1984

Operant Conditioning in the Water Snake (Nerodia sipedon)

Angela R. Deitz
Eastern Illinois University

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Operant Conditioning in

the Water Snake (*Nerodia sipedon*)

(TITLE)

BY

Angela R. Deitz

B.S. in Zoology

B.A. in Psychology

Eastern Illinois University, 1983

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science in Zoology

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1984

YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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ABSTRACT

Six subjects of *Nerodia sipedon* were tested for runway acquisition. Five of six subjects met criterion for runway learning, demonstrating a significant decrease over the 25 day testing period in mean latency and running times. Four subjects completed 170 trials in a T-maze to test two-choice discrimination and reversal learning. All subjects met criterion for the initial discrimination and one reversal while two subjects completed four reversals and one subject met criterion for six reversals. Results are similar or superior to those previously reported for reptiles and other non-human vertebrates.

ACKNOWLEDGEMENTS

I would like to express my graditude to my advisor, Dr. Michael A. Goodrich, for his guidance and suggestions at every stage of my development, research, and writing. His support and advice were invaluable.

I would also like to thank the other members of my graduate committee, Dr. William P. McGown. Dr. William S. James, and Dr. Richard D. Andrews. Their help in editing my manuscript was much appreciated.

Finally, I would like to thank William J. Taylor for his artwork and help in printing my manuscript.

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INTRODUCTION

A great deal of research has been done on learning in higher vertebrates (birds and mammals), with particular emphasis on the Norway rat and non-human primates. Many fewer studies have been made of the lower vertebrates, although it has been generally assumed that their learning capacity is limited. One group which has been especially overlooked is the Class Reptilia. As the evolutionary forerunner of the Aves and Mammalia, it is important to know how much a reptile can learn. It seems unlikely the learning potential of birds and mammals evolved since their divergence from the Reptilia.

The reptiles can be a difficult group to study. Special apparatus must be developed to assess the learning capacity of these animals. Even so, it is better to find out, as best we can, to what extent these animals can learn, than to make false assumptions with very little study.

Turtles were the first group of reptiles used to study conditioning and learning. Yerkes demonstrated in 1901 the ability of a single specimen of speckled turtle (Clemmys guttata) to solve two maze problems of increasing difficulty. Casteel (1911) used the midland painted turtle (Chrysemys picta marginata) to assess responses to patterns of different form in a series of two-choice discrimination experiments. Van Sommers (1963) trained nine red-ear turtles (Pseudemys scripta elegans) to depress a lever to secure air while submerged in water in an experimental chamber. Spigel (1965) used runway measurements in an attempt to demonstrate brightness discrimination in nine Chrysemys picta marginata. The variable results obtained were assumed to be due to a deficit in sensory capacity rather than learning capacity. Morlock, Brothers, and Schaffer (1968) required five eastern painted turtles (Chrysemys picta picta) to learn an under water E-maze to obtain access to air.

Lizards have also been subjects for experiments on operant conditioning. Brightness discrimination in eight collared lizards (Crotaphytus collaris) was studied by Vance, Richardson, and Goodrich (1965) with positive results. In 1965, Alkov and Crawford used heat and light as reinforcers to train 20 green iguanas (Iguana iguana) to develop runway acquisition. Krekorian, Vance, and Richardson (1969) conducted two-choice discrimination experiments with 20 desert iguanas (Dipsosaurus dorsalis) to show the importance of body temperature as a factor in learning. In 1969, Julian and Richardson conditioned six Dipsosaurus dorsalis on each of three mazes to show the relationship between temperature and learning. Kemp (1969) used disc pressing to assess thermoregulatory behavior in five Dipsosaurus dorsalis.

The caiman has been used in at least one learning study. Williams and Robertson (1970) used aversive training with 12 caimans who were trained to escape shock in a T-maze using brightness and spatial cues.

