

EVALUATION OF TECHNIQUES TO MEASURE VISUAL

ACUITY IN Etheostoma spectabile AND

Notropis lutrensis

By

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CHAPTER I

INTRODUCTION

A measurement of visual acuity of nonhuman species may be of value for several reasons. It permits comparison of the structure of the visual system with its function, it aids in the understanding of the ecological niche occupied by the species, and it aids in the understanding of the functional status of the nervous system when compared with a known, normal value for visual acuity of a species.

Methods to measure the visual acuity of nonhuman species fall into two major categories: operant conditioning techniques and optomotor-optokinetic techniques.

Operant conditioning techniques have been used to measure the visual acuity of several species of fishes and were the only techniques used prior to this investigation. These techniques seem adequate for most species, but the time involved in conditioning the test subjects to the criterion of correct response desired by the researcher is quite variable and frequently is a matter of months.

Optomotor-optokinetic techniques also seem adequate for several species of Peromyscus (Vestal, 1971; Vestal, 1973). In addition, the time required to condition test subjects is dispensed with; thus, the time required to measure visual acuity of test subjects is relatively short compared to the time required by the operant conditioning techniques. Although optomotor-optokinetic techniques have been used

to measure the visual acuity of several species of Peromyscus and the optomotor response has been investigated in several species of fish, these techniques have not been used to measure the visual acuity of any fish species.

Operant conditioning techniques and optokinetic-optomotor techniques measure different types of visual acuity, however. The optokinetic-optomotor techniques require a moving grid to which the test subjects respond by moving their eyes or body, and so, the value for dynamic visual acuity is measured. Operant conditioning techniques normally utilize a stationary grid and, thus, measure static visual acuity. The two types of acuity have been shown for some species to involve different portions of the retina.

Very few measurements of the visual acuity of fish species have been performed (Nakamura, 1968), and all of the measurements have been obtained by operant conditioning techniques. Nakamura (1968) determined static visual acuities of 5.5' of arc and 7.4' of arc for the skipjack tuna, Katsuwonus pelamis, and the little tunny, Euthynus affinis, respectively at an illumination of 170 lux. These values are comparable to the values for visual acuity obtained by other investigations of fish species.

Evaluation of the two categories of techniques to measure visual acuity is informative. Each has its own set of advantages and disadvantages. It is helpful to know as many of these as possible prior to utilizing these techniques. By knowing, much effort may be saved. This investigation examines the effectiveness of two operant conditioning techniques designed to measure the visual acuity of Etheostoma spectabile, the orange-throat darter, and the effectiveness of an optomotor technique

designed to measure the visual acuity of E. spectabile. Since the performance of E. spectabile was not as anticipated in Experiment IV, Notropis lutrensis, the red shiner, was also used. The dependability of the optomotor response by E. spectabile is also evaluated.

CHAPTER II

LITERATURE REVIEW

The study of vision and the visual system has prevailed over the past three thousand years (Polyak, 1957). Over such a period of time it is no surprise that an enormous amount of information relating to vision has accumulated. Fortunately, much of that information is available in the form of publications designed to review large portions of the literature of visual research. Some of these review publications include the following fields of knowledge: the phylogeny of the vertebrate visual system (Polyak, 1957), visual perception (Cornsweet, 1965; Graham, 1965; Murch, 1973), and the physiology of sensory systems, including the visual system (Uttal, 1972). An excellent work on vision in fish has been published by NATO (Ali, 1975).

For those interested in the study of visual acuity, specifically, the work by Riggs (1965) provides adequate basic information, and if more specific information is required, factors affecting visual acuity are documented. Stimulus intensity, stimulus duration, stimulus frequency, and interstimulus interval have been shown to affect visual acuity (Brown, 1972a; Brown, 1972b; Murch, 1973). Illumination has also been shown to have an effect on visual acuity (Nakamura, 1968; Fite, 1973). In addition, the state of adaptation to different day-lengths (Muntz and Northmore, 1973) and temperature (Thorpe, 1973) have been found to affect spectral sensitivity of fish, and conceivably,

these factors could affect visual acuity, as well.

Methods for Measuring Visual Parameters Other Than Acuity in Aquatic Species

Within the span of visual perception are several parameters other than acuity. Among these are included sensitivity, spectral sensitivity, and shape discrimination. All of these have been measured by operant conditioning techniques.

Shape discrimination capabilities have been investigated in the cephalopod, Octopus vulgaris (Sutherland, 1961) and in the fish, Tilapia macrocephalus (Hemmings and Matthews, 1965) by operant conditioning techniques. Spectral sensitivity has been investigated in the goldfish by an autonomic conditioning of heart and respiratory rate inhibition (Hester, 1968), and visual sensitivity of the fish, Scardinius erythrophthalmus (Muntz and Northmore, 1973) by operant conditioning techniques. Spectral sensitivity was measured in the goldfish, Carassius auratus and the clawed toad tadpole, Xenopus laevis by the utilization of an optomotor technique (Cronly-Dillon and Muntz, 1965).

Operant Conditioning Methods for Measuring Visual Acuity in Aquatic Species

Conditioned visual discrimination tasks have been used to measure the visual acuity of several aquatic and marine vertebrate species. Included is a relatively complete review of the literature dealing with such measurements.

Fish species which have been so investigated include: the freshwater minnow, Phoxinus laevis, capable of discriminating at a visual

angle of 10.7' of arc (Brunner, 1934); the marling convict fish, Microcanthus strigatus, capable of discriminating at a visual angle of 5' of arc (Yamanouchi, 1956); the cichlid, Aequidens portalegrensis (Baerends and Bennema, 1960); and two tuna species, Katsuwonus pelamis and Euthynus affinis, capable respectively of discriminating at visual angles of 5.5' of arc and 7.4' of arc (Nakamura, 1968).

Other aquatic species so investigated have all been marine mammals and include: the Pacific white-dised dolphin, Lagenorhynchus obliquegens, capable of discriminating at a visual angle of 6' of arc (Spong and White, 1971); the killer whale, Orcinus orca, capable of discriminating at a visual angle of 5.5' of arc (White et al., 1971); and the sea lion, Zalophus californianus, capable of discriminating at a visual angle of 8' of arc (Schusterman and Balliet, 1970).

The subjects used in these techniques are conditioned to discriminate between two visual targets. Targets previously used were vertically striped and horizontally striped (Nakamura, 1968), two lines and one line (Spong and White, 1971), and striped and gray (Schusterman and Balliet, 1970).

The motor tasks usually used were approaching to a target (Nakamura, 1968) or pressing a lever or button (White et al., 1971), but occasionally a novel test has been used, for instance, vocalization (Schusterman and Balliet, 1970).

The use of conditioned visual discrimination tasks to measure visual acuity has certain disadvantages. It may take thousands of conditioning trials to successfully condition a subject to perform the required task at a high level of correct response (Spong and White, 1971; White et al., 1971).

Optokinetic and Optomotor Methods for
Measuring Visual Acuity

Optokinetic and optomotor responses have been shown to be innate to a variety of animal species (Smith and Bojar, 1938; Rock et al., 1964; Shaw and Sachs, 1967). The distinction between "optokinetic" and "optomotor" is not definite and the two terms are often used interchangeably. When a distinction is made, however, "optokinetic" usually refers to eye movements in response to motion of a visual stimulus and "optomotor" usually refers to body movements in response to the motion of a visual stimulus (Fite, 1968).

Investigation has indicated that optomotor and optokinetic responses may be used to evaluate a variety of visual parameters and with more convenience than operant conditioning methods (Wallman, 1975). These optomotor and optokinetic responses have been used to measure spectral sensitivity in fish and amphibians (Cronly-Dillon and Muntz, 1965) and to measure visual acuity in Peromyscus species (Vestal and King, 1971; Vestal, 1973).

