groups was a spatial two-choice discrimination reversal in which a visual cue was correlated with the unreinforced response alternative. Previous research has indicated that in the limited number of operant tasks employed, food and mirror image reinforcement produced marked differences in behavior.

A secondary purpose of the study was to demonstrate a progressive improvement of performance in discrimination reversal tasks using fish. The majority of previous research has demonstrated that fish do not show a progressive improvement in discrimination reversal learning when food is used as reinforcement. Mirror image was also used in the present study not only to examine possible differences or similarities in behavior as a function of an alternative type of reinforcement but also because mirror image had not been previously used as a reinforcer in discrimination reversal learning paradigms.

Both food and mirror image subjects were tested employing methodological conditions which have been suggested to be important factors in maximizing discrimination reversal performance in fish. These methodological conditions were continuous trials training, unlimited correction procedures, increased distance between the response alternatives, and the location of reinforcement adjacent to that of the response mechanisms.

It was found that in the operant task used, food reinforcement subjects demonstrated a learning of the reinforcement contingencies in each discrimination problem. In contrast mirror image reinforcement subjects demonstrated only initial learning beyond which performance failed to reach the criterion level. The negative findings of the mirror image reinforcement group were discussed in terms of interference effects resulting from elevated arousal levels produced by mirror image presentation.

A progressive improvement in successive discrimination reversals for food reinforcement subjects was also observed. This improvement was attributed to the use of the methodological conditions previously suggested to maximize reversal performance in fish. An alternative hypothesis was also offered, stating that performance may have been a function of visual cues present in the test situation.

To my parents who gave everything for me.

ACKNOWLEDGEMENTS

The preparation of this manuscript during the past year of graduate study could not have been accomplished without the assistance of Dr. S. R. Goldstein. In this respect I thank Dr. Goldstein not only for his assistance as my thesis advisor, but also for the many hours of advice and consultation which he freely offered during the five years of my association with him. Special thanks must also go to Dr. R. P. Fisher of my Thesis Committee, for his helpful comments and insights concerning this manuscript, and to Mr. K. P. Sumpter (Glassblower, Science Faculty), who was instrumental in the construction of certain apparatus used in this study.

[1] This work was completed while the author was an Ontario Graduate Fellow at Lakehead University, Ontario, Canada.

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In spatial two-choice discrimination tasks, two response alternatives are present in the experimental situation, of which one is reinforced, and the other is unreinforced. During the original discrimination problem (R_0), the subject must learn to respond to the reinforced response alternative to a fixed criterion. Upon reaching this criterion, the discrimination problem is reversed and the subject must now learn to respond to the other response alternative until the same criterion is reached. Problem reversals continue until an asymptotic level of performance has been achieved.

It has been demonstrated with rats that the first problem following the original discrimination problem (R_0) is characterized by a sharp increase in errors (North, 1950). With subsequent reversals, learning of each new discrimination improves to the extent that rats will learn the reversal discrimination faster than they learned the original discrimination problem (Dufort, Guttman & Kimble, 1954). The slower learning (increased number of errors) following the initial discrimination may be characterized as a product of negative transfer. This is because rats demonstrate a preference for the previously reinforced response alternative until a new preference for the other response alternative (now reinforced) is progressively learned (Calhoun & Handley, 1973). With continued training in reversing between discrimination problems, the number of errors in responding to the previously reinforced response alternative progressively decreases. This regular decrease in errors per reversal constitutes progressive improvement in successive discrimination reversal tasks (SDR), and is characteristic of the performance of rats (e.g., Dufort, Guttman & Kimble, 1954; Gonzalez, Berger & Bitterman, 1966; Mackintosh, McGonigle, Holgate & Vanderver, 1968).

The behavioral effects of reversal learning tasks has been of interest to operant and comparative psychologists since Yerkes and Huggins (1903) demonstrated that crayfish show a minimal progressive improvement in the rate of learning to reverse between discrimination problems. For comparative purposes, other researchers have attempted to study reversal learning behavior in a number of animal species. Reversal learning has been shown using isopods (Thompson, 1957), ants (Schneirla, 1939), newts and terrapins (Seidman, 1949), pigeons (Schade & Bitterman, 1966), rats (e.g., Cowles, 1937; Gatling, 1952; Fritz, 1931; Krechevsky, 1932; North, 1950), monkeys (Harlow, 1944), and chimpanzees (e.g., Nissen, Reisen & Nowlis, 1938; Reisen, 1940). Research efforts using fish (e.g., African Mouthbreeders, <u>Tilapia macrocephala</u>) were originated by Wodinsky and Bitterman (1957) and Bitterman, Wodinsky and Candland (1958). Food reinforcement for an operant "pressing" response was used in these and subsequent studies.

Based on these initial attempts, Bitterman and his co-workers concluded that fish do not show a progressive improvement in SDR where the task employs either visual or spatial cues. Each new problem was just as difficult to learn as the previous problem. However, based on recent findings, the presence or absence of progressive improvement in fish has been a source of disagreement among a number of investigators.

Evidence for the Absence of Progressive Improvement in Fish

The majority of studies have indicated that fish do not demonstrate a progressive improvement in SDR (e.g., Behrend & Bitterman, 1967; Behrend, Domesick & Bitterman, 1965; Behrend, Jennings & Bitterman, 1968; Bitterman, 1965a, 1965b; Bitterman, Wodinsky & Candland, 1958; Gonzalez, Behrend & Bitterman, 1967; Warren, 1960, 1961). These studies showed that following the original discrimination, there is marked negative transfer effect similar to that demonstrated by rats. However, as reversals continue, there is no decrease in the number of errors per reversal, since subjects continually return to the previously reinforced response alternative. Failure to reduce errors indicates a failure to learn in reversal situations. In this respect Bitterman and his co-workers concluded "...that experiments on habit reversal tap an intellectual capability of higher animals that is not at all developed in the fish...[1965b, p. 96]." Bitterman (1965a) further states that "...there is no progressive improvement, but instead some tendency towards progressive deterioration as training continues...[p. 399]."

Evidence for Progressive Improvement in Fish

An interesting feature of research in this area is that although Bitterman and his co-workers have maintained that fish show no improvement in SDR, these authors also present evidence which supports the opposing hypothesis that fish can learn to reverse with increasing improvement between discrimination problems. For example, Behrend, Domesick and Bitterman (1965) state that:

> Although the majority of the individual curves are essentially flat (i.e., show no change in the difficulty of reversal as training continues), some rise and others fall. In two or three cases the individual curves have much the same shape as the curves for entire groups of higher animals which are said to show progressive improvement in habit reversal [p. 411].

This evidence suggests that fish are capable of progressive improvement, although occurrences of progressive improvement may be limited to a small number of subjects in any experimental group. Based upon this finding other researchers, independent of Bitterman's laboratory, have taken the position that fish are capable of progressive improvement in SDR, and that the source of improvement is a function of methodological conditions which provide an optimal environment for learning.

Using two-choice discrimination reversal tasks, Mackintosh and Cauty (1971), Setterington and Bishop (1967), and Squier (1969) demonstrated a progressive improvement in reversal learning. Other studies have also reported improvement in SDR but of a different nature. For example, Woodard, Schoel and Bitterman (1971), employing a unitary reversal paradigm, reported an improvement in SDR using goldfish (*Carassius auratus*). In a unitary situation, the discriminanda are presented singly and the latency or rate of response to either the positive or negative stimulus is measured. The authors found that improvement was atypical and not similar to performance observed in two-choice discrimination reversal learning. For example, progressive improvement in the unitary situation was characterized by a slowing of response to both the unreinforced response alternative (S^-) and the reinforced response alternative (S^+). Improvement in reversal stemmed from the greater change in response to S^- than in response to S^+. This pattern of improvement was not replicated by Woodard and Bitterman (1972).

As a result of these positive and negative findings, no firm conclusion can be made concerning the presence or absence of progressive improvement in SDR using fish. However, before concluding that the fish is capable or incapable of progressive improvement in SDR, one must "...be certain that optimal conditions for improvement have been considered [Setterington, 1967, p. 2]." In those studies reporting a progressive improvement in learning it is apparent that a number of methodological conditions have been isolated and used to enhance reversal performance.

Methodological Conditions in Progressive Improvement

Methodological conditions which have been used to facilitate progressive improvement in SDR may be divided into two categories. These are conditions which are specific to reversal learning and conditions which are non-specific, i.e., they will facilitate performance in any operant task.

Specific Conditions:

(a) Continuous trials. Based on the successful findings of Setterington and Bishop (1967), Bitterman (1969) suggested that the short inter-trial interval (2 sec) used in discrete trials training was the important factor in improved performance. A short inter-trial interval favours a win-stay, lose-shift strategy based on a carry-over from trial to trial of the sensory consequences (e.g., reinforcement) of response. With long inter-trial intervals (e.g., 10 sec), there does not appear to be any carry-over (Bitterman, 1969). Therefore, an inter-trial interval of 0 sec (i.e., continuous trials) would in all probability be most effective in facilitating performance if Bitterman's (1969) formulations are correct.

(b) Correction procedures. Setterington and Bishop (1967) suggested that an unlimited correction procedure, as opposed to non-correction, may facilitate SDR performance. This allows the subject to correct a wrong selection, by permitting the subject to make additional responses before the trial is terminated. Continuous trials training automatically allows a correction procedure to be in effect.

(c) Increased spatial discriminability. Setterington and Bishop (1967) also stated that an increased distance between response alternatives may allow increased discriminability and thereby enhance learning in spatial reversal tasks.

(d) Reinforcement proximity. Mackintosh and Cauty (1971) and Ames (1967) suggested that the location of reinforcement adjacent to that of the response mechanism is also a factor in improving performance in SDR. For example, rats and pigeons usually receive food reinforcement from a magazine located close to the response keys. In SDR studies using fish, however, reinforcement is usually dropped at the back of the tank, opposite the response keys (e.g., Bitterman, Wodinsky & Candland, 1958). Rats trained in analogous situations where food is dropped to the subject at the back of the cage, average nearly twice as many errors (Mackintosh & Cauty, 1971) as when food is presented adjacent to the response mechanism.

Non-Specific Conditions:

The progressive improvement in SDR reported by Squier (1969) may be attributed to the particular species of fish used (Oscars, *Astronotus ocellatus*) and the use of conditions which have been suggested to maximize general operant performance. However, these non-specific conditions must be considered as secondary in improving SDR performance, since they have also been present in the majority of studies reporting no improvement in reversal learning.

These non-specific conditions are (*a*) the use of large and non-confining tanks, (*b*) adequate visual stimulation in home tanks, since sudden exposure to visual stimuli can cause extreme reactivity to the point of behavioral disorganization, (*c*) the selection of a response task which facilitates responding on the part of the subject, (*d*) the elimination of inter-trial intervals in the dark (Marrone & Evans, 1966), and (*e*) increased visual discriminability between response alternatives by making each alternative different in visual appearance.

Although only a few studies employing fish have used increased visual discriminability, Hilgard and Bower (1966) and Kimble (1961) present evidence demonstrating that learning in mammals is greatly facilitated when there is more than one type of cue indicating which response alternative is correct or incorrect. For example, visual cues, when combined with spatial cues, maximize the rate of discrimination and reversal learning by rats (Restle, 1957) and by chicks (Warren, Brookshire, Ball & Reynolds, 1960). In such paradigms the two response alternatives are usually coloured white and black respectively and remain fixed in their locations, whereas the spatial position of reinforcement is successively reversed. Since the response alternatives become more discriminable due to the fact that they are each made to appear visually different to the subject, improved performance in SDR results. Based upon this evidence it is suggested that increased visual discriminability may also facilitate performance for fish in SDR tasks. However, Warren (1960) did not report a progressive improvement in spatial SDR using fish, when the visual cues (colour) were fixed as to location. Therefore the present study will employ visual cues in order to enhance response discriminability but in a manner different from such studies as Warren. In this respect a visual cue will be consistently correlated with either the reinforced or unreinforced response alternative and will not remain fixed in reference to spatial position.

Theoretical Approaches

The preceding discussion indicates that the presence or absence of progressive improvement in SDR using fish may be a function of the methodology used. Since improvement and non-improvement have been observed, all theories attempting to account for reversal learning in fish remain without conclusive empirical support. This is true of Warren's (1960) inhibitory model, Gonzalez, Behrend and Bitterman's (1967) proactive interference model and Mackintosh's (1969) attentional model.

Before a theoretical approach can be taken, the presence or absence of progressive improvement in reversal learning using fish must be firmly established. This may be accomplished in two ways. Since reversal learning in fish has been formally investigated using only four species of fish (viz., Mouthbreeders, Goldfish, Paradise fish, and Oscars), present research efforts must incorporate other fish species in SDR paradigms. This is because of the approximately 20,000 species of fish which exist (Brown, 1957), certain species may demonstrate a capacity for progressive improvement in SDR while others may not. For example, Gossette (1968) suggested that the presence of progressive improvement in some species and its absence in others is highly characteristic of birds. This finding may also be true of fish. Secondly, SDR in fish must also be examined under a wide range of reinforcement and experimental conditions, in order to observe if the presence or absence of progressive improvement is a function of methodological conditions. In this respect, it is suggested that use of the specific and non-specific methodological conditions as discussed will maximize performance in learning to reverse between discrimination problems.

Reversal Learning and Siamese Fighting Fish

Warren (1960) reported a pilot study in which Siamese Fighting Fish (*Betta splendens*) failed to demonstrate any form of progressive improvement in SDR. Warren's study and all other research efforts which have examined SDR in fish have used food as the only means of reinforcement. However, Siamese Fighting Fish are unique in that mirror image presentation can also serve as a reinforcer for this species (e.g., Thompson, 1963, 1968). Since mirror image has not been previously used in SDR tasks, it may be of value to examine the effects of this form of reinforcement as an alternative to food reinforcement.