The first study of operant conditioning in snakes was done in 1936 by Kellog and Pomeroy. Twelve water snakes (Natrix sipedon) were used in a multiple T-maze with escape from cold water used as motivation. The group as a whole did not meet criterion, although individual performances varied greatly. Wolfle and Brown (1940) did a similar study with eleven diamondback water snakes (Natrix rhombifera rhombifera) using a similar apparatus, but using escape from heat as the motivation as well as electric shock. Their results were also inconclusive.

The first attempt at operant conditioning using positive reinforcement was done by Crawford and Bartlett (1966) using eight grey rat snakes (Elaphe obsoleta spiloides). Subjects were tested for 14 weeks, after which time the experimental group showed better results than the control group, but no positive conclusions were made.

Crawford and Holmes (1966) used six yellow rat snakes (Elaphe quadrivittata) and two everglades rat snakes (Elaphe obsoleta rossalleni) in a two chamber compartment to test the response to vibratory stimulation. Although this was supposed to be an aversive stimulus, some animals responded positively. Even though there was a large variation between individuals, there was an overall decrease in average response time. In 1970, Kleinginna demonstrated that three indigo snakes (Drymarchon corais couperi) could be conditioned to operate a relay and press a key to obtain water reinforcement. In 1977, Schmitz and Goodrich found that garter snakes (Thamnophis sirtalis) could solve runway, T-maze and reversal problems. Burghardt, Wilcoxon, and Czaplicki (1973) used four Thamnophis sirtalis in an attempt to condition responses to certain foods using injected lithium chloride. Peretti and Carberry (1974) used an elevated Y-maze to successfully condition five Thamnophis sirtalis. In 1975, Fuenzalida and Ulrich tested five plains garter snakes (Thamnophis radix) in a water maze to attempt to condition escape. Limited success was obtained. Gavish (1979) demonstrated that four Malpolon monspessulanum could be conditioned to light. Kleinginna and Currie (1979), working with six Florida kingsnakes (Lampropeltis getulus floridana), demonstrated that these animals can maintain intermittant schedules of reinforcement (FR6 and FI30). In 1980, Kleinginna and Seamens, using three eastern kingsnakes (Lampropeltis getulus getulus), demonstated these animals can learn to press a lever to obtain water reinforcement.

No studies of learning set formation with reptiles have been completed. The purpose of this study was to recapitulate the study by Schmitz and Goodrich (1977) of Thamnophis sirtalis with a study of Nerodia sipedon, and to carry out an extended series of reversals to test for learning set formation.

RUNWAY EXPERIMENT

Subjects

Subjects for this study consisted of six water snakes, *Nerodia sipedon* . The water snake was chosen for this study because of its relative local abundance and the observation that these snakes feed more frequently than many other species, thus making daily testing with food reinforcement possible. Subjects were caught locally in Coles County, Illinois, and varied in length from 48 to 78cm. They were maintained in captivity for 1 to 23 months prior to testing. The individuals were maintained in the laboratory in a barracks of cages, with five individual chambers to a barracks (Fig.1). Each chamber had inside dimensions measuring 20cm x 18cm x 29cm with a vertically sliding glass door front. The cages were made of wood and painted black outside and light green inside. The floor of each chamber was lined with newspaper and water was provided at all times. The barracks cage was placed permanently on a table in the center of a room measuring 5.2m x 3.2m x 2.4m. The room had a large series of windows to one side. The natural photoperiod was maintained throughout the study. No artificial lights were used. Temperature was maintained between 24-26°C throughout the testing period.