The optomotor and optokinetic responses of several mammalian species have been studied and characterized (Smith and Bojar, 1938). The optomotor (s.s.) response of several fish species has been investigated, as well (Harden-Jones, 1963).

Optomotor and optokinetic response techniques have their own disadvantages, however. Small movements made by a subject in response to the motion of a visual stimulus may be overlooked by the observer (Vestal and Hill, 1972).

CHAPTER III

METHODS AND MATERIALS

The research was divided into four experiments. Experiment I and Experiment II were attempts to use a conditioned visual discrimination task to measure visual acuity in Etheostoma spectabile. The purpose of Experiment III was to test whether or not the optomotor response of E. spectabile changed over a relatively lengthy period of visual stimulation. Experiment IV was an attempt to measure the visual acuity of E. spectabile and the visual acuity of Notropis lutrensis by a technique which utilized the optomotor response of the subjects.

Subjects

The subjects of this investigation were fourteen Etheostoma spectabile, the orange-throat darter, seined from Salt Creek near Fairfax, Oklahoma, and five Notropis lutrensis, the red shiner. Three of the N. lutrensis were seined from Council Creek near Stillwater, Oklahoma, and two of unknown origin were obtained from aquaria at the Department of Zoology, Oklahoma State University, Stillwater, Oklahoma. The three N. lutrensis obtained from aquaria were a minimum of three years of age. The E. spectabile were estimated to be in excess of one year of age.

Subjects were maintained in separate 6 liter aquaria for a minimum of three weeks prior to the initiation of an experiment and throughout

the duration of an experiment. The aquaria were aerated by air stones, but were not filtered. Temperature was maintained at approximately 21° C with a photoperiod of approximately 14 hours. Subjects were fed a diet of frozen brine shrimp.

E. spectabile are known to prefer small, gravel-bottomed streams where they are usually found in riffle areas. They are rarely collected in streams with mud or silt bottoms in Oklahoma and are known to feed on a variety of insect larvae (Miller and Robison, 1973). In addition, darter species are known to orient towards food primarily through the sense of vision (Roberts and Winn, 1961).

N. lutrensis is the most common and widespread of the Oklahoma Notropis species and survives in a wide variety of habitats. It seems to do best in small streams and is generally least common in permanently clear, gravel-bottomed, high gradient streams (Miller and Robison, 1973).

Conditioning Procedure for Experiments I and III

In Experiment I and Experiment II the same procedure was used to condition the subjects to perform the same visual discrimination task, but in different condition apparatuses.

The task which was to be performed by the subjects was as follows: The subject was to approach a visual target consisting of two black, vertical lines and to avoid a visual target consisting of a single black, vertical line. The two visual targets subtended identical visual angles.

The task was performed correctly if the subject's eyes went beyond a small glass hurdle adjacent to the portion of the apparatus which contained the two-line visual target. In analysis, this was termed a

"type 3 response."

The task was performed incorrectly when a subject's eyes went beyond a small glass hurdle adjacent to the portion of the apparatus which contained the single-line visual target, termed in analysis a "type 1 response," or when the subject failed to cross either glass hurdle, termed a "type 0 response." A fourth type of response was observed when a subject began the trial with a type 1 response and subsequently responded correctly before the end of the trial. This type of response was termed a "type 2 response" in statistical analysis.

Either the latency to correct response (type 2 or type 3 response) was recorded in units of seconds or an incorrect response (type 0 or type 1 response) was recorded with a latency of 25 seconds, the total length of time from the beginning to the end of a single trial. The classification of response was recorded as type 0 through type 3 only in Experiment II. In Experiment I, type 2 and type 3 responses were recorded as "correct" and type 0 and type 1 responses were recorded as "incorrect."

The conditioning procedure began by placing a subject in the starting chamber of the apparatus (Figure 1; Figure 2). Overhead fluorescent lights provided illumination. After a pause of one minute, a 100 Watt, Cool White, incandescent light was turned on directly over the center of the apparatus, as a cue to the subject indicating that the trial had begun. Simultaneously, the partition or partitions which separated the subject from the visual targets were removed and the timing was started. If the subject responded with a type 3 response in less than 5 seconds, the 100 Watt light was turned off and the latency of correct response was recorded. If the subject responded with a