Mirror image as reinforcement. Mirror image is considered a reinforcer since it will maintain behavior contingent upon mirror image presentation in an operant task. This effect may be due to the fact that mirror image acts as a releasing stimulus for aggressive behavior in the form of frontal and lateral displays (Simpson, 1968). The opportunity to engage in aggressive activity has been considered rewarding (e.g., Lorenz, 1961).

Although mirror image has been demonstrated to be a reinforcer, the problem arises as to whether or not mirror image reinforcement will support behavior in complex tasks such as SDR. Siamese Fighting Fish have been shown to operate effectively on continuous reinforcement schedules (e.g., Goldstein, 1967; Hogan, 1967; Thompson, 1963), but under more complex schedules such as fixed ratio (Hogan, Kleist & Hutchings, 1970) and variable interval (Goldstein, 1971), mirror image reinforcement is only marginally effective. Therefore it is not known how Siamese Fighting Fish will perform in SDR conditions for mirror image reinforcement which also serves as a stimulus for the release of intra-species aggression.

It has been demonstrated, however, that an opportunity to respond for stimuli which release aggression can be employed as an effective reinforcer in SDR using mammals. Tellegen, Horn and Legrand (1969) found that mice demonstrated progressive improvement in spatial reversal when reinforcement was the opportunity to attack a "victim mouse." Reinforcement in the form of food or an opportunity for aggression does not appear to produce any major differences in mammals (e.g., mice) with respect to progressive improvement in SDR. This conclusion, however, may not be representative of Siamese Fighting Fish.

Support for this position is given by Hogan, Kleist and Hutchings (1970) who tested two groups of Siamese Fighting Fish under various fixed ratio conditions using food and mirror image reinforcement, respectively. The results demonstrated that although the tasks were identical for both groups, the performance of these groups differed markedly. It was found that as the fixed ratio increased for the mirror image reinforcement group, the total number of responses per 12 hr session remained almost constant, while the number of reinforcements per session decreased. In the food reinforcement group, as the fixed ratio increased, the total number of responses per 12 hr session increased while the number of reinforcements remained almost constant. The authors concluded that "...these results suggest that the two reinforcers may depend on different mechanisms for their effect...[p. 356]." Therefore, it is highly probable that mirror image and food reinforcement may also produce different results in spatial reversal tasks.

<u>Comparability of mirror image and food reinforcement</u>. Although two studies have compared food and mirror image reinforcement (Hogan,

1967; Hogan, Kleist & Hutchings, 1970), a problem arises as to whether or not performance under both types of reinforcement may be accurately compared. For example, performance differences between food and mirror image reinforcement may be a function of differential reinforcement magnitudes. However it has been shown that the magnitude of reinforcement does not change behavior in food or mirror image reinforcement conditions (e.g., Behrend, Domesick & Bitterman, 1965; Hogan, Kleist & Hutchings, 1970). Differences between food and mirror image reinforcement in SDR may therefore be a qualitative function of the type of reinforcement used as Hogan, Kleist and Hutchings (1970) have suggested.

Purpose of study. The purpose of this study was two-fold. The first objective was to examine the behavioral effects of mirror image and food as two different reinforcers in identical operant tasks using Siamese Fighting Fish (*Betta splendens*). The operant task employed was spatial SDR in which a visual cue was correlated with either the reinforced or unreinforced response alternative. Concurrent with the first objective, the second objective was to determine whether or not a progressive improvement in learning to reverse between problems was present in the SDR situation as described. Both reinforcement groups were also tested under conditions (specific and non-specific) which had been previously employed to facilitate discrimination reversal performance in fish.

Method

Subjects

Twelve experimentally naive male Siamese Fighting Fish (*Betta splenden*) were used. Plate 1 is representative of the appearance of

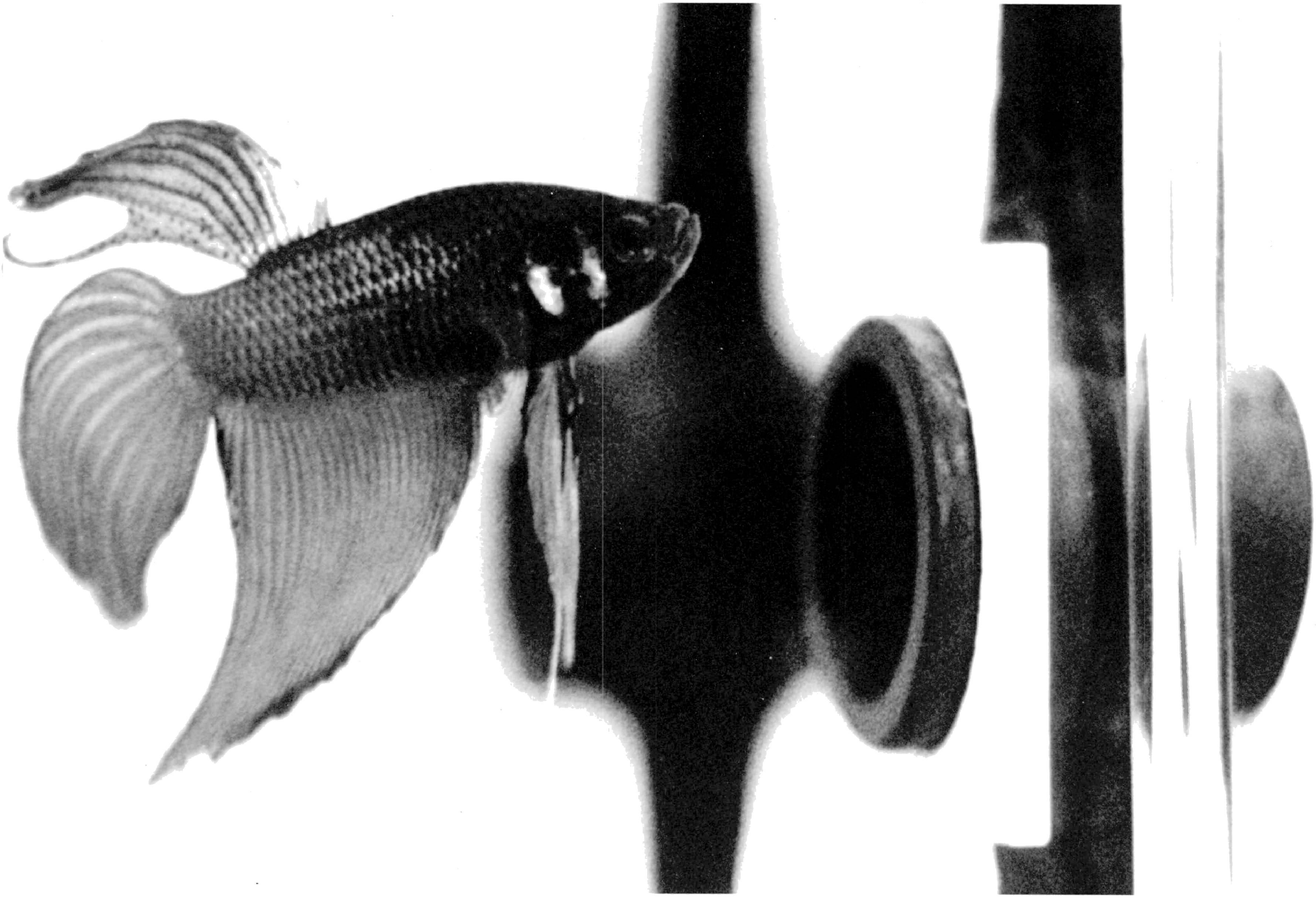

Plate 1. Male Siamese Fighting Fish.

this species. All subjects were sexually mature and were obtained from a Florida (United States), commercial dealer. One subject died following 28 days of testing.

Apparatus

Large non-confining 21-litre aquariums individually housed each subject. These aquariums were situated on shelves and were exposed to daily laboratory activity in order to avoid any form of visual deprivation. However the tanks were spaced far enough apart to ensure that all subjects were visually isolated from each other.

Two separate aquariums (30 x 25 x 25 cm) shown in Figs. 1 and 2 served as the operant tanks. Each operant tank was provided with a gravel depth of approximately 8.5 cm. Each home tank had a white opaque glass cover, continuous aeration being provided by a portable pump via external filters. Glass wool and charcoal were used as filtration agents. In order to maintain constant temperatures, an aquarium heater (Supreme Heatmaster) was placed in each operant tank in a corner opposite the response mechanisms.

The response mechanisms for each operant tank (Figs. 1 and 2) consisted of two funnel-shaped glass swimming rings which were 3.5 cm in length. The diameters of both rings were 2.5 cm at the largest opening and 2.0 cm at the smallest opening. The distance between the response mechanisms was 9.0 cm. Each swimming ring was covered with a black non-reflective Varathane plastic coat and was provided with an embedded photo-diode and light source. Electrical cables connecting the photo-diode system to the programming equipment were placed through

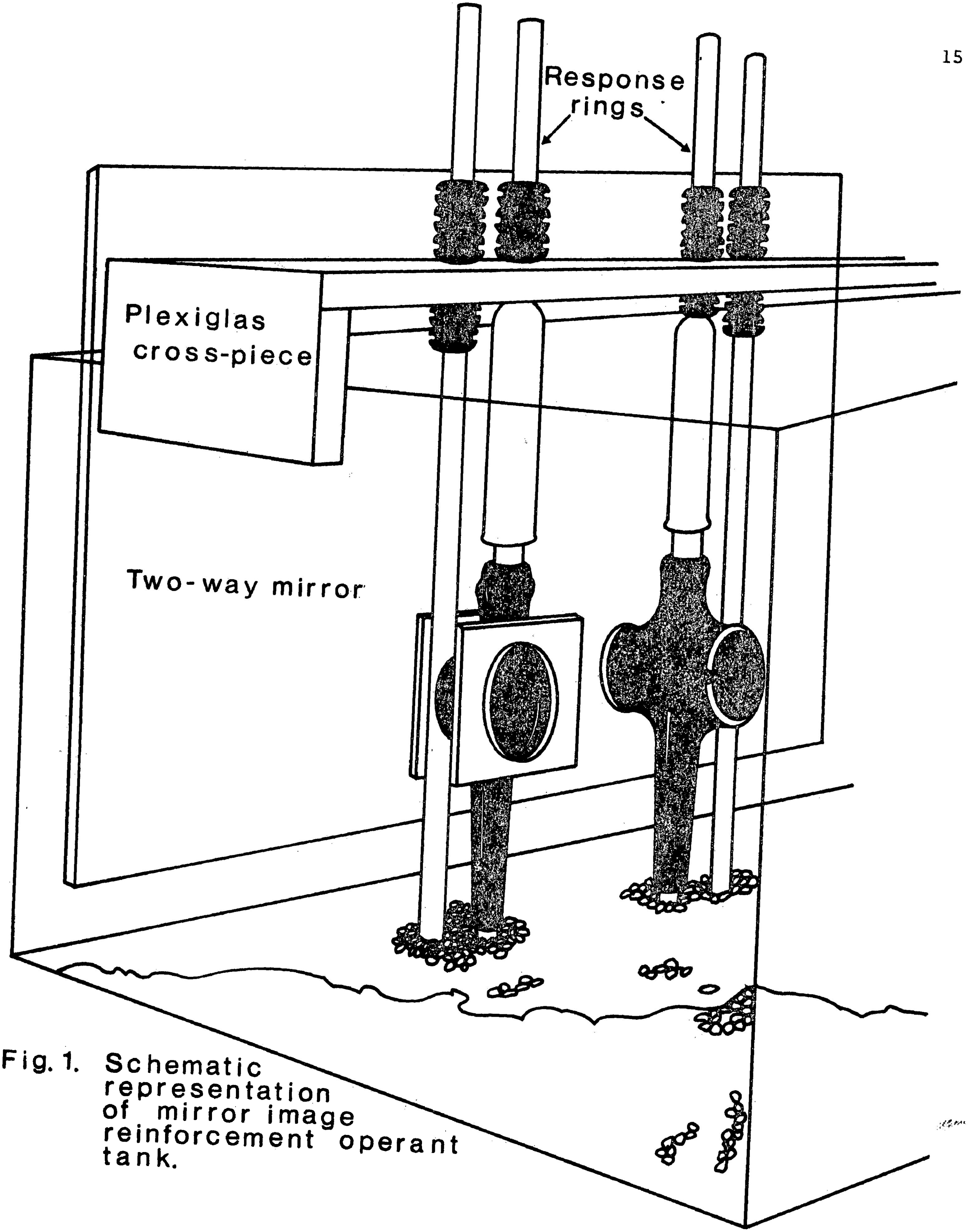

Fig. 1. Schematic representation of mirror image reinforcement operant tank.