Apparatus

The runway consisted of an alley measuring 58cm x 18.5cm x 26.5cm. The width of the runway and goal box were equal to the width of the individual cages. The goal box and alley were both made of wood and painted black outside and light green inside, the same as the

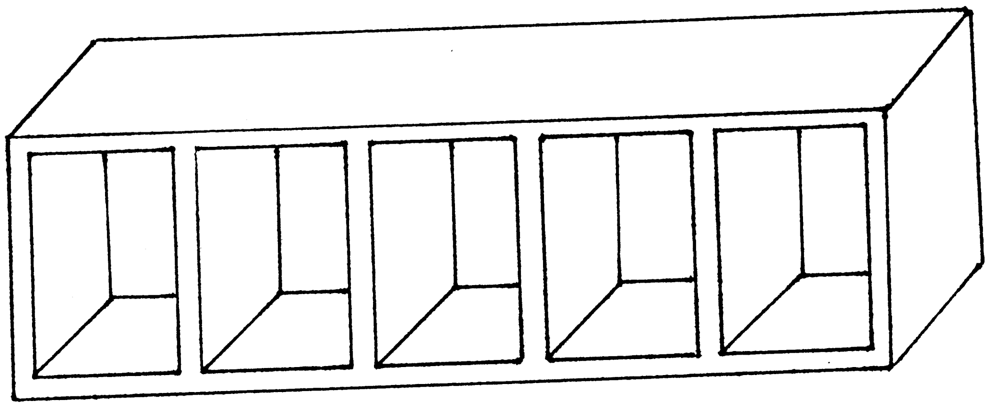

Fig 1: Barracks-type housing for five *Nerodia sipedon*.

home boxes (Fig.2). The runway and the goal box were covered with removable pieces of glass to prevent escape of subjects during testing. A strip of plastic 17.5cm x 1mm x 2cm was glued at the end of the alley and 2cm inside the goal box, to prevent visual identification of the contents of the goal box.

Procedure

Six subjects were evaluated in the runway experiment. For seven days prior to the beginning of the experiment each subject was fed one minnow daily to assure that the hunger drive did not become satiated with this feeding frequency. Testing began on February 21, 1984 and ended on May 2, 1984, with one trial per day being given to each subject at the same time each day. At irregular intervals during testing there were one or two day periods during which testing was suspended, but the animals were still fed one minnow each day. Reinforcement throughout the study consisted of a variety of species of locally collected minnows. Small minnows or larger ones cut in half were used; the approximate size range of the daily reinforcement was geared to the relative size of each snake. The minnows were killed just prior to each days testing by placing them in hot tap water so that their movement would not provide an extra variable in the testing. Freshly killed minnows were readily accepted by the snakes. If a subject did not respond, or did so incorrectly, it was given the same ration of food each day after it was returned to its home box, equalizing hunger drive in all subjects as much as possible.

One hour prior to testing, the newspaper lining and water bowls were removed from all subjects' cages. At the start of a testing