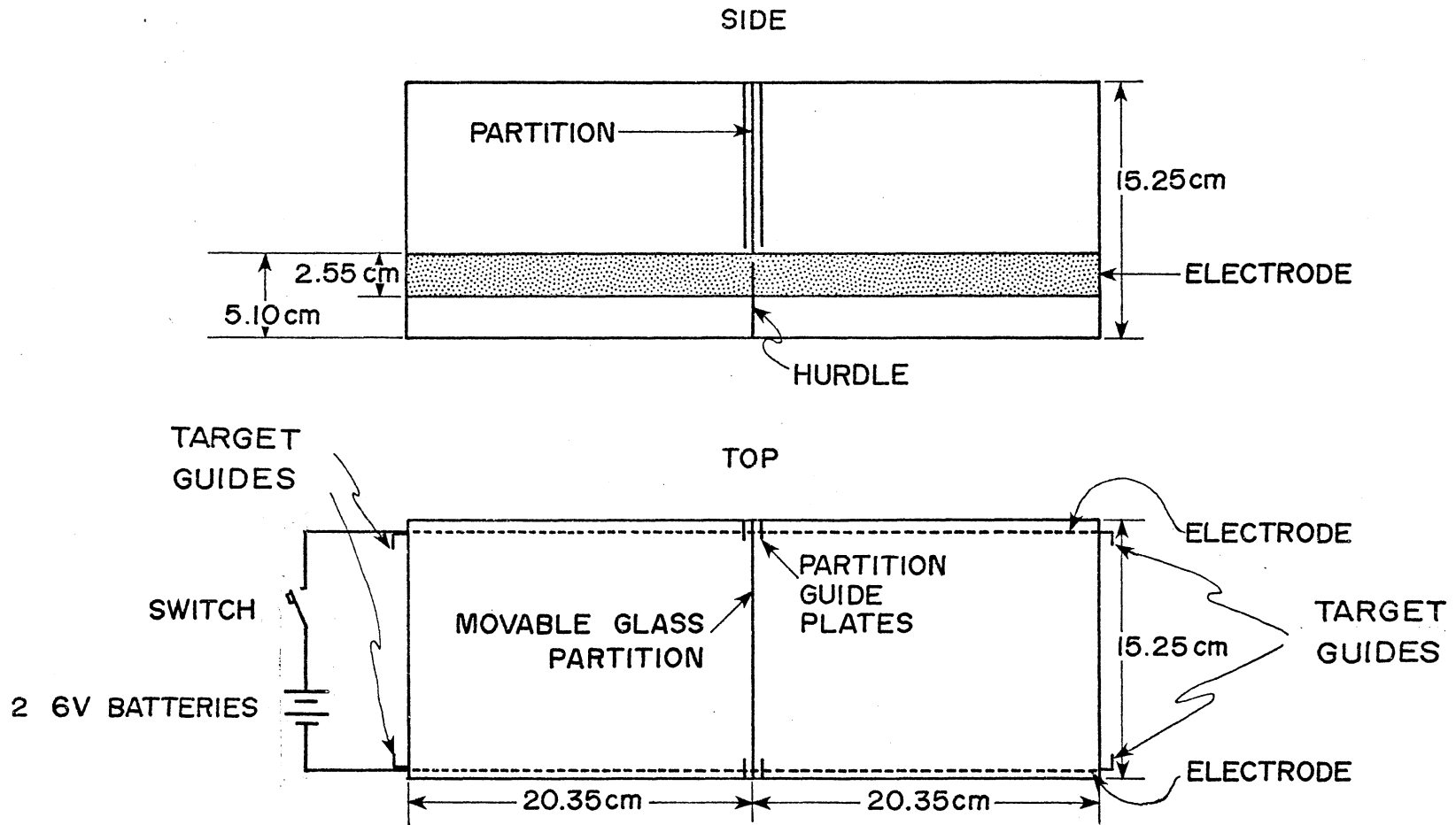


Figure 1. Apparatus for Conditioning in Experiment I

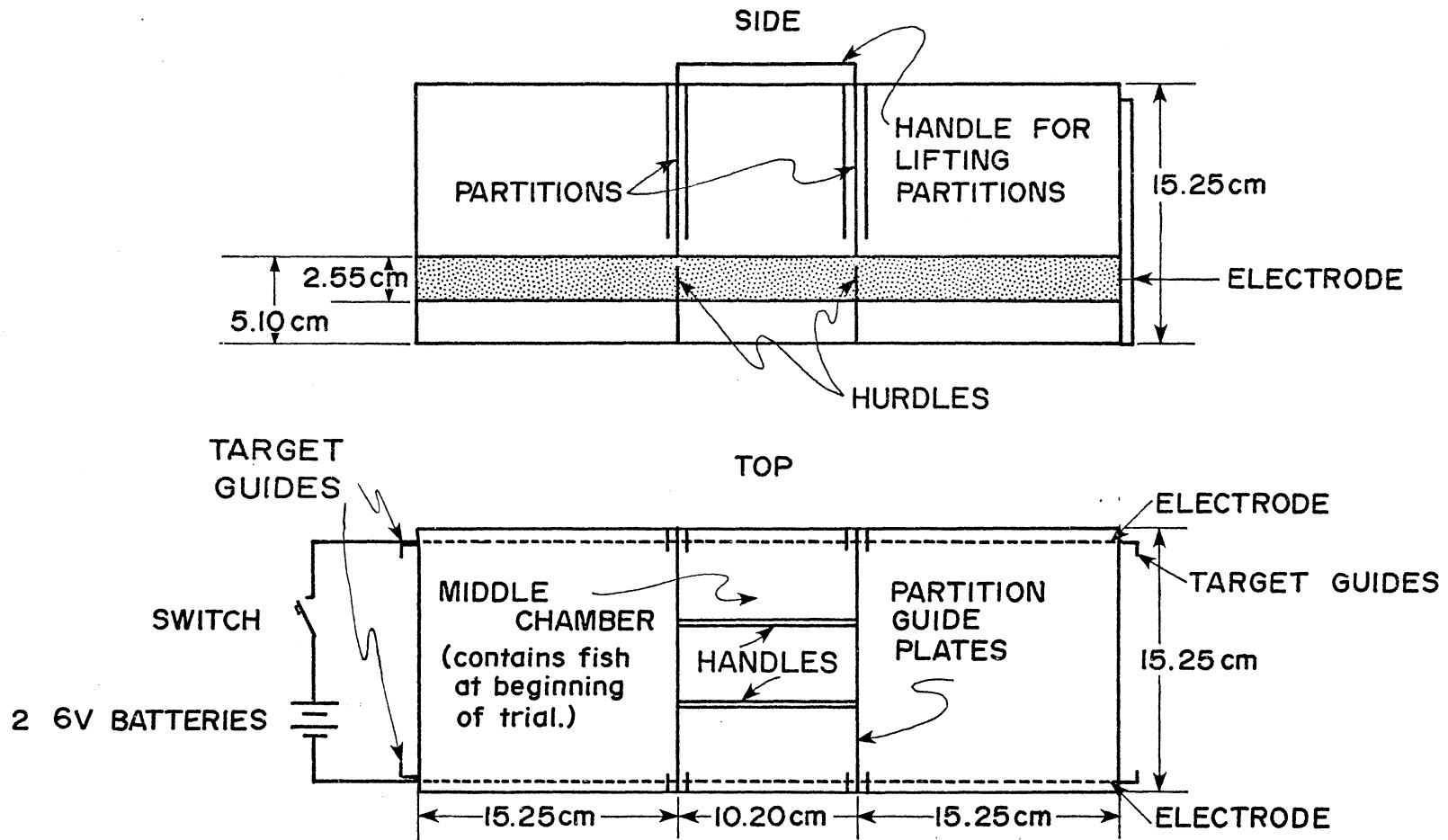


Figure 2. Apparatus for Preconditioning in Experiment I

type 1 response (crossing the wrong hurdle) the subject was shocked momentarily by 12 volts of direct current and was shocked at subsequent 5 second intervals until the subject crossed the correct hurdle or until 25 seconds had elapsed since the beginning of the trial, the maximum length of time for any trial. If the subject responded with a type 0 response (crossing neither hurdle) for 5 seconds, the subject was shocked with 12 volts of direct current and was shocked at subsequent 5 second intervals until the subject crossed the correct hurdle or until 25 seconds had elapsed since the beginning of the trial or until the subject crossed the incorrect hurdle. If the subject crossed the incorrect hurdle, the procedure was the same as if the subject had initially crossed the incorrect hurdle.

The procedure was repeated ten times per day on each of five subjects for the duration of the experiment.

Experiment I

Five Etheostoma spectabile were the subjects to be conditioned in Experiment I. A set of fifty pretraining trials was performed by each subject in the apparatus shown in Figure 2. The pretraining trials always began with the subject placed in the section of the apparatus opposite to the section of the apparatus containing the two-line target. In the remainder of the experiment the conditioning procedure previously described in this section was used.

On the day following the completion of the pretraining trials, a set of eighty trials per subject was begun using the apparatus shown in Figure 1, and the set was completed eight days later. The section in which the two-line target was placed was determined by a random selection at the beginning of each day.

Experiment II

Twenty four days after the completion of Experiment I, Experiment II was begun using the apparatus shown in Figure 3 and the same conditioning procedure as described previously. Subjects for Experiment II were 7 naive E. spectabile and the 5 E. spectabile which had been subjects for Experiment I. Five of the seven naive subjects completed a set of 140 trials per subject (14 days). The remaining two died early in the set. Four of the experienced subjects from Experiment I performed a set of 260 trials per subject (26 days) and one experienced subject was discarded because of consistently poor performance in the conditioning trials.

Optomotor Procedure for Experiment III and Experiment IV

The apparatus used in both Experiment III and Experiment IV is illustrated in Figure 4. The apparatus consisted of a rotating styro-foam disc on which a cylindrical visual acuity grating was placed (Figure 5). The disc was rotated by a spring-driven kymograph motor at 6 revolutions per minute. Directly above the disc was placed a side mask and bottom mask of cardboard which prevented the subject from seeing any movement other than that of the visual acuity grating. The cylindrical, clear glass tank in which the subjects were placed was suspended on the central axis from the top of the encasing cylinder. The subjects were observed by means of a periscope placed directly over the tank. The luminance in the tank was measured to be 0.23 lux.

After each subject was placed within the central tank, one to five minutes was allowed for recovery from some of the effects of handling.