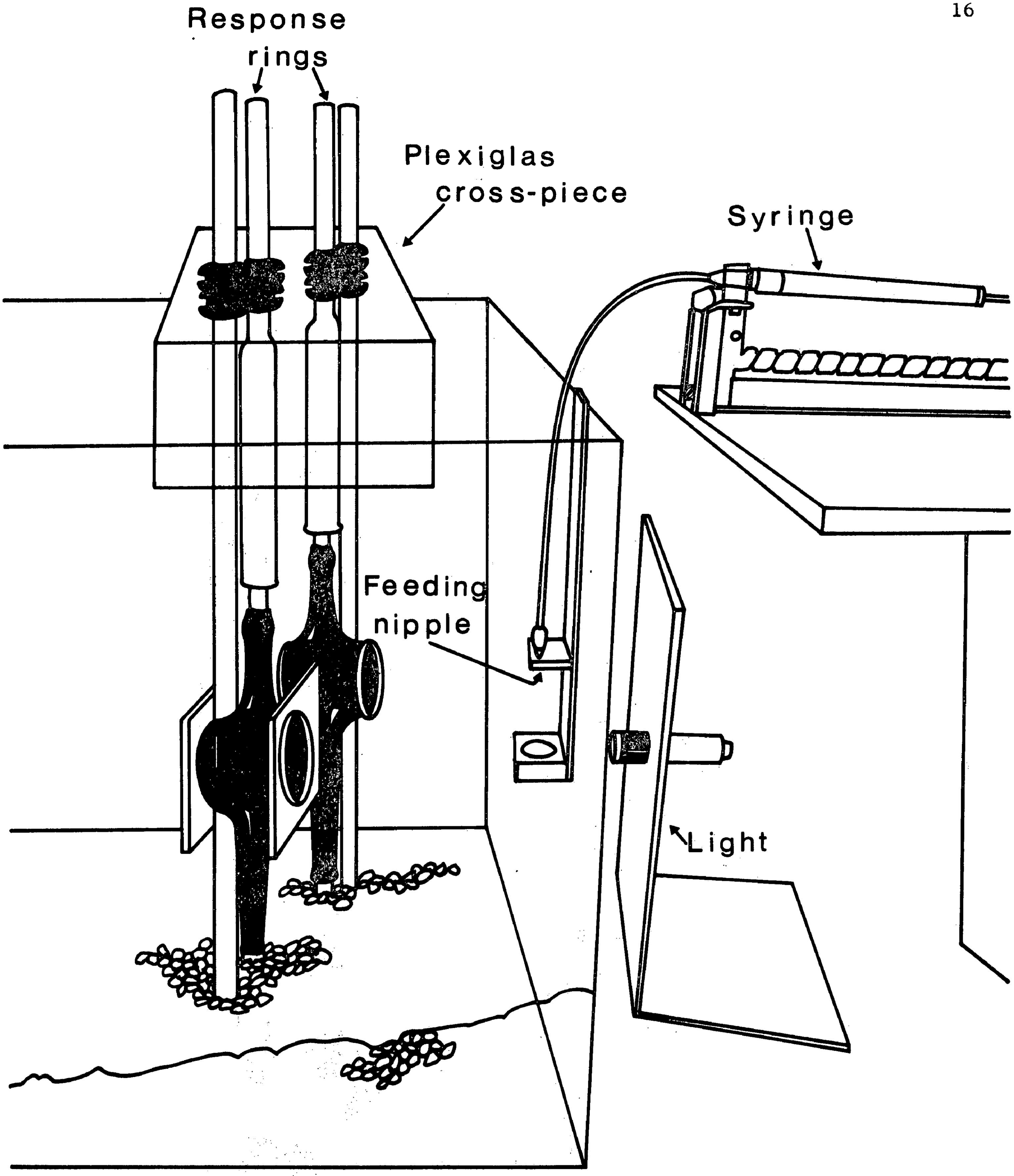

Fig. 2. Schematic representation of food reinforcement operant tank.

two glass tubes positioned at right angles to each swimming ring. To allow access to the respective photo-diodes and light source, the tubes were detachable since they were connected to the rings by high pressure glass vacuum seals. These tubes extended to the top of the tank and were attached to a transparent Plexiglas cross-piece that supported the two swimming rings in each tank. The bottoms of the swimming rings were placed approximately 5.0 cm above the gravel surface. In addition, square white plates (4.5 x 4.5 cm) were placed over both ends of one of the rings in each operant tank. These plates served as the visual cue. A hole was bored in each respective plate equal to the diameter of the ring's widest and narrowest openings. The plates did not interfere with the subjects' entry or exit from that particular swimming ring in either the mirror image or food reinforcement operant tank.

A two-way mirror (30.5 x 30.5 cm) was located on the exterior side of the short axis of one operant tank, with a frosted 60 w bulb in a fan-cooled box enclosure mounted directly behind the mirror. The two-way mirror acted as transparent glass when the light was on, and as a true mirror when the light was off. The side of each swimming ring nearest the two-way mirror was placed 7.0 cm away from that surface. A small 28 v light (1.0 cm in diameter) was also placed in the center of the mirror, resting between the mirror and the tank wall.

On the exterior end of the food reinforcement operant tank, a motor driven glass syringe was mounted horizontally and at right angles to the tank wall. A plastic tube with inside diameter of 0.15 cm connected the syringe and cylindrical feeding nipple which was centered against the inside tank wall widthwise, and was approximately 7.5 cm above the gravel surface. A 28 v light was also positioned directly behind the feeding nipple exterior to the tank. The syringe was driven by a modified Gerbrands student cumulative recorder and acted as the feeding unit which delivered food in the form of a liver paste.

The operant program was maintained automatically by a BRS Digi-Bit unit. In the mirror image reinforcement tank, interruption of the photo-diode system for a correct ring swimming response deactivated the light bulb for a 20 sec period. This allowed the two-way mirror to act as a true mirror. Selection of the incorrect response alternative did not result in mirror image presentation. The mirror duration was fixed at 20 sec and could not be prolonged by the subject either sitting within the ring (continued interruption of photo-diode system) or making additional responses during that period. The 28 v light was also activated during mirror image presentation, but not when an incorrect response was made.

In the food reinforcement tank, food was presented to the subject only when the correct response alternative had been selected. At the onset of the correct response the light directly behind the feeding nipple was activated for a period of 10 sec. The light did not appear if the incorrect ring was selected.

For both food and mirror image reinforcement groups, reinforced and non-reinforced responses were recorded on separate event counters and on a six-channel Gerbrands event recorder, of which four channels were used. Remote surveillance of both operant tanks was accomplished through the use of two video-tape cameras with monitors.

Procedure

The 12 subjects were randomly assigned to two groups of six members each and were individually housed at a temperature of 26 ± 1.1 C°. both in the home and experimental tanks. The mirror image reinforcement group was maintained on equal sized portions of chicken liver and were fed following each experimental test period. The only food allowed the food reinforcement group was that received in each daily session, and which consisted of liver paste. Data from previous pilot studies had indicated that chicken liver was superior to the majority of other commercially available foods, including other forms of liver (e.g., beef) in maintaining operant behavior in Siamese Fighting Fish.

To reduce intra-subject variability over continued training, the same order of subject testing was maintained for each experimental day. Training for mirror image or food reinforcement was continuous with no experimenter imposed inter-trial intervals. This allowed a correction procedure to be in effect. A nylon mesh net was used to transport subjects from the home tanks to the operant tanks.

Pre-training: Mirror image reinforcement group. In order to allow each subject to acquire the ring swimming response without the formation of a left-right position habit, a single ring was used. This ring was centered in the tank width-wise where the two response alternatives (rings) were later placed. Following five manual presentations of the mirror image, each subject was allowed to acquire the ring swimming response without training by the experimenter (self-shaping). Self shaping occurred when each subject interrupted the photo-diode system in exploring the ring, resulting in automatic mirror image presentation. All subjects were allowed 20 responses per day for six consecutive days.

Discrimination training: Mirror image reinforcement group. On the seventh day, both rings were introduced into the operant tank. Half of the mirror image reinforcement group was conditioned through selective reinforcement to use the left swimming ring; the remaining subjects were conditioned to use the right swimming ring. This constituted the original discrimination problem (R_0). The white stimulus cue plates (visual cue) were always mounted on the unreinforced ring in order to make that ring more visually discriminable from the other ring.

A criterion performance of at least 17 correct choices out of 20 responses within one day constituted R_0 learning. After this criterion level was met, the first reversal problem was administered, in which the previously reinforced choice was now unreinforced. Training continued until 13 additional reversals per subject had been completed following the original discrimination problem. The same performance criterion of 17 out of 20 correct responses was required in each reversal problem before the subject was given the next problem. Each of the subjects progressed at its own pace.

If a subject did not demonstrate reversal behavior, training continued for 50 consecutive days. This insured that the absence of reversal behavior was not a function of lack of practice. Upon the completion of the last discrimination problem (problem 13) or 50 day period, exinction procedures (non-reinforcement) were then instituted. Extinction was terminated after subjects had responded continuously for a 10 hr period or when subjects had failed to respond during a 1 hr interval.

Following extinction, all subjects were reintroduced into the experimental situation for another three discrimination problems or 10 consecutive days of training if subjects did not demonstrate reversal behavior. The white stimulus cue plates were now mounted on the reinforced ring instead of the unreinforced ring. This was done in order to determine the nature of the control exerted on reversal behavior by the stimulus plates (non-specific condition).

Pre-training: Food reinforcement group. Each subject was magazine trained by presenting .008 c.c. of food when the subject was in the immediate area of the feeding nipple. Since it was found that subjects would not self-shape to swim through the ring after a reasonable period of time (10 hr), each subject was trained through successive approximations to perform the ring swimming response. All other procedures were identical to that in pre-training for mirror image reinforcement.

Discrimination training: Food reinforcement group. The training procedures for food reinforcement subjects in discrimination training were identical to those of the mirror image reinforcement group, except that food reinforcement was used.

Results

The data were based on 564 experimental sessions (11,280 trials).

Although training was continuous and not discrete within sessions, each response was defined as a trial. One subject in the mirror image reinforcement group died during discrimination training. The performance scores for this subject were included in the analyses for only the first 8 days of training. Standard analyses of variance for trend (Edwards, 1968) were performed on all data in accordance with statistical procedures established by Setterington (1967) in relation to reversal learning.

The results indicated that subjects using food as reinforcement learned to reverse spatially between the response alternatives (Fig. 3), whereas subjects using mirror image as reinforcement did not reach criterion for the reversal of a discrimination beyond the first reversal problem (Fig. 4). Food reinforcement subjects also demonstrated an improvement (reduction of errors) in performance over the 13 problems following the original discrimination (total of 14 problems). Due to the presence of reversal behavior in food reinforcement subjects, and its absence in mirror image reinforcement subjects, no comparative statistical examination between groups was possible. The analyses, therefore, are based on the performance for the 14 discrimination problems for the food reinforcement group prior to extinction, and the performance of the mirror image group for 50 consecutive days following Day 8 of training (Fig. 5). No further reversals occurred beyond Day 8 of training for mirror image reinforcement subjects.

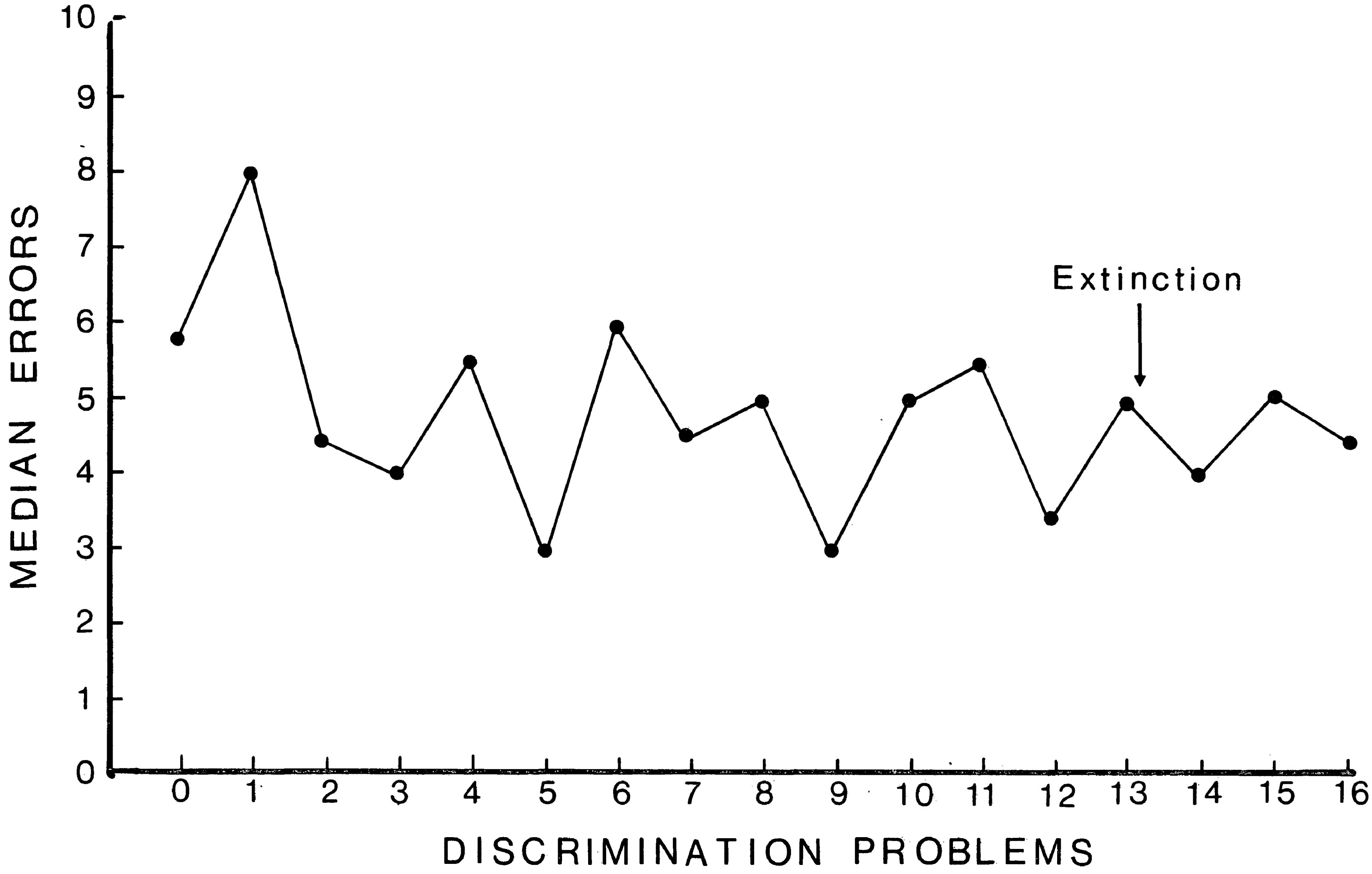

Fig.3. Medians of median error per fish per discrimination problem (Food reinforcement group).