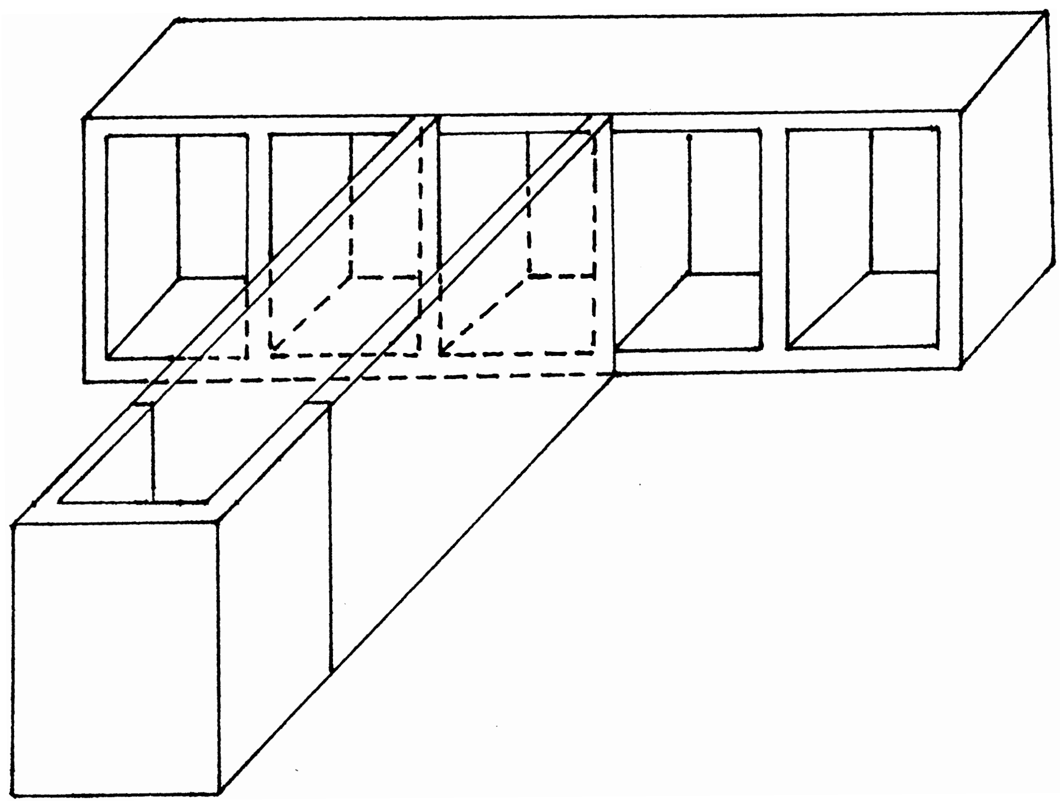

Fig 2: Runway apparatus, in position, used for first series of experiments with *Nerodia sipedon*.

session, the alley with goal box attached was moved up against the glass front of a subjects home box. Minnow reinforcement was placed near the back of the goal box. The sliding glass door of the snakes home cage was raised with a vibrating motion so that it rattled and a stop watch was started. This would provide a cue to the snake that a session was beginning and that the glass barrier had been raised. The snake was observed through a crack between the home box and alley. When the snake's nose crossed over the edge of the home box into the alley, the first stop watch was stopped and the latency time recorded, and a second stop watch was simutaneously started. When the snake crossed over the edge from the alley into the goal box, as observed through a crack between the alley and goal box, the second stop watch was stopped and running time was recorded. Subjects were then given time to eat the minnow reinforcement before being guided back to the home box using a wooden meter stick. If the minnow was not located and eaten, a trial was not recorded. A time of ten minutes was set as the maximum for latency and running time. If a subject failed to come out of the home box in ten minutes or failed to reach the goal box after exiting the home box within this specified time limit, the testing session was ended and a trial was not recorded. Incomplete trials and those where reinforcement was not taken were less than 1% in all subjects.

Results

Five subjects completed 25 trials in the runway experiment. One subject failed to meet this criterion. The mean latency times of the five subjects that did complete the runway experiment are shown in

Fig.3. There is a general trend toward decreasing latency times but sharp fluctuations are present. Fig.4 shows mean running times of the five subjects. This shows a sharp initial drop, followed by a gradual reduction in the length of time it took to travel the runway from trial 1 to trial 25. Because one of the subjects (#3) was sporadic in responding to testing, sometimes refusing to exit the home box and sometimes staying in the box for long times before coming out, its latency times were highly variable. When the latency times of the other four subjects were averaged a more continuous decrease from trial 1 to trial 25 was observed (Fig.5). The running time results of these four subjects were also averaged (Fig.6). This relationship was much the same as the average for all five subjects.

Discussion

When the subjects of the study were naive, they had no knowledge that the end of the runway contained food. Their initial reasons for leaving the home box and traveling down the runway may have been any one of several possibilities. They could have been exploring a new aspect of their environment or escaping from the home box or some still other unknown motivation. If learning had not taken place at all, latency and running times would not have decreased in a significant way.

The data collected show a definite decrease over 25 trials in both latency and running times. I conclude that subjects learned that reinforcement could be found at the end of the runway and thus more readily left the home box and more rapidly ran the course.