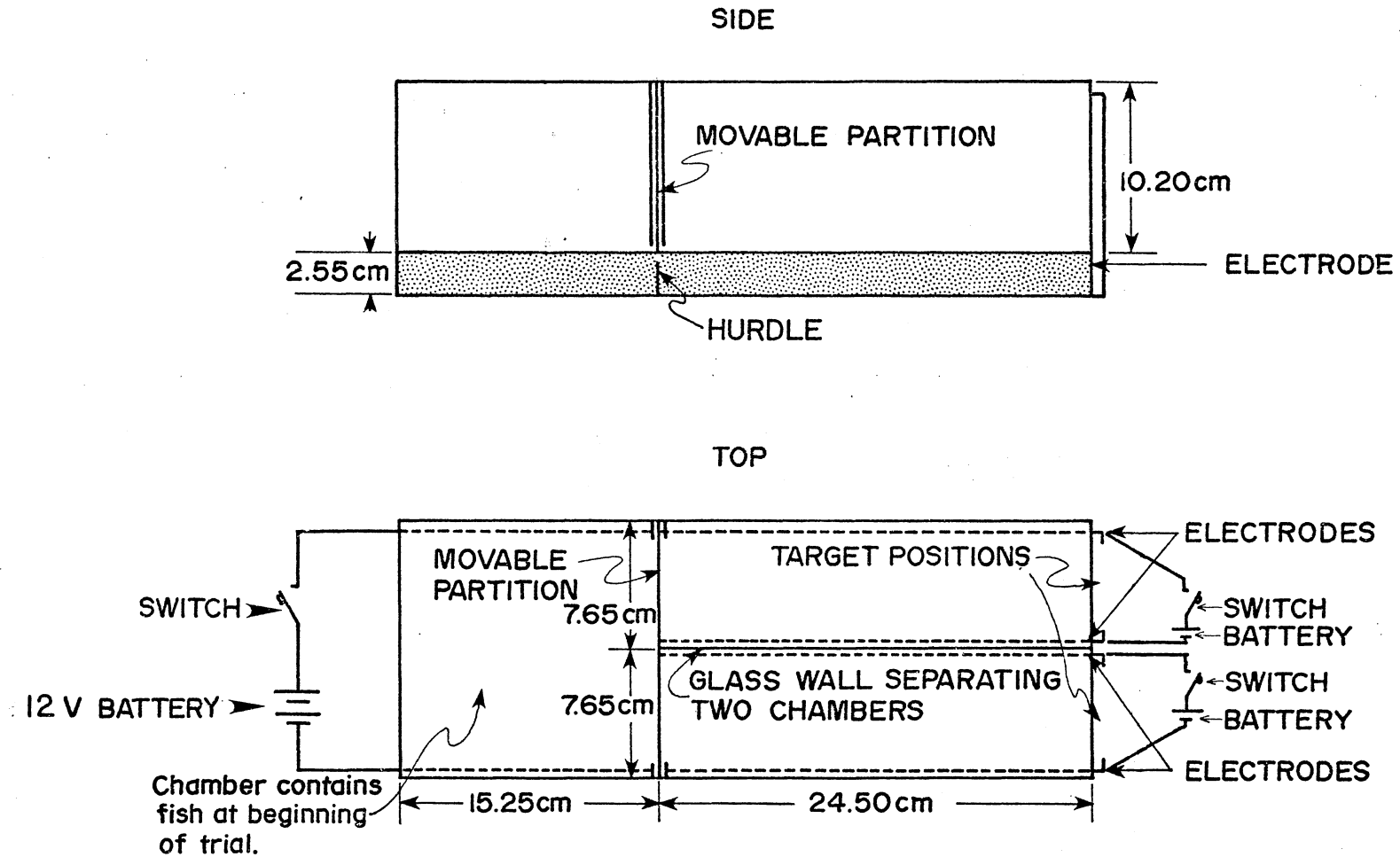


Figure 3. Apparatus for Conditioning in Experiment II

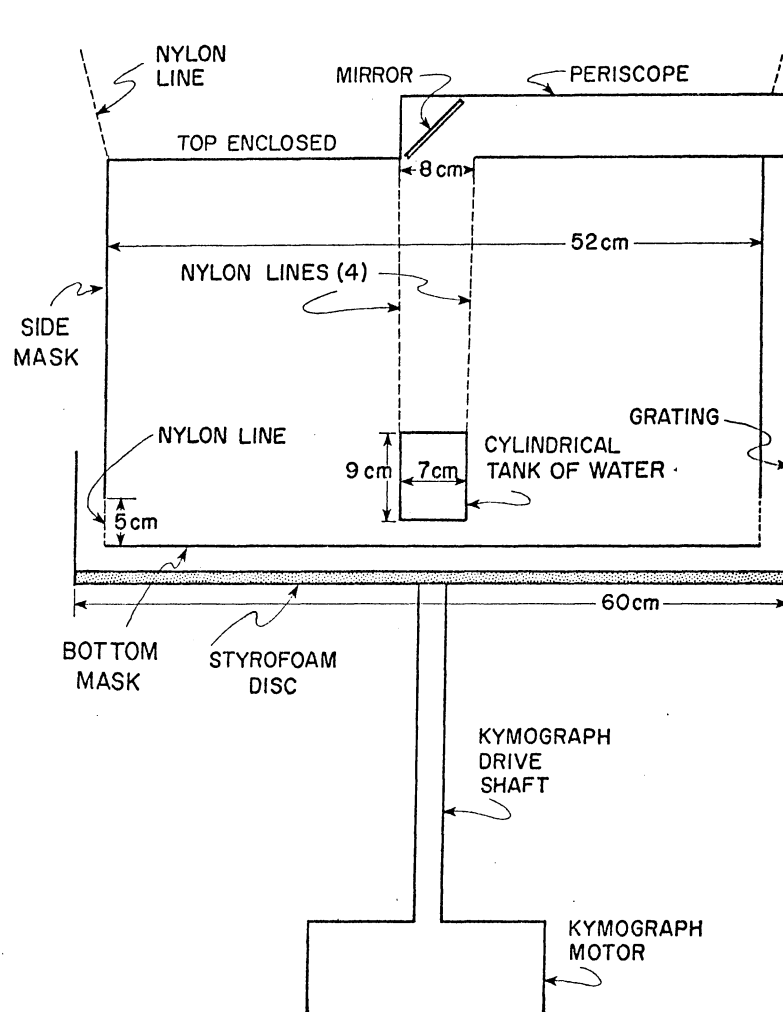


Figure 4. Optokinetic-Optomotor Apparatus for Experiments III and IV

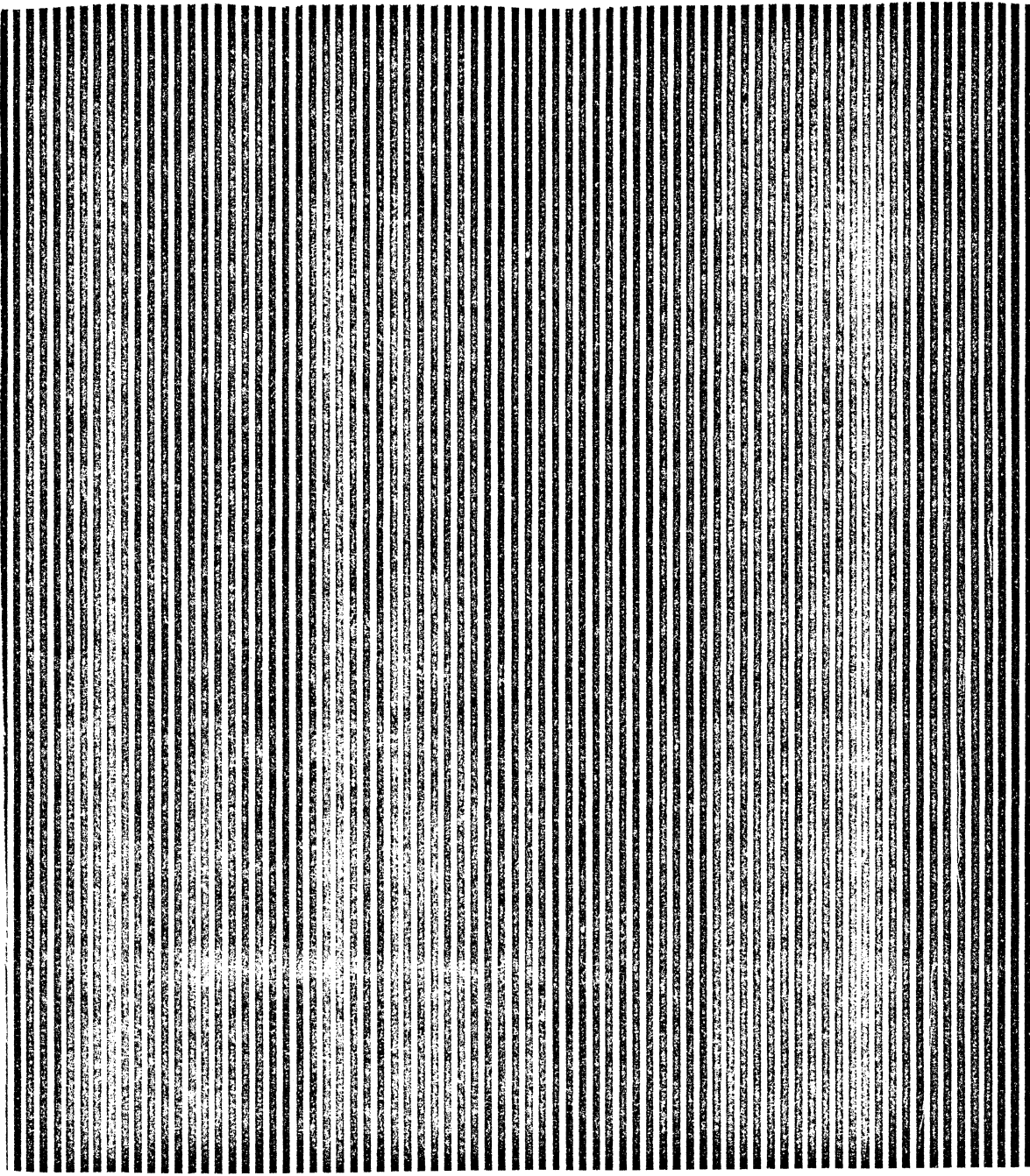


Figure 5. Example of Visual Acuity Grating Used in Experiments III and IV

When the subject appeared calm, the kymograph motor was turned on. When the subject exhibited a whole-body optomotor response, the kymograph motor was turned off and the latency of response was recorded. A trial continued for a maximum of 60 seconds from the starting of the motor.

The whole-body optomotor response in both Etheostoma spectabile and Notropis lutrensis is characterized in two distinct ways. The response most often observed was the subject swimming in a circling motion, keeping its head moving in the direction in which the visual acuity grating was moving. Rarely, this response would be preceded by a momentary swimming in the opposite direction.

The second and somewhat less common type of whole-body optomotor response by these two species was characterized by a bending movement of the body so that the head is moved in the direction of the movement of the visual acuity grating. The subject then straightened its body and immediately repeated the previous pattern of motion.

Experiment III

Experiment III was an attempt to determine if the whole-body optomotor response in E. spectabile was altered in its latency or frequency when subjects were exposed to a set of 100 trials per subject designed to elicit that response.

Subjects for this experiment were five E. spectabile. The procedure followed was that described above. Each of the five subjects performed a set of ten trials per day for ten days. The visual acuity grating consisted of alternating black and white lines of equal width. Each line subtended a visual angle of 6.67' of arc.

Experiment IV

Experiment IV was an attempt to measure the visual acuity of Etheostoma spectabile and Notropis lutrensis via the whole-body optomotor response. The procedure used was identical with that previously described for optomotor techniques, except for the gratings used.

Etheostoma spectabile. Five naive E. spectabile were used in the first part of Experiment IV. Each subject performed ten trials per day, each day with a different size of visual acuity grating. Five gratings were used, beginning with the largest and changing progressively to the smallest. In addition to these, a blank white visual target of coarse grade paper and a grating with approximately 1 cm wide lines were used.

The visual acuity gratings had lines which subtended visual angles, in descending magnitude, of approximately 6' 40" (#1), 4' 27" (#2), 3' 15" (#3), 2' 9" (#4), and 1' 2.5" (#5).

Notropis lutrensis. The only modification of method for N. lutrensis was a change in the order of presentation of targets so that visual acuity grating Number 4 was shown first, size 5 was shown next, and target size 3 was shown last. Target sizes 1 and 2 were not used for N. lutrensis since the visual acuity was measured effectively by using target sized 3, 4, and 5.

Data Analysis