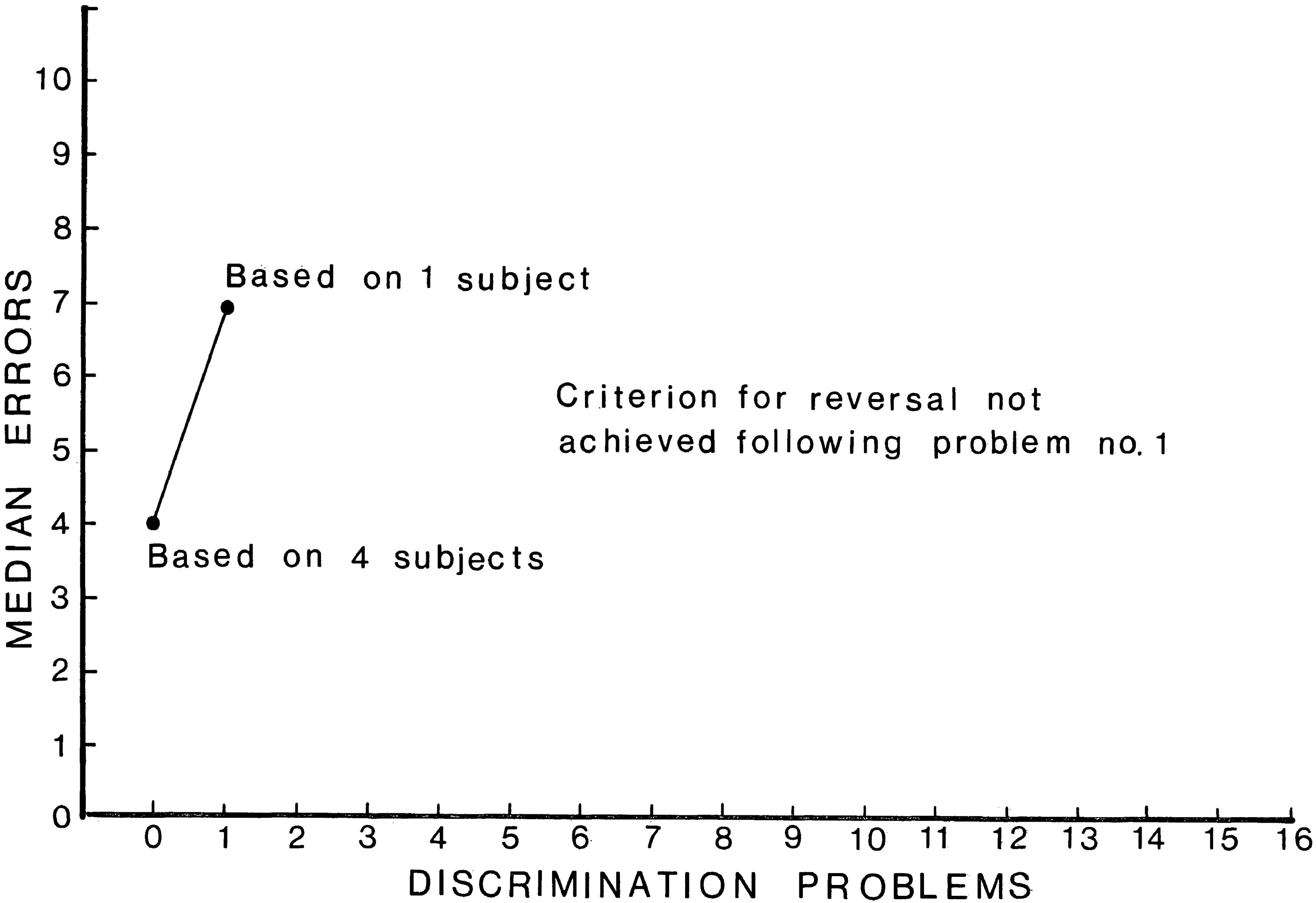

Fig.4. Median errors per discrimination problem (Mirror image reinforcement group).

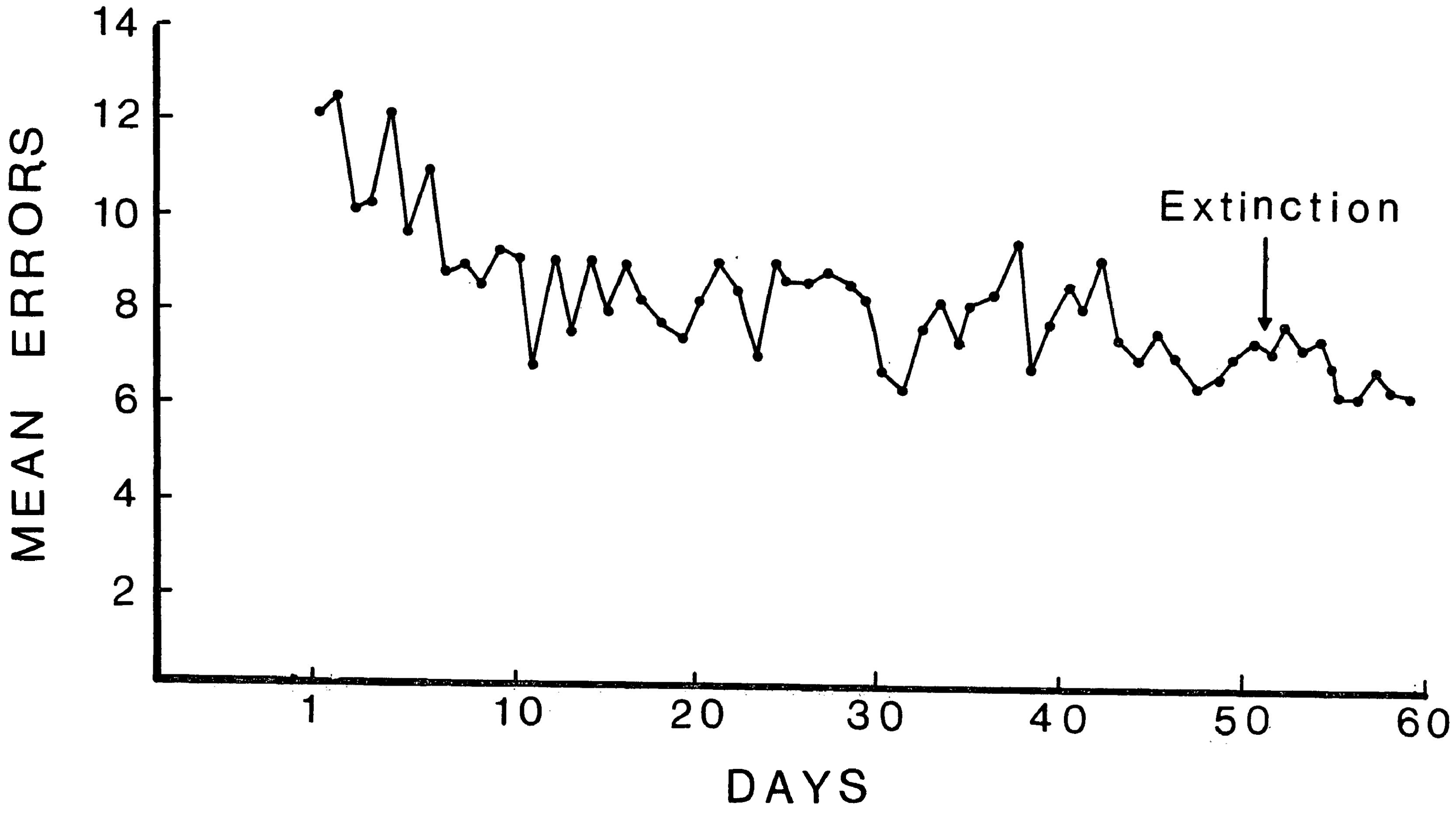

Fig.5. Mean errors per test day for mirror image reinforcement group following completion of problems R_0 and R_1.

Performance Across Problems for Food Reinforcement Subjects

Two measures were taken to ascertain the performance of the food reinforcement group: median errors per reversal (Bitterman, Wodinsky & Candland, 1958), and mean number of trials to achieve criterion (three errors or fewer) for each discrimination problem (Warren, 1960). Figures 3 and 6, respectively, demonstrate a significant improvement in performance across problems as measured by median errors (F = 2.609, df = 13/65, $p < .01$) and mean number of trials to criterion (F = 4.568, df = 13/65, $p < .01$).

This improvement is also observed in an examination of the average performance (repetitive errors) within each 20-trial session between discrimination problems. Figure 10 shows a significant reduction in the number of repetitive errors between groups of discrimination problems. (F = 2.714, df = 6/30, $p < .05$). Repetitive errors are defined as the number of consecutive errors following the occurrence of any given error per block. The New

Also evident in Figs. 3 and 6 is the characteristic negative transfer effect (increase in errors in problem R_1), beyond which performance improved to approximately the fourth discrimination problem. A Newman-Koules test also showed that there was a significantly greater level of errors in problems R_0 and R_1 than in all succeeding problems. Problems R_2 to R_{13} demonstrated no significant differences.

Another measure of improvement in reversal learning was also used for the food reinforcement group in the form of mean initial errors per discrimination problem (Fig. 7). An initial error is defined as an incorrect response which occurs on the first trial of

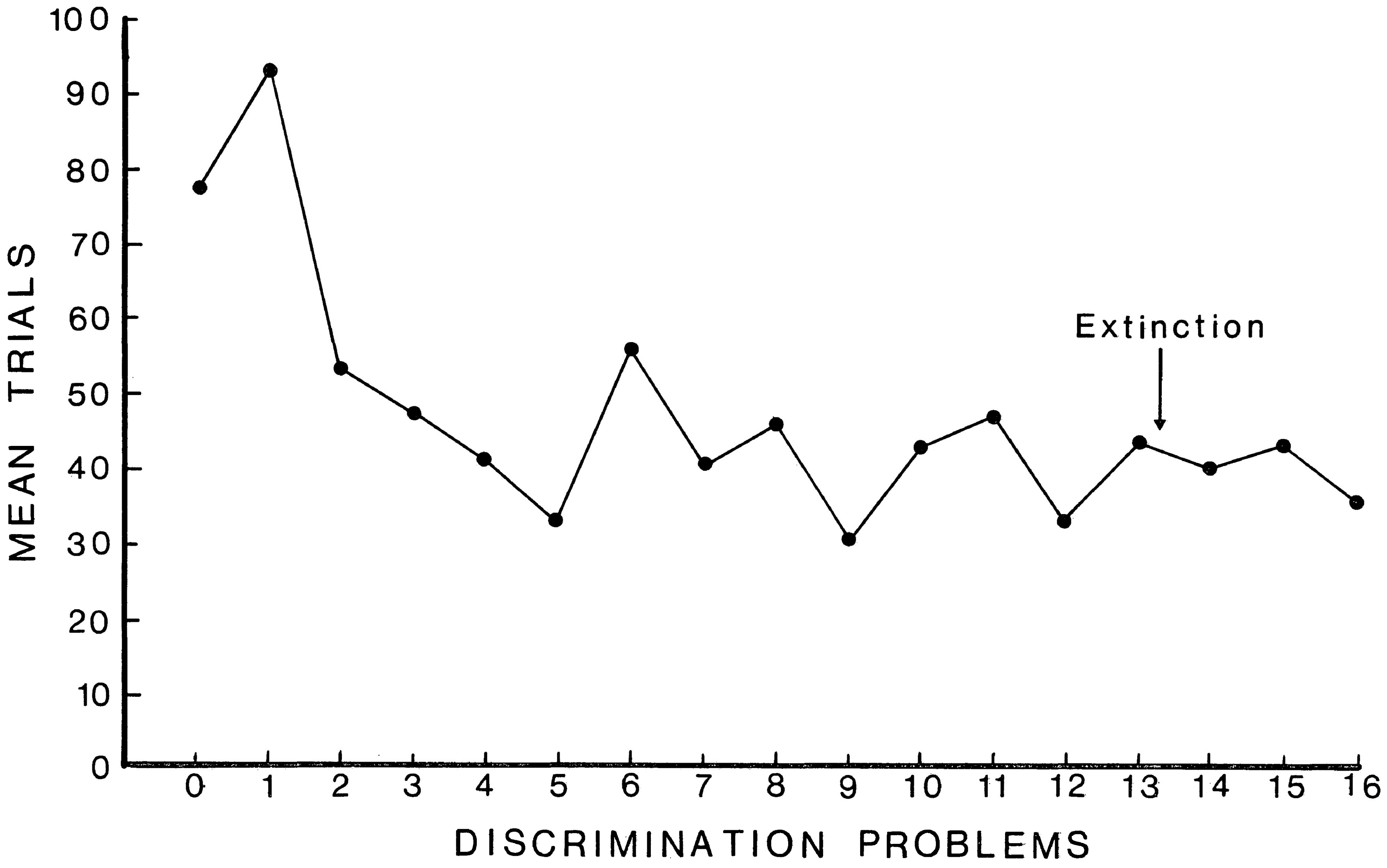

Fig. 6. Mean trials to criterion per discrimination problem (Food reinforcement group).