Data from all four experiments were analyzed by use of a Statistical Analysis System program on an IBM 360 digital computer located on the campus of Oklahoma State University. Linear regressions, means, standard deviations, orthogonal polynomials, and F values of the latencies of correct response were calculated for all subjects in each experiment.

CHAPTER IV

RESULTS

Experiment I

The mean latencies of correct response by the Etheostoma spectabile subjects in this experiment varied from 14 seconds to 21 seconds with a pooled mean latency of 16 seconds (Table I). All subjects were pretrained.

TABLE I
MEAN LATENCY OF CORRECT RESPONSE AND VARIANCE OF
LATENCY OF SUBJECTS IN EXPERIMENT I

Subject	Mean Latency	Variance
1	14.2125	9.5448
5	14.3875	17.1147
7	18.2125	11.1247
11	15.4000	7.4632
13	21.4500	4.6440
All	16.2083	10.8084

Analysis of latencies of correct response in the trials within days indicated that two subjects performed at significantly different

latencies of correct response over trials within days of the experiment. It was also noted that the regression of the latencies of correct response for all subjects was positive, indicating that the latency of correct response tended to increase during the 10 trials of a single day for each of the subjects (Table II).

TABLE II
LINEAR REGRESSION COEFFICIENTS AND LEVEL OF SIGNIFICANCE
OF CHANGE IN LATENCY OF CORRECT RESPONSE
WITHIN DAYS FOR EXPERIMENT I

Subject	Slope of Regression	Intercept	Level of Significance
1	0.0159	14.1409	0.0663
5	0.3553	12.7886	0.1900
7	0.9841	13.7841	0.0076
11	0.4788	13.2455	0.0093
13	0.0606	21.1773	0.8576

Analysis of latency of correct response over the days of the experiment showed that no subject demonstrated significant change in latency of correct response over days (Table III).

Analysis of the latencies of correct response over days by orthogonal polynomials showed no significant linear or quadratic trends in the latencies of correct response. One subject exhibited a significant cubic trend (Table IV).

TABLE III
 LINEAR REGRESSION COEFFICIENTS AND LEVEL OF SIGNIFICANCE
 OF CHANGE IN LATENCY OF CORRECT RESPONSE
 OVER DAYS FOR EXPERIMENT I

Subject	Slope of Regression	Intercept	Level of Significance
1	0.5158	11.8929	0.3211
5	-0.4036	16.2036	0.5506
7	0.3964	16.4286	0.4704
11	-0.1952	16.2786	0.6596
13	2.0200	16.4000	0.1710
All			0.4220

TABLE IV
 ORTHOGONAL POLYNOMIAL ANALYSIS RESULTS FOR CHANGE
 IN LATENCY OF CORRECT RESPONSE OVER DAYS,
 EXPERIMENT I

Trend	Subject	Trend Coefficient	Level of Significance
linear	1	0.5155	0.3280
linear	5	-0.4036	0.3996
linear	7	0.3964	0.5043
linear	11	-0.1952	0.5634
linear	13	2.0200	1.0000
quadratic	1	0.2780	0.2962
quadratic	5	-0.0089	0.9688
quadratic	7	-0.2161	0.4691
quadratic	11	-0.1310	0.4462
quadratic	13	-1.2000	1.0000
cubic	1	-0.1179	0.3924
cubic	5	0.3477	0.0380
cubic	7	0.1280	0.4238
cubic	11	0.2081	0.0652
cubic	13	1.4000	1.0000

Experiment II

Analysis of the effect of pretraining showed significant difference between the latencies of correct response of pretrained subjects and subjects not pretrained at the 0.01 level of significance. Significant differences between the latencies of correct response of different subjects were exhibited at the 0.001 level of significance. No significant effect of sex or sex-pretraining interaction was shown.

The mean latencies of correct response were adjusted for number of days of training. These adjusted mean latencies ranged from 12 seconds to 17 seconds for the experienced subjects and from 18 seconds to 22 seconds for the naive subjects. The pooled mean latency was 16.7080 seconds (Table V).

TABLE V

ADJUSTED MEAN LATENCY OF CORRECT RESPONSE, VARIANCE OF LATENCY,
AND PRETRAINING STATUS OF SUBJECTS IN EXPERIMENT II

Subject	Adjusted Mean Latency	Variance	Pretraining Status
1	13.9907	20.2692	pretrained
3	12.0753	17.4675	pretrained
5	17.4223	10.9779	pretrained
11	12.8946	16.3353	pretrained
2	20.9223	9.0534	none
4	20.6151	9.9377	none
6	21.3223	11.5989	none
8	18.0043	11.4460	none
12	22.6008	6.5729	none
All	16.7080	13.8933	

The change in latency of correct response over trials within days was demonstrated to be significant for only one subject. In addition, the slope of the linear regression of the latencies was shown to be negative for the responses of only two subjects (Table VI).

TABLE VI
LINEAR REGRESSION COEFFICIENTS AND LEVEL OF SIGNIFICANCE OF
CHANGE IN LATENCY OF CORRECT RESPONSE WITHIN DAYS
FOR EXPERIMENT II

Subject	Slope of Regression	Intercept	Level of Significance
1	0.1779	13.6286	0.9099
3	-0.2797	14.6810	0.1067
5	0.6918	14.2238	0.3626
11	0.4654	11.0905	0.9934
2	-0.1922	22.5857	0.6210
4	0.3000	19.5714	0.8306
6	0.0623	21.5857	0.0760
8	0.2429	17.1143	0.0430
12	0.2247	21.9714	0.0869

The average change in latency of correct response of all subjects over days of the experiment was not shown to be significant, but the change in latency by three individual subjects was significant. The slopes of the regressions of each of the three subjects was negative, indicating a decrease in the latency of correct response (Table VII).

The same three subjects mentioned above demonstrated significant linear trends with negative slopes. Two of these three subjects

demonstrated significant quadratic trends, indicating that the slope of the change in latency changed significantly during the conditioning process (Table VIII).

TABLE VII
 LINEAR REGRESSION COEFFICIENTS AND LEVEL OF SIGNIFICANCE OF
 CHANGE IN LATENCY OF CORRECT RESPONSE OVER DAYS FOR
 EXPERIMENT II

Subject	Slope of Regression	Intercept	Level of Significance
1	-0.1606	15.5188	0.1850
3	-0.3187	15.7375	0.0076
5	0.0853	17.3890	0.7047
11	-0.2585	15.7431	0.0222
2	0.0831	20.9055	0.6888
4	-0.4604	24.6747	0.0479
6	-0.2431	23.7516	0.3029
8	-0.0136	18.6152	0.9457
12	-0.0081	23.2681	0.9626
All	-0.2078		0.5350

TABLE VIII
 ORTHOGONAL POLYNOMIAL ANALYSIS RESULTS FOR CHANGE IN LATENCY
 OF CORRECT RESPONSE OVER DAYS FOR PRETRAINED SUBJECTS,
 EXPERIMENT II

Trend	Subject	Trend Coefficient	Level of Significance
linear	1	-0.1606	0.1952
linear	3	-0.3187	0.0015
linear	5	0.0853	0.7108
linear	11	-0.2585	0.0052
quadratic	1	0.0118	0.5195
quadratic	3	0.0503	0.0009
quadratic	5	0.0786	0.2371
quadratic	11	0.0495	0.0006
cubic	1	0.0021	0.4576
cubic	3	-0.0014	0.4981
cubic	5	0.0017	0.9248
cubic	11	0.0017	0.3702

Over days, the only subject exhibiting a significant trend of latency of response was subject four, which resulted in a significant ($p < 0.05$) linear decrease in latency of response (Table IX).

TABLE IX
 ORTHOGONAL POLYNOMIAL ANALYSIS RESULTS FOR CHANGE IN LATENCY
 OF CORRECT RESPONSE OVER DAYS FOR SUBJECTS NOT PRETRAINED,
 EXPERIMENT II

Trend	Subject	Trend Coefficient	Level of Significance
linear	2	0.0831	0.6328
linear	4	-0.4604	0.0347
linear	6	-0.2431	0.2487
linear	8	-0.0136	0.9471
linear	12	-0.0081	0.9597
quadratic	2	0.1030	0.0538
quadratic	4	0.0554	0.3177
quadratic	6	0.0987	0.8587
quadratic	8	0.0095	0.8581
quadratic	12	0.0534	0.2520
cubic	2	-0.0216	0.1426
cubic	4	0.0290	0.0856
cubic	6	0.1265	0.4824
cubic	8	0.0210	0.1609
cubic	12	-0.0203	0.1392

Experiment III

The mean latencies of correct response by the subjects in Experiment III ranged from 5.65 seconds to 22.35 seconds. The pooled mean latency of correct response was found to be 10.59 seconds (Table X).

TABLE X
MEAN LATENCY OF CORRECT RESPONSE AND VARIANCE OF LATENCY
OF CORRECT RESPONSE FOR EXPERIMENT III

Subject	Mean Latency	Variance
1	11.80	38.5511
2	22.35	136.1478
3	6.35	11.3533
4	6.81	17.0250
5	5.65	6.5923
All	10.59	39.7807

No subject exhibited significant change in latency of correct response over trials within days. The slope of the linear regressions of latency of correct response was found to be negative for three of the five subjects within days (Table XI).

The average change in latency by all subjects over days was not found to be significant. The change in latency of correct response was demonstrated to be significant by three of the five subjects, and the slopes of the regressions of latency were negative in all cases (Table XII).

TABLE XI

LINEAR REGRESSION COEFFICIENTS AND LEVEL OF SIGNIFICANCE OF
CHANGE IN LATENCY OF CORRECT RESPONSE WITHIN DAYS
FOR EXPERIMENT III

Subject	Slope of Regression	Intercept	Level of Significance
1	-0.3212	21.0733	0.099
2	-0.5048	27.3800	0.212
3	0.0406	12.7800	0.896
4	-0.1012	9.4667	0.692
5	0.0285	9.3096	0.683

TABLE XII

LINEAR REGRESSION COEFFICIENTS AND LEVEL OF SIGNIFICANCE OF
CHANGE IN LATENCY OF CORRECT RESPONSE OVER DAYS
FOR EXPERIMENT III

Subject	Slope of Regression	Intercept	Level of Significance
1	-1.6861	21.0733	0.0389
2	-0.9145	27.3800	0.4968
3	-1.1691	12.7800	0.0136
4	-0.4830	9.4667	0.3187
5	-0.6648	9.3067	0.0465
All	-0.9835	16.0013	0.7820

The same three subjects which showed significant change in latency over days exhibited significant linear trends in change in latency over days. Two of these three subjects also exhibited significant quadratic trends in change in latency over days (Table XIII).

TABLE XIII

ORTHOGONAL POLYNOMIAL ANALYSIS RESULTS FOR CHANGE IN LATENCY
OF CORRECT RESPONSE OVER DAYS FOR EXPERIMENT III

Trend	Subject	Trend Coefficient	Level of Significance
linear	1	-1.6861	0.0365
linear	2	-0.9145	0.5527
linear	3	-1.1691	0.0015
linear	4	-0.4830	0.1328
linear	5	-0.6648	0.0340
quadratic	1	-0.4561	0.1162
quadratic	2	-0.1909	0.7511
quadratic	3	0.3473	0.0061
quadratic	4	0.3973	0.0111
quadratic	5	0.1616	0.1458
cubic	1	0.0308	0.7747
cubic	2	-0.0857	0.7308
cubic	3	-0.0396	0.2973
cubic	4	-0.0688	0.1806
cubic	5	-0.0564	0.2053

Experiment IV

The change in latency of correct response over different target sizes was not demonstrated to be significant for Etheostoma spectabile subjects in this experiment. The change in latency of correct response over target sizes was demonstrated to be significant at the 0.0003 level for Notropis lutrensis subjects.