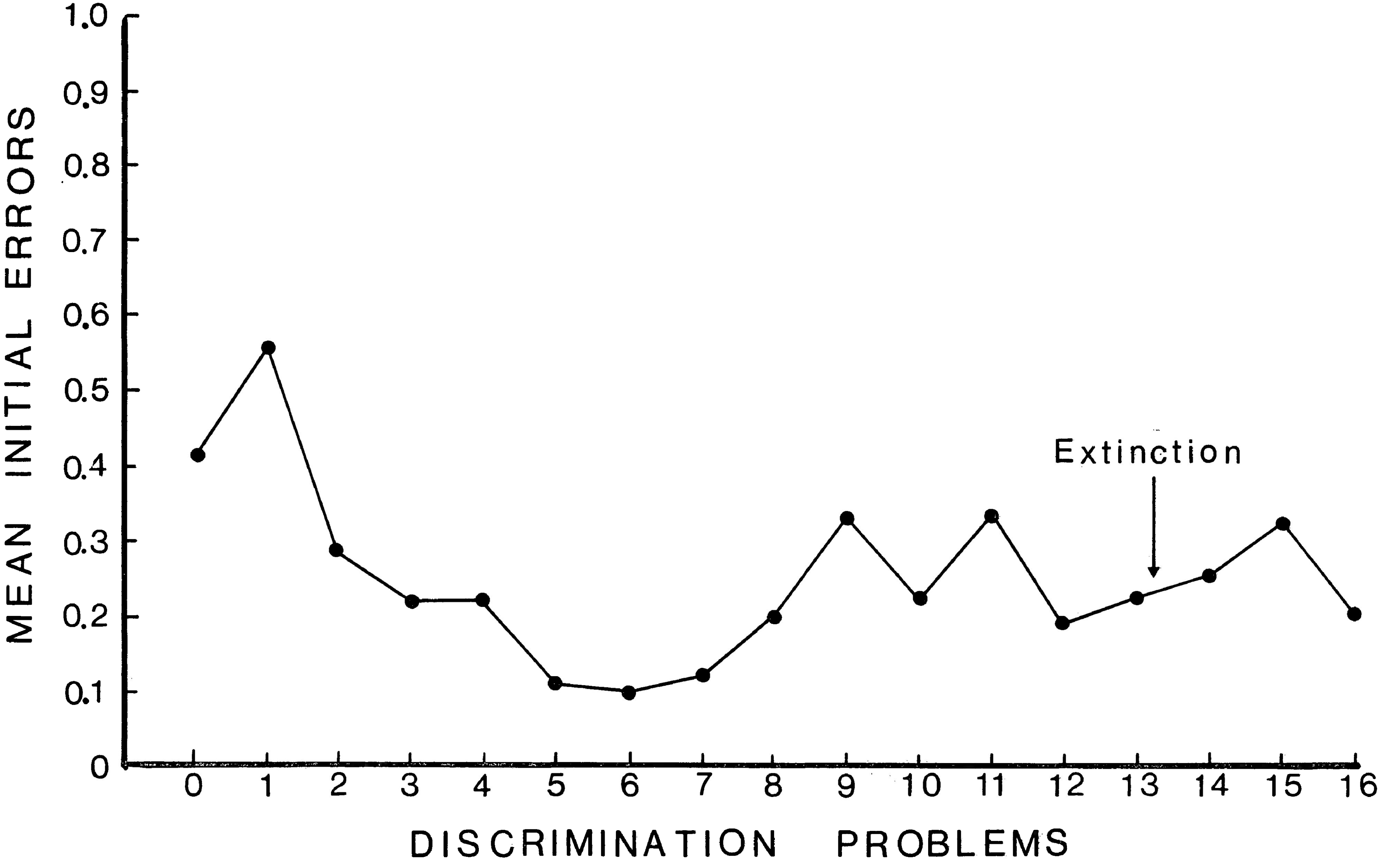

Fig.7. Mean initial errors per discrimination problem (Food reinforcement group).

each experimental session. The food reinforcement group did not demonstrate a significant reduction of initial errors across all problems (F = .612, df = 13/65, $p > .25$ NS), although a decrease in initial errors across the first 6 to 7 problems is evident. The overall non-significant effect is not wholly atypical, since Setterington (1967) also reported that one of his experimental groups did not show a significant decrease in the mean number of initial errors across discrimination problems. Although mirror image reinforcement subjects did not demonstrate reversal behavior, these subjects also showed no significant decrease in initial errors (Fig. 8) across five 10-day blocks (F = 2.463, df = 4/16, $.05 < p < .10$ NS). However, this may only be considered marginally non-significant as a downward trend in the reduction of errors is evident. No direct comparison could be made with the results for the food reinforcement group since the data for mirror image reinforcement subjects was based upon errors per day, and no subject reached the necessary criterion for reversal over the 50 day period.

Performance Across Days for Mirror Image Reinforcement Subjects

Subjects using mirror image as reinforcement did not demonstrate reversal behavior beyond problem R_1. Of the six mirror image subjects, only four subjects learned the original discrimination with one of these subjects also completing the first reversal (R_1). Training for problems R_0 and R_1 required a total of 8 days beyond which no further reversals occurred (i.e., no subject reached the criterion of three or fewer errors per session). Since four subjects completed R_0 and only

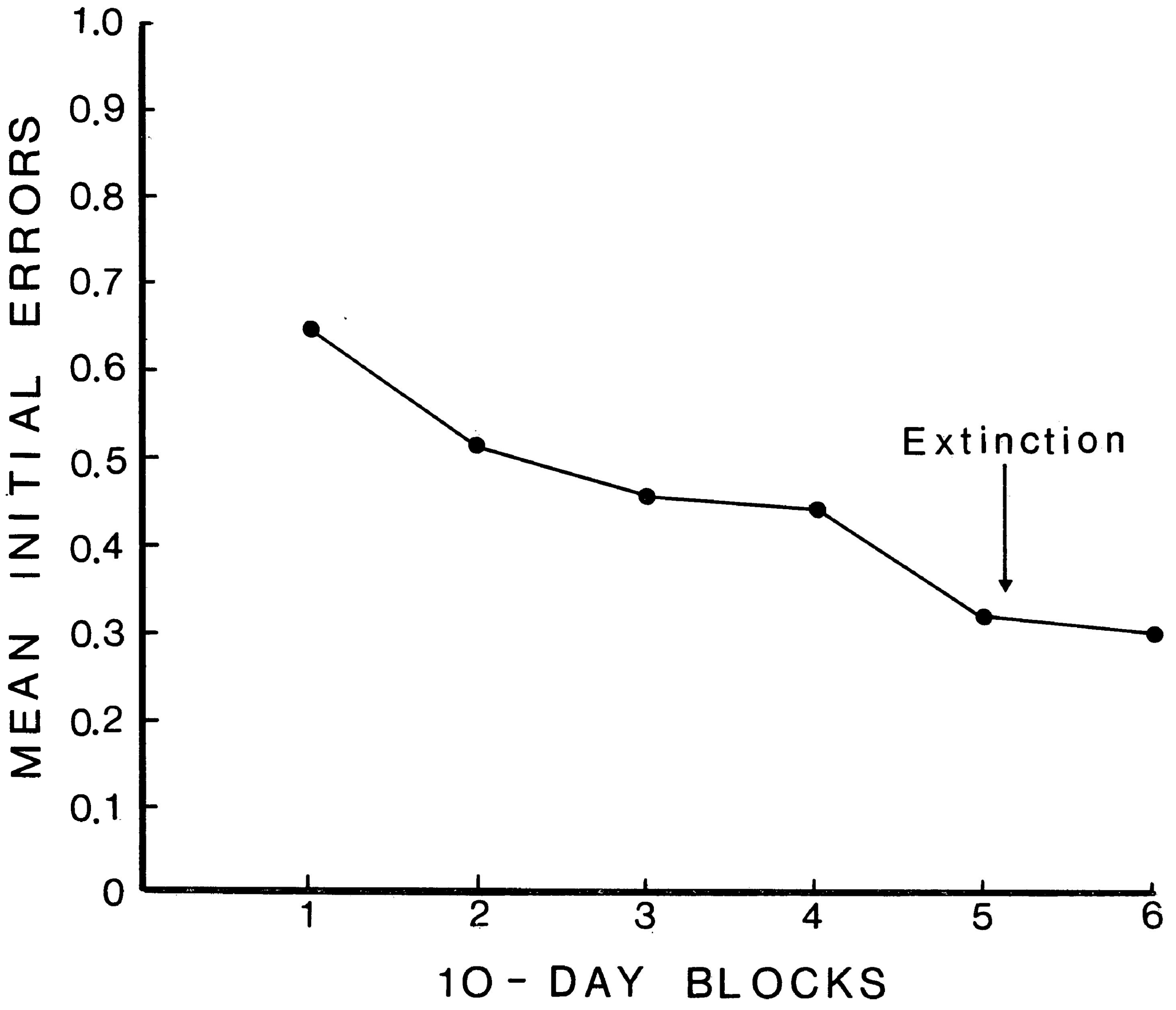

Fig.8. Mean initial errors per 10-day block (Mirror image reinforcement group).

one subject completed R_1, use of a statistical comparison between both problems could not be readily justified.

In order to account for the possibility that mirror image reinforcement subjects may have learned to reverse with additional training, these subjects were tested on the spatial discrimination problem for 50 consecutive days prior to extinction. Figure 5 indicates a significant decrease in the mean errors per day ($F = 2.016$, $df = 49/196$, $p < .01$); however, this did not reach the required criterion necessary before the reversal of a discrimination problem would be allowed. This improvement is also evident in repetitive errors for mirror image reinforcement subjects, since Fig. 12 indicates a significant decrease in repetitive errors across blocks of days ($F = 7.000$, $df = 4/16$, $p < .01$). The majority of this improvement of this improvement appears to occur during the first 10 days of training.

Performance Within Problems for Food Reinforcement Subjects

To further investigate reversal performance for food reinforcement subjects, a within problems analysis was performed. The advantage of this method of analysis is that it allows an examination of the average performance within each 20 trial session (4 blocks of 5 trials) per discrimination problem.

Figures 9 and 10 illustrate the mean number of errors and the mean number of repetitive errors within sessions for groups of two discrimination problems (Discrimination Problems 0 & 1, 2 & 3, 4 & 5, 6 & 7, 8 & 9, 10 & 11, 12 & 13). Based on both criteria, food

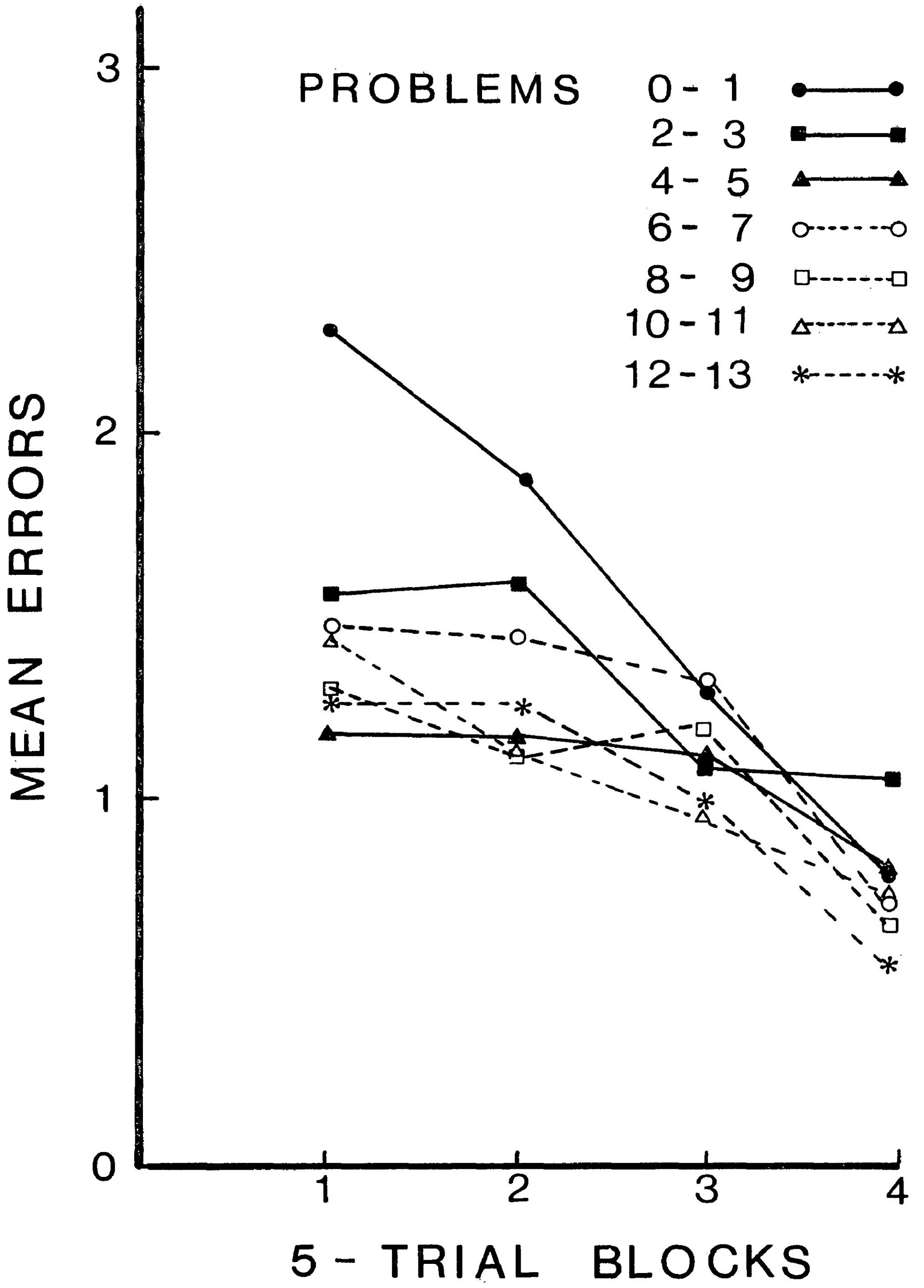

Fig. 9. Mean errors per 5-trial block between groups of discrimination problems (Food reinforcement group).