Linear, quadratic, cubic, and quartic trends in change in latency of correct response over target size were not found to be significant for E. spectabile subjects. A significant linear trend in change in latency of correct response over target sizes was exhibited by N. lutrensis subjects. The quadratic trend in change in latency, however, was not found to be significant for N. lutrensis (Table XIV).

TABLE XIV

ORTHOGONAL POLYNOMIAL ANALYSIS RESULTS FOR CHANGE IN LATENCY
OF CORRECT RESPONSE OVER DAYS FOR EXPERIMENT IV

Species	Level of Significance			
	Linear Trend	Quadratic Trend	Cubic Trend	Quartic Trend
<u>E. spectabile</u>	0.0942	0.3785	0.5614	0.3217
<u>N. lutrensis</u>	0.000009	0.06258	-----	-----

The mean latency of correct response by each subject for each target size further shows that there was little change caused by a change in target size. Further, the mean latency of correct response to the smallest target size, number five, by the subjects ranged from 3.2 seconds to 33.3 seconds, which is well within the time limit of a single trial (Table XV). The pooled mean latency of correct response to different target sizes by E. spectabile subjects shows a slight, but not sufficient, increase in latency of response to decreasing target size (Table XVI).

TABLE XV

MEAN LATENCIES OF CORRECT RESPONSES FOR INDIVIDUAL
Etheostoma spectabile TO DIFFERENT TARGET SIZES,
EXPERIMENT IV, MEAN LATENCIES (SECS.)

Subject	Target Size				
	1	2	3	4	5
1	7.8	2.0	3.0	19.6	33.3
2	15.1	13.5	24.4	10.4	17.2
3	3.3	10.8	4.9	6.5	7.1
4	7.3	6.8	8.0	4.8	3.2
5	3.1	6.1	5.2	3.7	16.4

TABLE XVI

POOLED MEAN LATENCIES OF CORRECT RESPONSES FOR DIFFERENT
 TARGET SIZES BY Etheostoma spectabile AND
Notropis lutrensis IN EXPERIMENT IV

Target Size	Mean Latency (<u>E. spectabile</u>)	Mean Latency (<u>N. lutrensis</u>)
1	7.32	-----
2	7.84	-----
3	9.10	8.30
4	9.00	23.87
5	15.44	57.33
6	10.14	-----
7	2.56	-----

The mean latency of correct response to different target sizes by Notropis lutrensis subjects exhibits a large increase in latency of correct response with decreasing target size. The mean latencies of correct response to the smallest target size ranged from 57.33 seconds to 60.00 seconds (no response), suggesting that the N. lutrensis subjects were unable to discriminate the lines of the smallest size target (Table XVIII).

The pooled mean latency of correct response by N. lutrensis subjects to different target sizes also showed a marked increase in latency of correct response to decreasing target size, further suggesting that N. lutrensis subjects were not able to discriminate the lines of the smallest target (Table XVI).

The percentage of the trials in which the optomotor response was displayed by E. spectabile subjects changed little except when tested with target size 5 (Table XVII). This was not the case, however, for the N. lutrensis subjects whose percentage of response with target size changed drastically from target size 3 to target size 4, and again from target size 4 to target size 5. Four of the five N. lutrensis subjects failed to respond in any of the trials with target size 5, and the other responded only once during the set of thirty trials with that target size (Table XIX). This indicates that the N. lutrensis subjects were not able to discriminate the lines of the grating of target size 5.

TABLE XVII

PERCENTAGE OF OPTOMOTOR RESPONSES TO DIFFERENT
TARGET SIZES BY Etheostoma spectabile

Subject	Target Size				
	1	2	3	4	5
1	100	100	100	100	70
2	90	100	90	100	80
3	100	100	100	100	100
4	100	100	100	100	100
5	100	100	100	100	100

TABLE XVIII

MEAN LATENCIES OF CORRECT RESPONSE FOR INDIVIDUAL
Notropis lutrensis AT DIFFERENT TARGET SIZES,
EXPERIMENT IV, MEAN LATENCIES (SECS.)

Subject	Target Size		
	3	4	5
1	21.70	56.37	60.00
2	6.90	51.37	60.00
3	7.40	36.43	60.00
4	9.20	59.30	60.00
5	8.30	23.87	57.33

TABLE XIX

PERCENTAGE OF OPTOMOTOR RESPONSES AT DIFFERENT TARGET
SIZES BY Notropis lutrensis, EXPERIMENT IV

Subject	3	4	5
1	90	10	0
2	100	23	0
3	100	47	0
4	100	3	0
5	100	73	7

CHAPTER V

DISCUSSION

Experiment I

In this experiment, little progress was made towards reaching the required criterion of an average latency of correct response of less than five seconds. The large mean latencies, lack of significant change in latency, and positive slopes of linear regressions of latencies do not encourage further use of this technique.

Experiment II

The mean latencies of correct response were shorter in this experiment than those obtained for subjects in Experiment I. Particularly, the mean latencies of those subjects used in Experiment I and subsequently used in Experiment II were shorter than those obtained in Experiment I and those obtained for naive subjects used in Experiment II. This suggests that the technique used in Experiment I was, at least, effective as pretraining. The difference between the latencies of correct response of pretrained and not pretrained subjects was significant. Therefore, a pretraining period is apparently advantageous in terms of shorter time required for conditioning.

Operant Conditioning Techniques

The methods used in Experiments I and II require that conditioning proceeds to the point when the subjects achieve an average latency of correct response of less than five seconds. If an attempt is made to measure visual acuity before this point is reached the results are thrown into doubt by the punishment required at five second intervals to cause the subject to respond. Extrapolation of the linear regressions of latencies of correct response in Experiment II shows that the most promising of the subjects with a significant linear trend in change of latency might reach the necessary latency criterion in $3\frac{1}{4}$ days and the least promising subjects might never reach the necessary latency criterion. Thirty-four days is comparable to the length of time required for conditioning in some other techniques utilizing reward systems (Spong and White, 1971; White et al., 1971) but is also much greater than some (Nakamura, 1968; Muntz and Northmore, 1973; Thorpe, 1973). Whether the difference in number of trials required for conditioning is due to difference in technique or differences in the learning capabilities of different species or individuals is not evident.

Two factors are highly probable flaws in operant conditioning techniques, as utilized in this investigation. The schedule of punishment in both techniques occasionally caused subjects to be punished upon entering the starting chamber after a type 1 response and may have been, thereby, conditioned to avoid exploratory behavior in the conditioning apparatus, since such behavior involved reentry to the starting chamber in many cases (Figure 2; Figure 3).

The second flaw is fundamental to any conditioning technique using punishment systems. At low levels of strength, punishment may,

paradoxically, cause effects similar to reward (Church, 1963). The effect of punishment on behavior is unpredictable. It may fail to cause avoidance behavior and its cessation may not cause approach behavior.

On the basis of the results of this investigation, modification of the operant conditioning techniques utilized here is necessary due to the projected length of time needed to train subjects adequately. A reward system of conditioning may be more advantageous in terms of time required for conditioning than a punishment system. A punishment system may yet be made to work, however. By constructing a conditioning apparatus so that a subject is not required to traverse the starting chamber to approach the correct target after approaching the incorrect target, exploratory behavior would not be as likely to be inhibited. The association of exploration with punishment would be reduced. A more flexible schedule of punishment during the conditioning trials could also be beneficial. By punishing the subject only when the subject is not moving towards the correct target would simplify the conditioning process, and would prevent conditioning the subject to avoid the correct target along with the incorrect target.