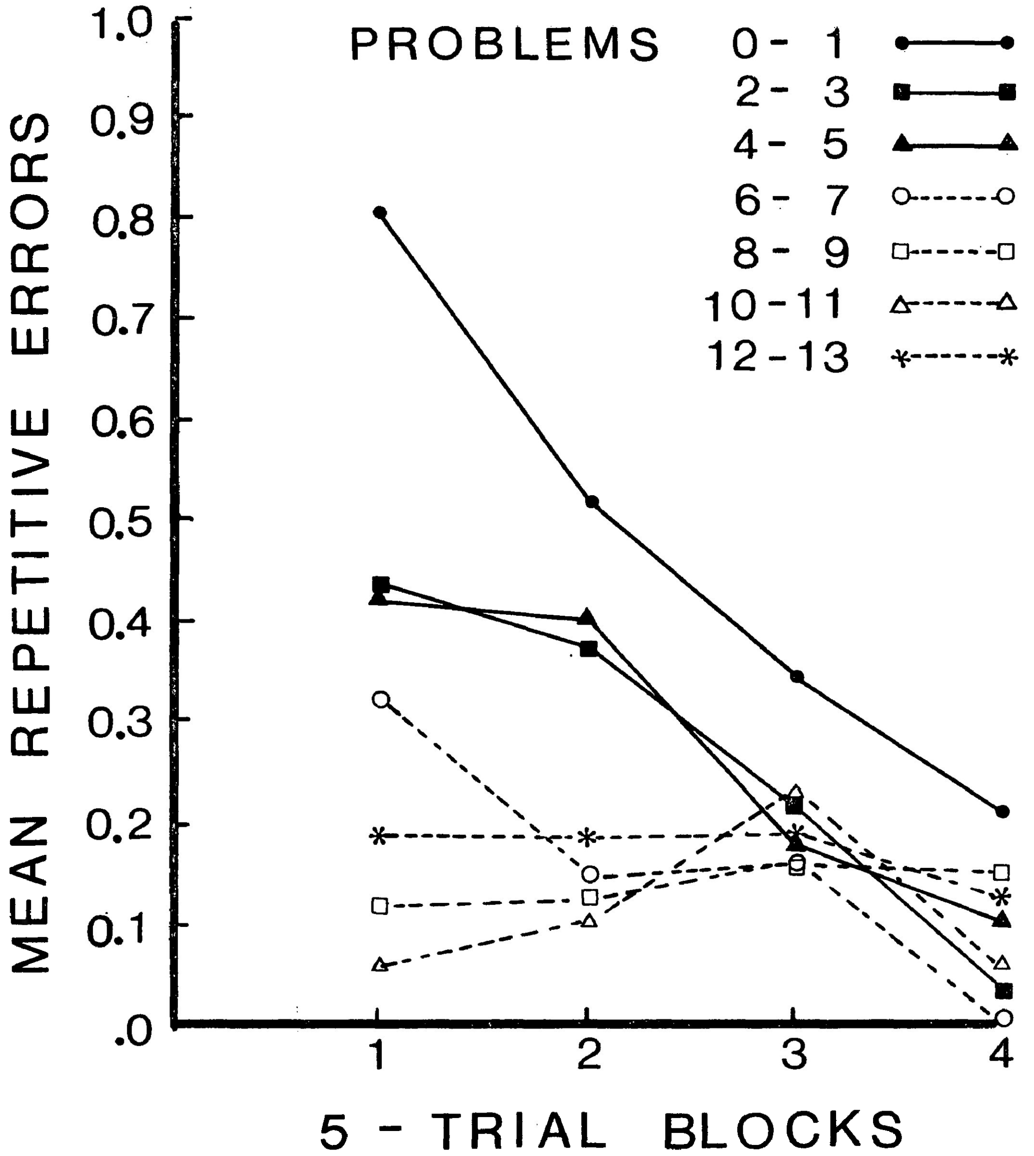

Fig.10. Mean repetitive errors per 5-trial block between groups of discrimination problems (Food reinforcement group).

reinforcement subjects demonstrated a learning trend (progressive reduction of errors) within discrimination problems for mean number of errors within sessions (F = 31.714, _df_ = 3/15, $p < .01$) and for repetitive errors within sessions (F = 15.666, _df_ = 3/15, $p < .01$).

Performance Within Days for Mirror Image Reinforcement Subjects

Figures 11 and 12 show the within session performance of mirror image subjects for blocks of days (Days 1-10, 11-20, 21-30, 31-40, 41-50). Mirror image reinforcement subjects did not demonstrate any significant improvement in performance within sessions as measured by mean errors (F = 2.439, _df_ = 3/12, $.10 < p < .25$ NS) or mean repetitive errors (F = 1.776, _df_ = 3/12, $.10 < p < .25$ NS). Although there is a general reduction in errors and repetitive errors over blocks of days, it is possible that the absence of learning within sessions may account for the finding that no subjects reached the criterion necessary for the reversal of the discrimination problem.

Within-Across Problems Analyses: Food Reinforcement Subjects

The data for food reinforcement subjects was analyzed with reference to performance between problems and performance within problems. However, Figs. 9 and 10 combine both sets of data and demonstrate differences in the rate of learning within problems as a function of training. The significant Within x Between Problems interaction supports the conclusion that the majority of learning takes place early in training (e.g., problems 0-1, 2-3) with little change

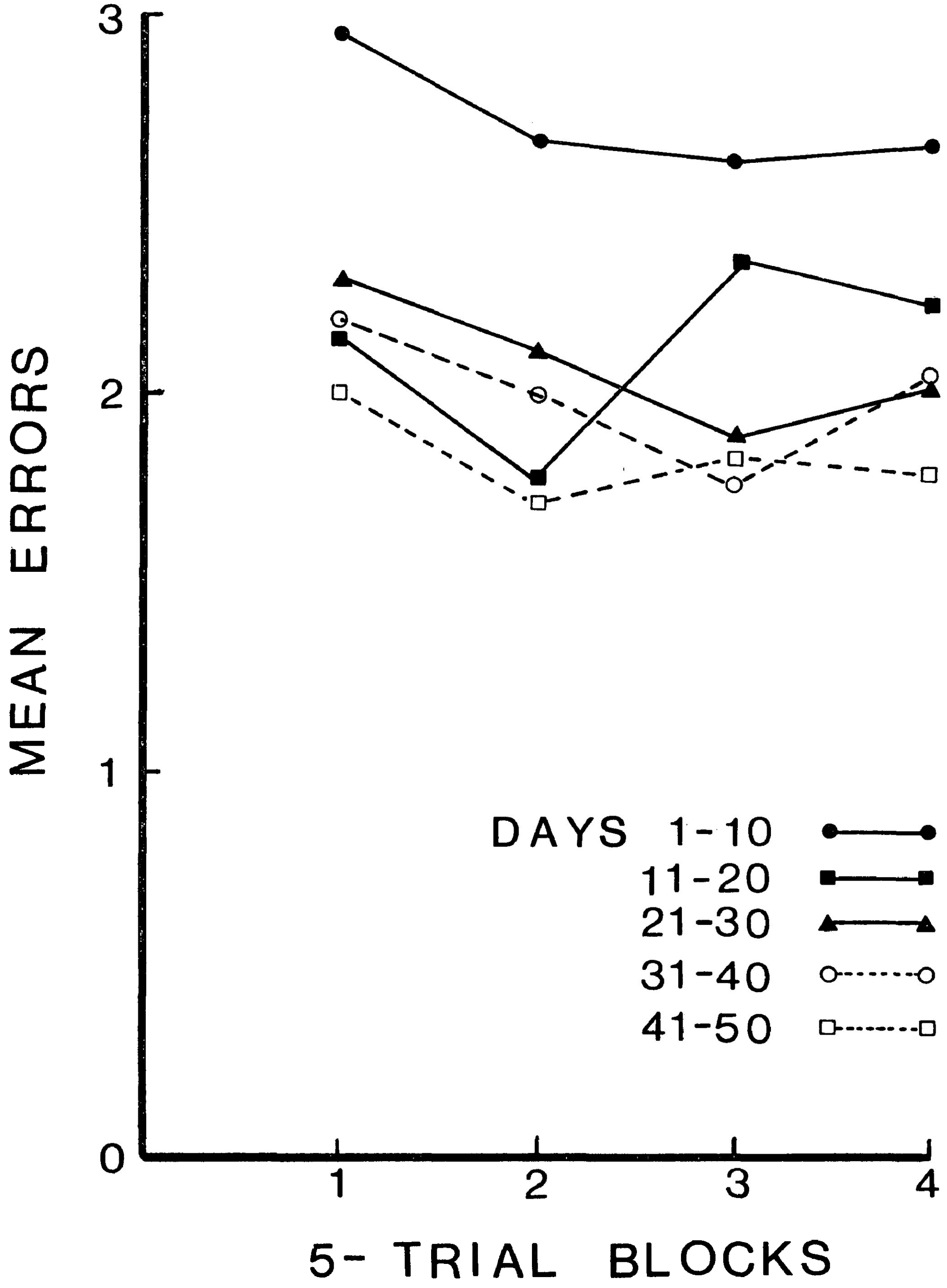

Fig. 11. Mean errors per 5-trial block between groups of days (Mirror image reinforcement group).

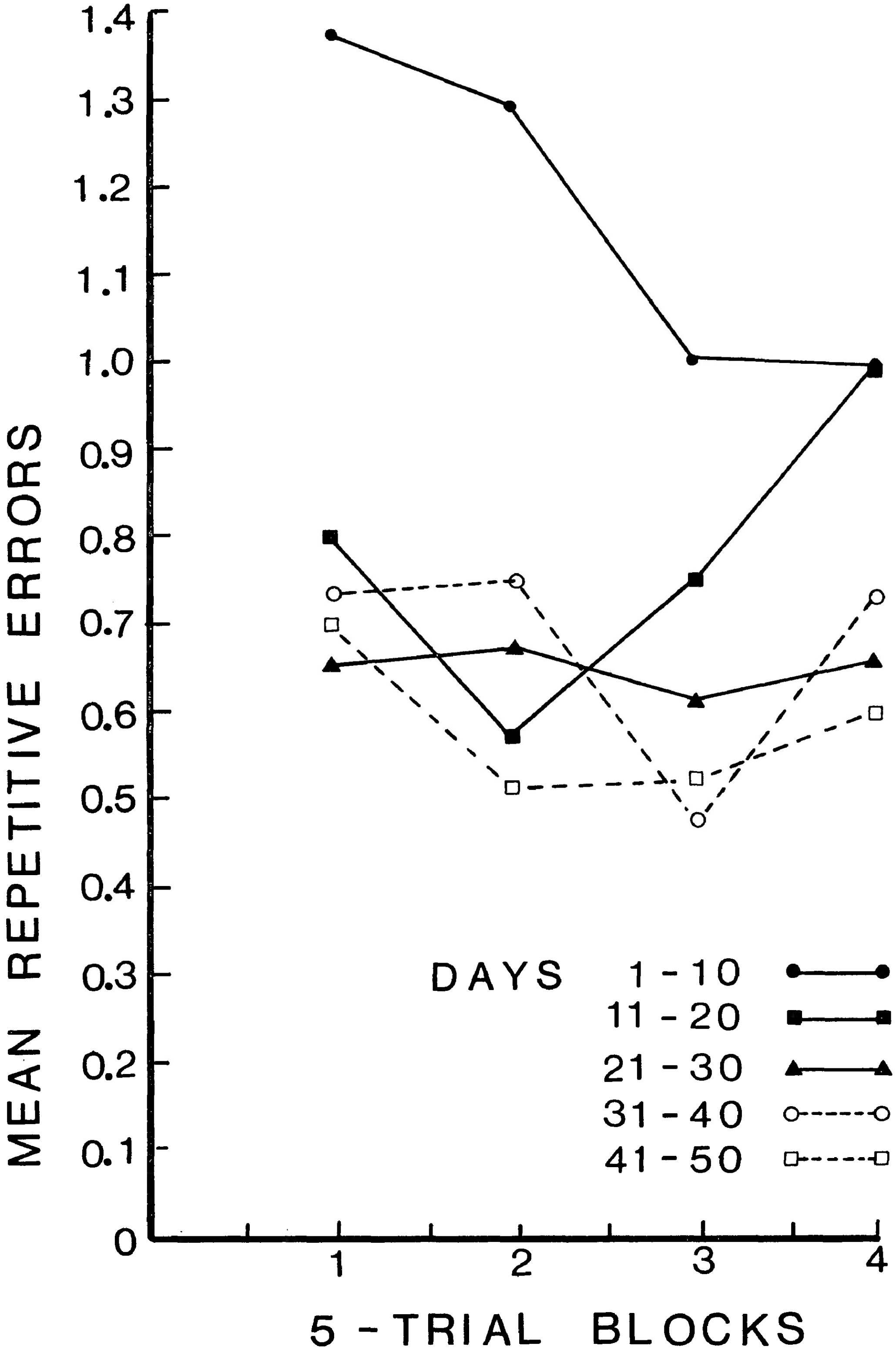

Fig.12. Mean repetitive errors per 5-trial block between groups of days (Mirror image reinforcement group).

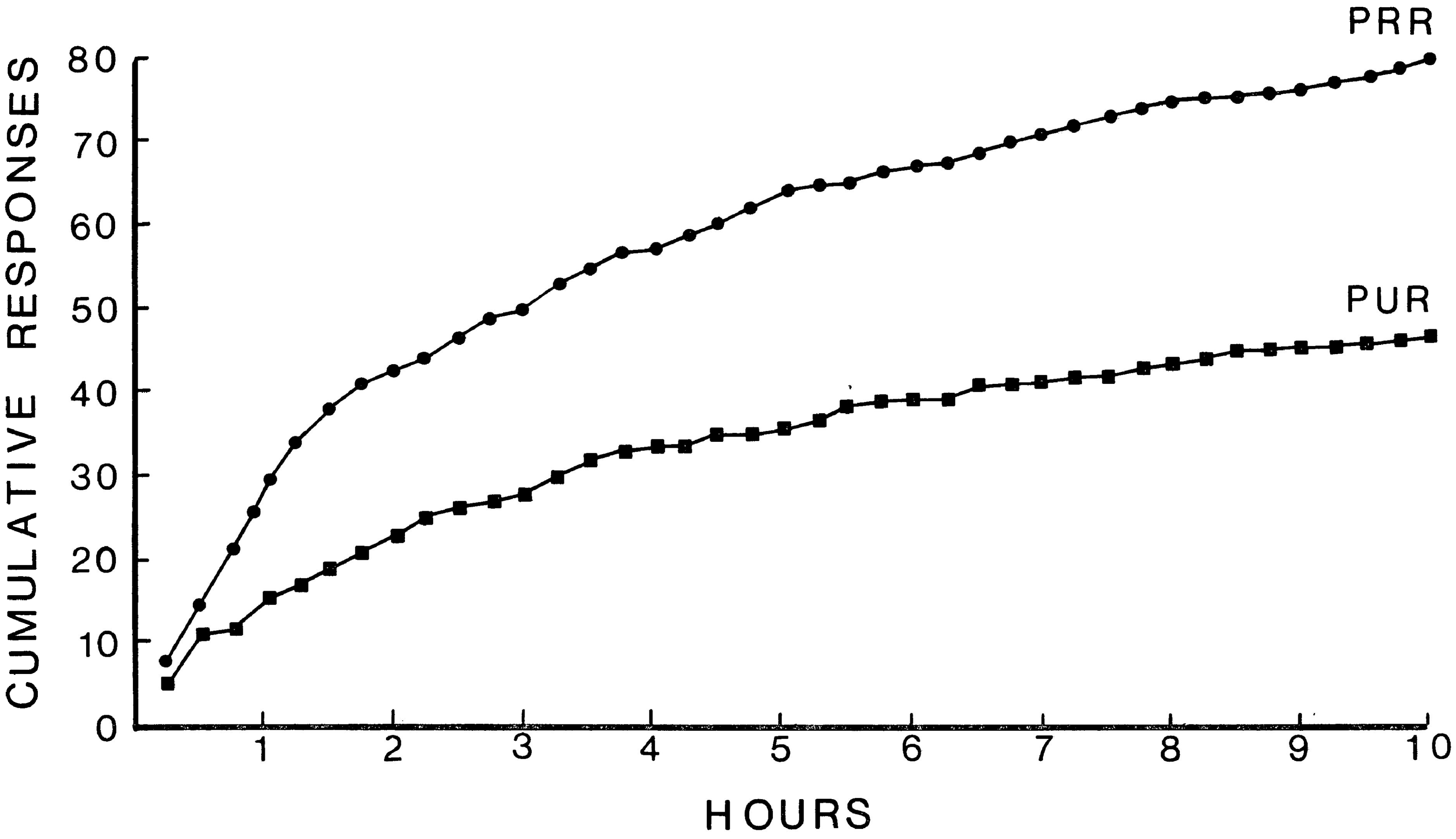

Fig.13. Mean cumulative responses during extinction to the previously reinforced and previously unreinforced rings (Food reinforcement group).

occurring in the remaining problems (problems 4-13), for mean errors (F = 1.762, df = 18/90, $p < .05$) and repetitive errors (F = 1.895, df = 18/90, $p < .05$).

Within-Across Days Analyses: Mirror Image Reinforcement Subjects

The finding of differential rates of learning as a function of training was not representative of mirror image reinforcement subjects (Figs. 11 & 12). These subjects demonstrated similar rates of learning early and late in training as indicated by mean errors (F = .312, df = 12/48, $p > .25$ NS) and repetitive errors (F = .545, df = 12/48, $p > .25$ NS).

Extinction

Figures 13 and 14 illustrate response differences between food and mirror image reinforcement subjects to the previously reinforced ring (PRR) and the previously unreinforced ring (PUR). All subjects responded for the full 10 hr time limit during extinction in which the visual cue was correlated with the previously non-reinforced ring as in prior training. At the end of the 10 hr period, subjects in both groups made more responses to the previously reinforced ring ($\overline{X}$ = 79.9 responses) than to the previously unreinforced ring ($\overline{X}$ = 42.3 responses). For both groups, there was an initial preference for the previously reinforced ring.

However mirror image and food reinforcement subjects did demonstrate differences in the rate of response to both the previously reinforced and previously unreinforced rings during extinction.

For example, food reinforcement subjects initially demonstrated a high rate of response, which decreased regularly for both rings over the extinction period. In contrast, mirror image subjects did not show a similar decrease in the rate of response during extinction, but rather demonstrated a relatively constant rate of response to both rings for the full 10 hr of extinction.

Reversal of Visual Cue

As previously indicated, the food reinforcement subjects were tested on three additional problems following extinction; however, the white stimulus cue plates were now correlated with the reinforced ring and not the unreinforced ring, as in the previous 14 discrimination problems. Analysis of variance of the data from the three problems following extinction and the three problems prior to extinction (Figs. 3, 6 & 7) indicated no significant differences between the two groups of problems for median errors ($F = .296$, $df = 1/5$, $p > .25$ NS), trials to achieve criterion ($F = .034$, $df = 1/5$, $p > .25$ NS), and initial errors ($F = .093$, $df = 1/5$, $p > .25$ NS). These exceptionally low F values (< 1.00) strongly indicate that the consistent association of the visual cue with a particular response alternative did not facilitate learning of discrimination problems.

This is further supported by the results for the mirror image reinforcement subjects who were tested for 10 consecutive days following extinction with the visual cue reversed in the same manner. Figures 5 and 8, representing mean errors per day and mean initial errors per 10-day block, respectively, demonstrate no negative transfer

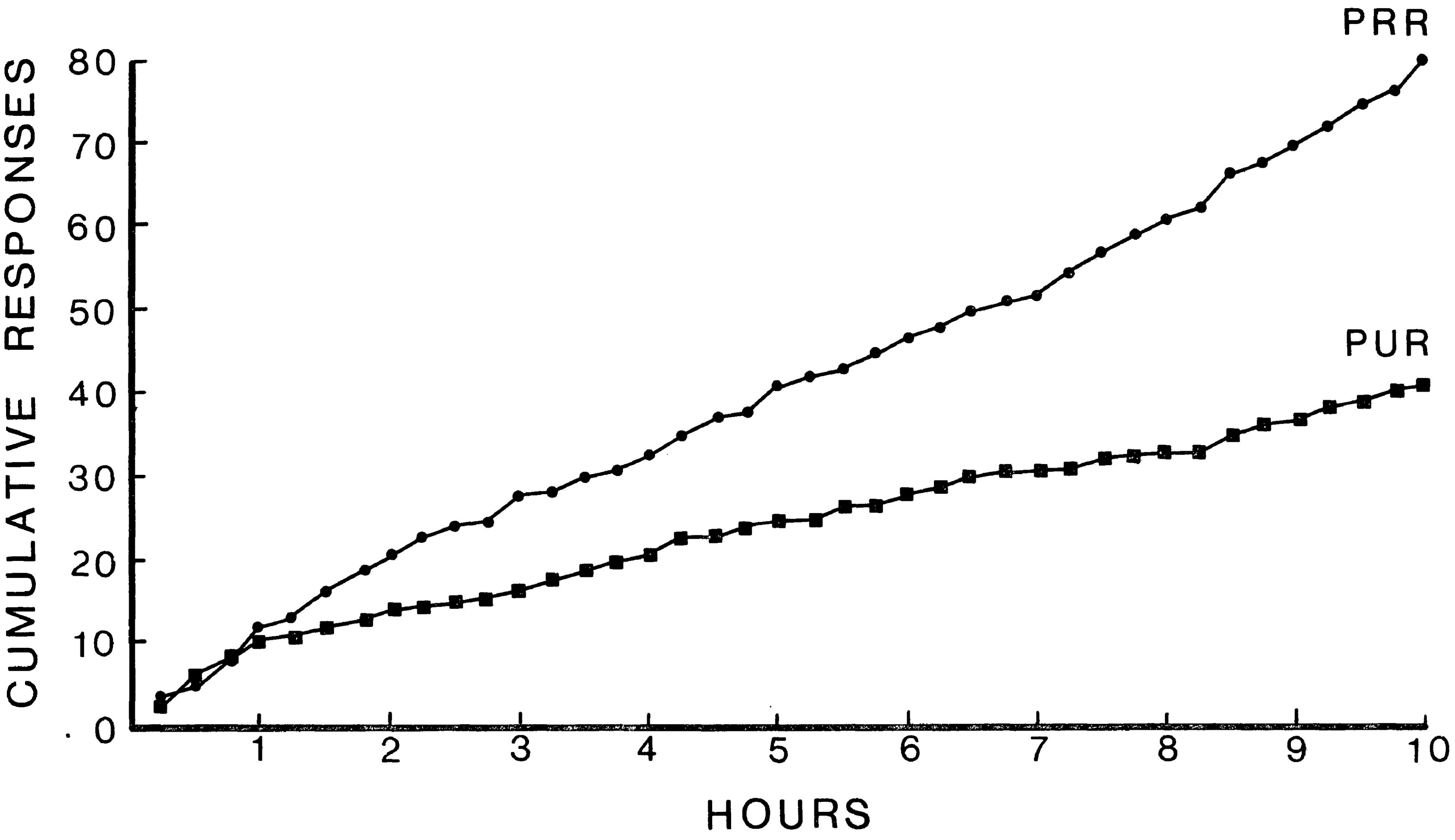

Fig.14. Mean cumulative responses during extinction to the previously reinforced and previously unreinforced rings (Mirror image reinforcement group).

following extinction. Statistical analysis indicated no significant difference in the mean number of errors between the 10 days prior to, and following extinction ($F = 1.149$, $df = 1/4$, $p > .25$ NS) or for initial errors during the same period ($F = .043$, $df = 1/4$, $p > .25$ NS).

Summary

The results indicated that subjects using food as reinforcement learned to reverse between discrimination problems. These subjects also demonstrated an improvement in performance (reduction of errors) across successive problems. Subjects maintained by mirror image reinforcement did not reach the required criterion for the reversal of a discrimination beyond the second problem. In this case the original discrimination problem was learned by only four of the six subjects, of which only one learned the second discrimination problem. Differences in the results between mirror image and food reinforcement subjects were also evident in the extinction typography for both groups. Food reinforcement subjects showed a progressive and expected decrease of responding during the extinction period, as opposed to mirror image subjects who showed a relatively constant rate of responding. Both food and mirror image reinforcement groups demonstrated a response preference for the previously reinforced ring as opposed to the previously unreinforced ring. In addition, reversal of the visual cue from the unreinforced ring to the reinforced ring produced no changes in behavior.

Discussion

The effectiveness of food reinforcement and the relative ineffectiveness of mirror image reinforcement, in controlling behavior as demonstrated by the present study, provides supporting evidence for similar findings by Hogan (1967) and Hogan, Kleist and Hutchings (1970). Although each of these experiments differed as to learning task, it is evident that in all cases mirror image did not facilitate learning to the same extent as did food. The congruence of this finding under widely disparate conditions, lends support to the concept that there may be a qualitative difference between food and mirror image reinforcement. In this respect the failure of Siamese Fighting Fish to demonstrate a comparable level of learning under mirror image reinforcement conditions remains a complex issue as Hogan, Kleist and Hutchings (1970) have suggested.

In an attempt to answer why Siamese Fighting Fish did not demonstrate reversal behavior for mirror image reinforcement as opposed to food reinforcement, four hypotheses may be considered. These hypotheses are (*a*) procedural variations, (*b*) mirror image as a weak reinforcer, (*c*) different internal mechanisms responsible for the reinforcing effects of mirror image and food, and (*d*) interfering effects of elevated arousal level.

First, differences between mirror image and food reinforcement groups may be attributed to procedural variations since mirror image subjects acquired the ring swimming response through self-shaping, whereas food reinforcement subjects acquired the ring swimming response through successive approximations. Although this remains a possibility, it is highly unlikely, since these shaping procedures were used only to allow the subject to make the "first" response. Beyond this point all procedures were automated and identical in 6 subsequent days of pre-training using one ring followed by discrimination training using two rings.

Second, another possible explanation is that mirror image reinforcement represents a weaker reinforcing agent than does food, and thereby does not support behavior. For example, Kraeling (1961) showed that rats learned significantly slower for a reinforcement of low concentration sugar solution (weak reinforcer) than for a high concentration sugar solution (strong reinforcer). If mirror image is a weak reinforcer then the obtained results are to be expected. However, evidence has been presented which clearly indicates that mirror image is as strong, if not a stronger reinforcer than food. Hogan (1961, 1967) showed that in a runway situation Siamese Fighting Fish swam as fast or faster on some trials for mirror image reinforcement than for food. Also, during extinction, mirror image reinforcement subjects demonstrated no evidence of a decline in response rate to both the PRR and PUR during a 10 hr period. During the same period the food reinforcement subjects indicated a progressive and expected decline in the rate of responding. Kimble (1961) states that the stronger the reinforcer, the greater the rate of responding in extinction, which would suggest that mirror image is a superior reinforcer in comparison to food. The absence of a progressive decline in response rates for mirror image subjects during extinction corroborates earlier findings by Goldstein (1971), Rnic (1973), and Turnbough and LLoyd (1973). Based on these findings, the hypothesis that subjects did not learn to reverse for mirror image reinforcement because mirror image is a weak reinforcer, cannot be supported.

Third, an alternative hypothesis has been offered by Hogan (1967) in order to account for the general difference in performance between food and mirror image reinforcement. Hogan states that "...it seems quite likely that display and food represent two kinds of reinforcers that may well depend for their effects on different mechanisms... [p. 359]." Since Hogan does not specify the nature of these "different mechanisms" this model is not specific enough to explain the results obtained in the present study.