Experiment III

The results of this experiment indicate that the latency of the optomotor response does not increase with continued exposure to visual stimuli designed to elicit the response. Hence, the results obtained in measurement of visual acuity by use of the optomotor response are not invalidated by the effects of habituation, at least for Etheostoma spectabile.

The optomotor response required no conditioning of E. spectabile and has been shown to be innate in several other species (Rock et al., 1964).

Three subjects were shown to have significant decreases in latency over the days of the experiment. These three subjects also exhibited significant linear trends in change of latency and one of the three exhibited a significant quadratic trend in change of latency. This may indicate that these subjects become more responsive to the visual stimuli because of an increasing familiarity of the environment of the optomotor apparatus. These data also suggest a possibility that the subjects may have been inadvertently conditioned to respond with decreased latency. If the visual stimuli were noxious to the subjects they may have associated the cessation of the visual stimuli with their response and, thus, responded with decreasing latencies. This possibility throws some doubt on visual acuity data obtained through this technique. Subjects, if conditioned to respond during the trials, may respond to auditory cues associated with the optomotor trials instead of the visual stimuli.

Experiment IV

Etheostoma spectabile

As the threshold of the dynamic resolution acuity is approached, the latency of the optomotor response should increase (Vestal, 1975). Although no significant trends in change of latency with change of target size were demonstrated, the linear trend was significant at the 0.10 level (Table XIV), and, further, the pooled mean latency of optomotor response is larger for the smallest target size than that for any other target size (Table XVI). Though this evidence is not

significant at the critical level of 0.05, it does, at least, suggest that latency is increasing as the threshold of dynamic resolution acuity is approached.

As the threshold of the subjects' dynamic resolution acuity is approached, the frequency of optomotor response (expressed as percentages) in other measurements which utilized the optomotor response ranged from 25% for Peromyscus species (Vestal, 1975) to 80% for goldfish and clawed toad tadpoles (Cronly-Dillon and Muntz, 1965). Only two subjects reached the 80% criterion and these reached the criterion during trials with the smallest target size. If the 80% criterion is a valid one, the threshold of dynamic resolution acuity of these two subjects is approximately 1' 2.5" of arc at a distance of 26.5 cm.

Notropis lutrensis

Results obtained for N. lutrensis exhibit a significant linear trend in change of latency of the optomotor response with change of target size and this trend is evidently an increase of latency of the optomotor response with decreasing target size (Table XVII). Thus, these data support the hypothesis that as the threshold of dynamic resolution acuity is approached the latency of the optomotor response increases.

In addition to the trend of increase in latency of the optomotor response, the percentage of optomotor responses over trials with decreasing target sizes is shown to decrease dramatically. If a criterion of 80% response to a given target is used as the determinate of the threshold of dynamic resolution acuity, all subjects possessed thresholds of approximately 2' 9" of arc at 26.5 cm from the target. Even if the 25% criterion is used, three of the subjects reached

threshold at the 2' 9" level and the remaining two reached threshold at approximately 1' 2.5".

Optomotor Techniques

Optomotor techniques have been shown in this investigation to have advantages. The time required to measure visual acuity is approximately 7 days. The optomotor response is easily recognized by the observer. The optomotor response requires no conditioning and it does not, apparently, decrease due to habituation.

There are some disadvantages, however. Subjects may have been inadvertently conditioned to respond to the auditory stimuli associated with the optomotor technique, such as the sound of the kymograph motor. Therefore, the subjects may have continued responding even though the threshold of acuity had been passed in Experiment IV. This problem was ameliorated for N. lutrensis subjects by beginning the optomotor technique with a small target size, but results obtained for E. spectabile are in more doubt, particularly since E. spectabile subjects responded quite well to a blank white target.

Obtaining an optically perfect target is extremely difficult, and imperfections in a visual target would alter results obtained for visual acuity. If possible, a projected visual acuity grating as described by Wallman (1975) should be used instead of a paper target.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Attempts were made to measure the visual acuities of Etheostoma spectabile and Notropis lutrensis. E. spectabile, the orange throat darter, was used in two operant conditioning techniques, both using punishment and no reward, and in a technique to test for habituation of the optomotor response. Both E. spectabile and N. lutrensis were used in an optomotor technique designed to measure visual acuity.

Results obtained from the two operant conditioning experiments were somewhat ambiguous. Subjects were not conditioned to the required latency of frequency of correct response criteria. The extrapolation of the small, but significant decrease (Table VI) in latency of correct response obtained in Experiment II to the required latency is comparable to the length of time required to condition other species to a high criterion of correct response (Nakamura, 1968; Spong and White, 1971; White et al., 1971). Thus, the technique used in Experiment II is probably adequate to measure the visual acuity of E. spectabile, but it requires considerable time in conditioning the subjects.

The technique in Experiment II would probably be improved by decreasing the size of the starting chamber (Figure 2) so that the subject is more likely to cross a hurdle, and by using a food reward system in conjunction with the punishment system.

Experiment III was designed to detect changes in the optomotor response over time. Results indicated that habituation to the visual stimuli did not occur and the latency of response to the visual stimuli decreased significantly in three of the five subjects of the experiment. The latency of response to the visual stimuli decreased in the cases of the other two subjects, but the decrease was not significant. The causes of these decreases in latency of response are highly conjectural but may include an increased familiarity of the subjects to the optomotor apparatus and an inadvertent conditioning of the subjects to respond.

Experiment IV was designed to measure the dynamic resolution acuity of Etheostoma spectabile and Notropis lutrensis subjects by use of the optomotor response. A measurement of the dynamic resolution acuity of E. spectabile was not obtained, possibly because of lack of a sufficiently small visual acuity grating or to an inadvertent conditioning of the subjects to respond to the auditory stimuli associated with the apparatus of the optomotor technique. The latter possibility is most likely in view of the continuing response of the subjects to a blank white visual target. Some increase in latency of response suggests that the threshold of dynamic resolution acuity was being approached by the subjects, however.

The dynamic resolution acuity of N. lutrensis subjects was measured. The possibility of conditioning the subjects to respond to the auditory stimuli associated with the optomotor technique was avoided by beginning with the small visual targets presumed to subtend a visual angle near the threshold of acuity for the subjects. At a threshold criterion of 25% response, three of the N. lutrensis subjects reached the threshold

of dynamic resolution acuity at a visual angle of 2' 9". The remaining two subjects reached threshold at a visual angle of 1' 2.5". These values are lower than reported for static resolution acuity of other fish species. The range of static resolution acuities for other fish is from 5' minimum visual angle (Yamanouchi, 1956) to 10' 24" minimum visual angle (Brunner, 1934). Dynamic visual acuity is normally lower than static resolution acuity (Brown, 1972b), making the disparity between the results of this investigation and results reported elsewhere even larger. This disparity may be factual or may be the result of the refraction of light and distortion brought about by the air-glass interface and the glass-water interface present in the optomotor apparatus. The technique would be improved by the use of a projected visual acuity grating such as that described by Wallman (1975) and the air-glass and glass-water interfaces removed from the optomotor apparatus. The optomotor technique used in this investigation is workable, however.

Since a numerical value for the dynamic resolution acuity of E. spectabile subjects was not obtained, quantitative comparisons between the dynamic resolution acuities of E. spectabile and N. lutrensis are impossible. However, change in mean latency associated with change in target size (Table XVI) and significance of linear trend in change in mean latency associated with change in target size (Table XIV) show some indication that the threshold of dynamic resolution acuity of E. spectabile was approached, and the threshold is smaller than that of N. lutrensis (1' 2.5" - 2' 9").

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VITA - 2

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