The fourth hypothesis is that mirror image may produce high levels of arousal which subsequently interfere with the learning of an operant task. For example, Hogan (1967) observed that "...the heightened excitability of fish that have recently displayed (Hogan, 1961) makes a fish swimming for display more distractable. During training sessions for display, fish were observed to pay attention to and to attack small pieces of dirt in the water which would normally be passed unnoticed [p. 359]." In the present study this was characterized by an elevated activity level within the operant tank for a number of minutes following the termination of mirror image reinforcement. Fish subjected to mirror image reinforcement appear to be more distractable than are fish which are subjected to food reinforcement (Hogan, 1967). This may be due to an increased level of excitement (arousal), as a result of aggressive display.

In this respect, Ferster (1958) has shown that increased arousal levels seriously disrupt the attentive processes to relevant cues resulting in poor discrimination learning in primates. Additional evidence demonstrating that high arousal levels interfere with task learning has been given by Belanger and Feldman (1962), Broadhurst (1957), Brush (1957), Lindsley (1957) and Stennett (1957). The interfering effects of elevated arousal levels on discrimination learning can best be understood by an examination of the components of discrimination learning as proposed by Mackintosh (1969), Sutherland (1964), and Zeaman and House (1963). These authors state that the subjects must learn to attend to the relevant dimension of the experimental situation, as well as learning what value of that dimension is rewarded. Attention to the relevant cues in SDR and the learned consequences of previous trials will, in part, determine the subject's choice on a given subsequent trial.

Detection of the relevant cue is the critical factor in discrimination learning tasks such as SDR. However, as Ferster (1958) has shown, increased arousal level interferes with the subject's ability to detect these cues resulting in poor performance. It is these hypothesized high arousal levels in response to mirror image stimulation by Siamese Fighting Fish, which may account for the absence of reversal behavior under mirror image reinforcement conditions. Subjects using this form of reinforcement fail to attend to those cues necessary for successful performance in SDR.

The finding of poor performance for mirror image subjects also bears a strong relationship to the Yerkes-Dodson law (1908) from which the arousal concept was later derived. The Yerkes-Dodson law states that learning of difficult tasks are easiest when motive strength (i. e., arousal) is low and that learning of simple tasks are easiest when motive strength is high. This parallels the findings of the present study concerning food and mirror image reinforcement. During pre-training with one ring, it was found that mirror reinforced subjects learned the response more readily than food reinforced subjects. However the reverse effect occurred when task difficulty was increased by the addition of a second response alternative. These data are readily accounted for by the assumption that mirror image engenders higher levels of arousal than food. In this respect the results of both reinforcement groups are in congruence with the Yerkes-Dodson law.

Although the arousal interpretation is consistent for the data from Day 8 onward for mirror image reinforcement subjects, it does not explain why four subjects successfully learned the original discrimination with one subject also learning the first reversal problem. No definite hypothesis can be offered for this finding. However, it was observed that the successful performance for subjects during this period may be more "artifactual" than a product of true learning. For example, problems R_0 and R_1 (8 days of training) represented novel environmental stimuli for subjects following pre-training. Subjects were therefore observed to select and remain in the vicinity of one of the discrimination rings (reinforced) and not to explore the other. This resulted in what may be termed a "fixation" in responding to one ring. This was observed in the finding that an overall mean of 5.4 errors per session were recorded for the 8 days of training in which problems R_0 and R_1 were completed. Following Day 8, the mean error rate per day increased dramatically to a mean score of 12 errors per session. This indicated that as the subject became more familiar with the experimental situation as a function of time, there was a dissipation of the "fixation" effect and more responses were made to the other ring. This resulted in significantly greater error scores.

If the mirror image reinforcement subjects had actually learned the reinforcement contingencies in problems R_0 and R_1, it would be safe to assume that the same level of performance would follow beyond this point, with a subsequent decrease in errors as training progressed. Since this did not occur, the performance of subjects during the first two discrimination problems may not necessarily be attributed to progressive learning of the task. Following this point, performance does not reach criterion due to the interfering effects of elevated arousal levels. However, due to the significant decrease in errors per day over the succeeding 50 days, it does indicate that subjects were learning the reinforcement contingencies, although this learning was minimal. It may also be assumed that since Siamese Fighting Fish show habituation to mirror image presentation (Baenninger, 1966; Clayton & Hinde, 1968) there may also be a reduction of arousal with continued training. Such a reduction in arousal would further tend to facilitate learning during the 50 day period following problems R_0 and R_1.

In addition, the statistical analysis of the present study, also appears to indicate a progressive improvement in the rate of reversal learning for food reinforcement across discrimination problems. If this interpretation is accurate, then the finding of progressive improvement warrants a re-examination of the position that fish are incapable of "learning to learn" in SDR situations for food reinforcement (Bitterman, 1965b). However, a criticism may be directed at this study, stating that subjects did not in fact learn to reverse based on spatial cues, but rather learned only to avoid the response alternative with the visual cue as this cue was consistently correlated with non-reinforcement. Since the design of the study does not allow a resolution of which cues (spatial or visual) were utilized by the subjects, clarification of this issue can only be accomplished through future research. For example, the visual cue may be first associated with the non-reinforced alternative for a number of discrimination problems, and then reversed to the reinforced response alternative. If no change in behavior occurs, then further support would be added to the view that subjects were not employing the visual cue but rather spatial cues in reversal learning. A similar design would be to correlate the visual cue with one of the response alternatives for a number of discrimination problems, and then discontinue the use of the visual cue. No changes in behavior between both conditions would also contribute additional evidence for the possible ineffectiveness of visual cues in controlling discrimination learning. A third method of investigation in order to observe the effects of the visual cue, would be to test two groups simultaneously. One group would be tested with no visual cues present in the experimental situation, whereas the other group would be tested with the visual cue consistently correlated with either of the response alternatives. Differences or similarities in the average performance between both groups would provide additional evidence as to the effects of the visual cue.

Although it is possible that the visual cue controlled reversal learning in the present study, evidence is provided demonstrating that learning was controlled by spatial cues. These are discussed as follows.

First, a pilot study (Appendix A) showed that reversal of the visual cue from the non-reinforced alternative to the reinforced alternative following seven discrimination problems, produced no significant changes in operant performance ($F = .639$, *df* = 1/1, $p > .25$ NS). This provides strong evidence that subjects were not utilizing the visual cue in learning the reinforcement contingencies of the reversal problem, since a negative transfer effect following visual cue reversal would be expected if subjects were employing visual cues as an indicator of which ring was reinforced or unreinforced.

In addition, as demonstrated by Figs. 3, 6 and 7, the finding of no significant differences in behavior following cue reversal is further substantiated where reversal of the cue followed an extinction period. Although no behavioral differences were observed, this argument may be questioned, by the fact that cue reversal took place following extinction, which may have neutralized the effect of prior training and the association of the visual cue with the unreinforced response alternative. However, when considered in context with the pilot study, this does not appear probable.

Second, the contention that subjects had only to learn the relatively "simple task" of avoiding the ring with the visual cue may not be able to account for the finding that mirror image reinforcement subjects failed to demonstrate any significant learning in the discrimination situation. That is, if food reinforcement subjects were able to learn the operant task based on visual cues, it would also be expected that mirror image reinforcement subjects should demonstrate a comparable level of learning if an avoidance of a visual cue was the only learning required. However, it is acknowledged that differences between food and mirror image could be pronounced to the extent that differences between reinforcers may still be evident in relatively simple tasks.

These arguments dealing with visual cue reversal and task simplicity argue against solely a visual cue discrimination interpretation, but do add further support to a spatial cue interpretation especially when considered in a phylogenetic perspective. For example, the effectiveness of one cue in controlling behavior and the relative ineffectiveness of another cue within the same task is not totally without precedent. As Gilbert (1969) states, there is enough evidence to suggest that there is a phylogenetically determined predisposition on the part of an organism to come under the control of some dimensions (stimulus cues) rather than others. This is termed an "attending hierarchy" by Baron (1965), where certain types of cues are given priority over others. In otherwords, certain types of cues are more salient to the organism depending on the species used. In this respect, as the work of Bitterman (1965b) has shown, fish may be more responsive to spatial cues rather than visual cues. This also appears to be true of performance in spatial as opposed to visual reversal tasks (Bitterman, Wodinsky & Candland, 1958), providing additional support to the position that reversal performance in the present study was based on spatial cues.

In addition recent experimental efforts dealing with the feature negative effect (Jenkins & Sainsbury, 1969) and the spatial separation of cue and response (Stollnitz, 1965), contribute to the position that the visual cue as employed in the present problem did not influence discrimination learning. In this respect, Jenkins and Sainsbury (1969) has provided empirical evidence showing that when a distinctive feature (i.e., visual cue) is correlated with the positive response alternative then the visual cue tends to maximize discrimination performance. However, as in the present study, when the same visual cue was correlated with the negative response alternative, no observable effects concerning discrimination performance was evident (feature negative effect). As Hearst (1969) states "...the great majority of subjects in their feature-negative groups showed absolutely no evidence of learning this successive discrimination, even after 24 training sessions [p. 16]." The analogous use of the visual cue in both studies, strongly suggests that such cues, when correlated with the negative response alternative weakens control over behavior in discrimination learning.

Secondly, Stollnitz (1965) has also shown in monkeys, that when a visual cue is separated from the response by distances as small as .50 - .75 in. discrimination performance is sharply impaired. The same finding may also be representative of the present study in which the visual cue was also separated from the response. That is, the visual cue in the form of the white plate encircling the ring was separated from the response of swimming through the ring by a distance of approximately 1.5 cm (0.6 in). According to Stollnitz's findings, this separation between cue and response should serve to destroy the effectiveness of the visual cue in learning the discrimination task. This is especially true in a phylogenetic sense, for if monkeys demonstrate difficulty in discrimination training with small separations between cue and response, then it would be expected that fish would reflect at least an equal difficulty.

Therefore, the general conclusion based upon evidence found within this study and in related areas as discussed, strongly points to the position that spatial cues and not visual cues were responsible for discrimination reversal learning. However, a firm conclusion can only be reached, when the specific effects of the visual cue as used in the present study have been specifically determined.

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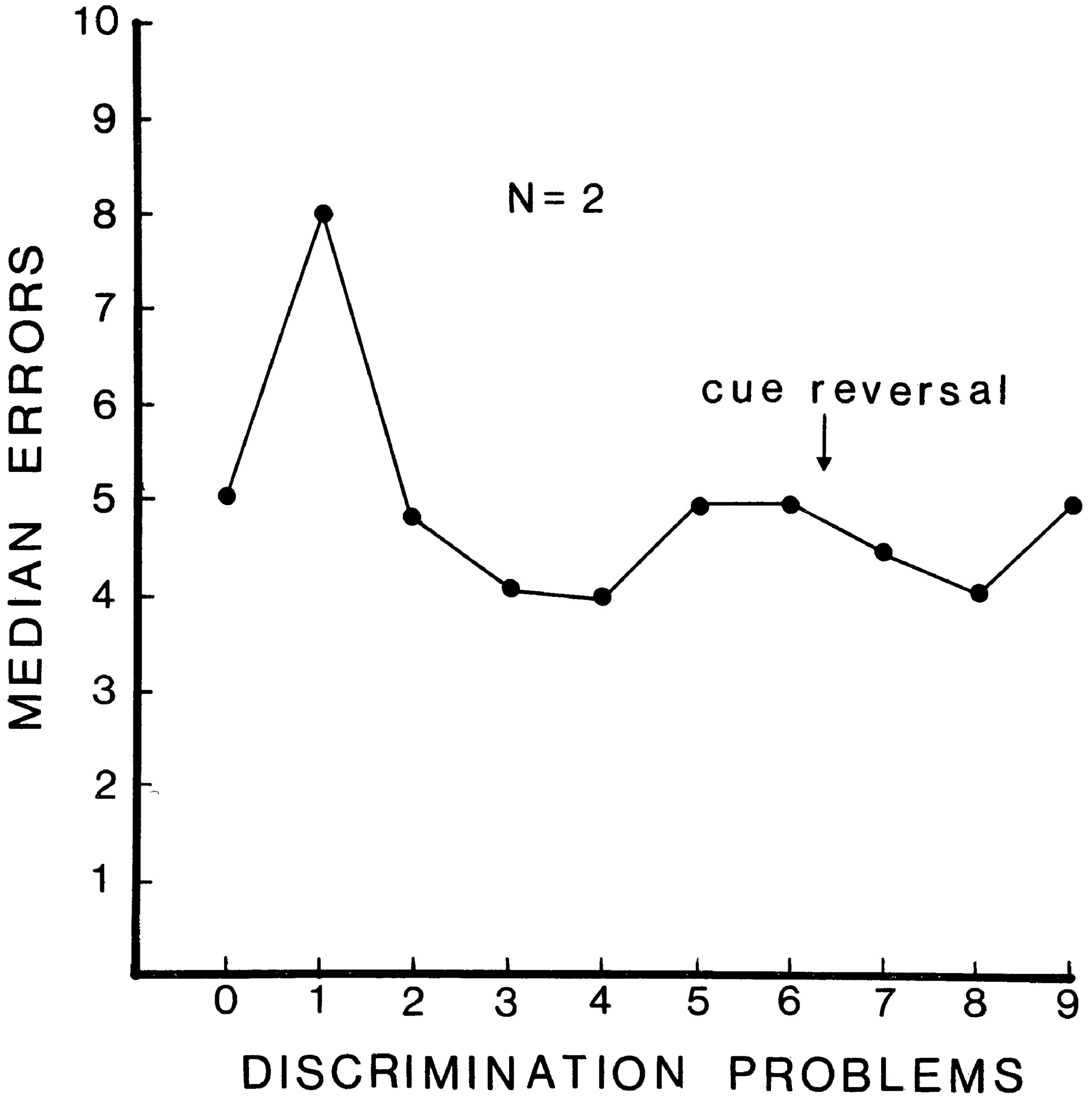

Appendix A. Medians of the median error per fish per discrimination problem (Food reinforcement group).