Over the 25 trials, most subjects learned that the rattle of the

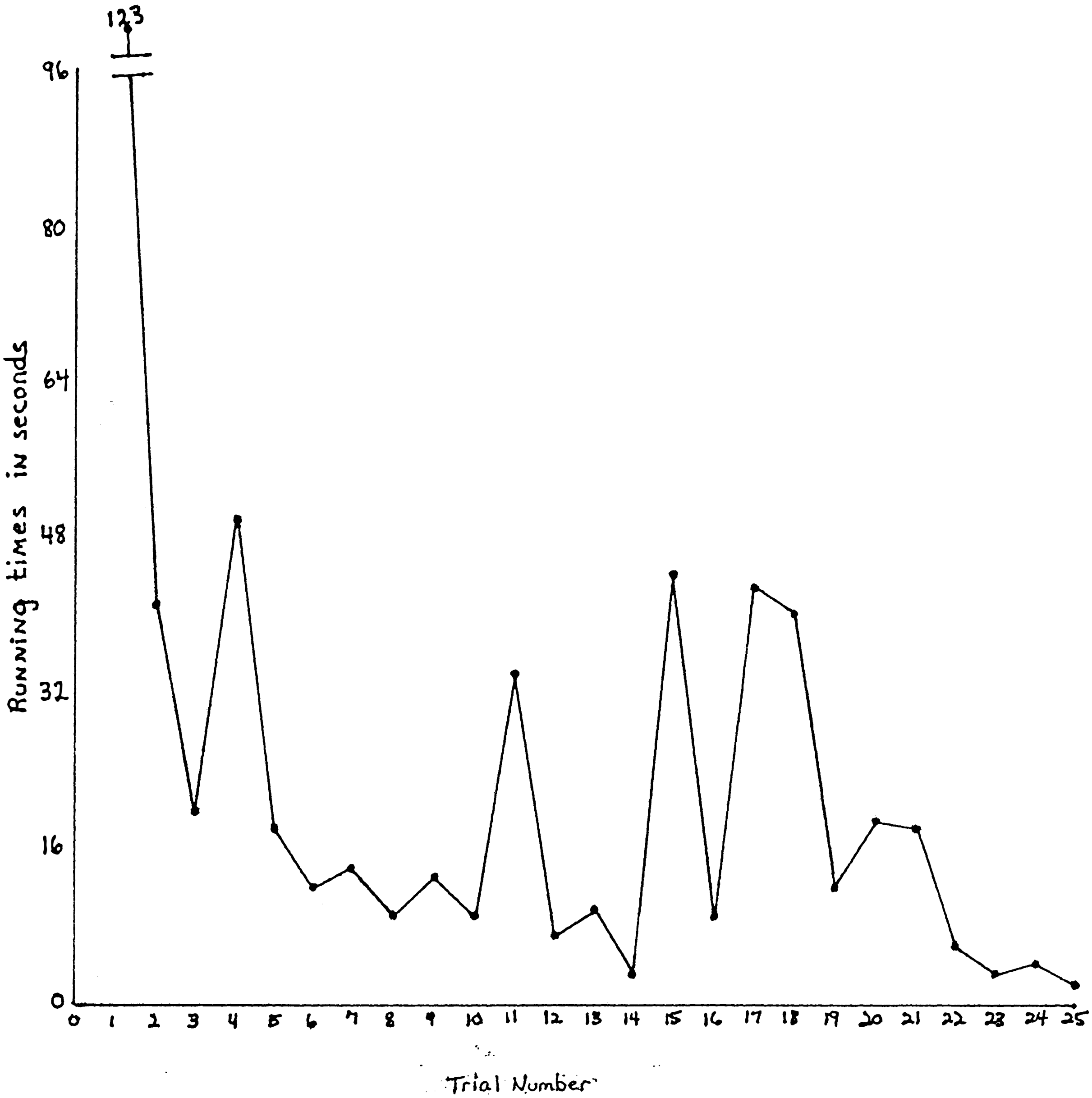

Fig 3: Mean latency times for runway experiment with five Nerodia sipedon.

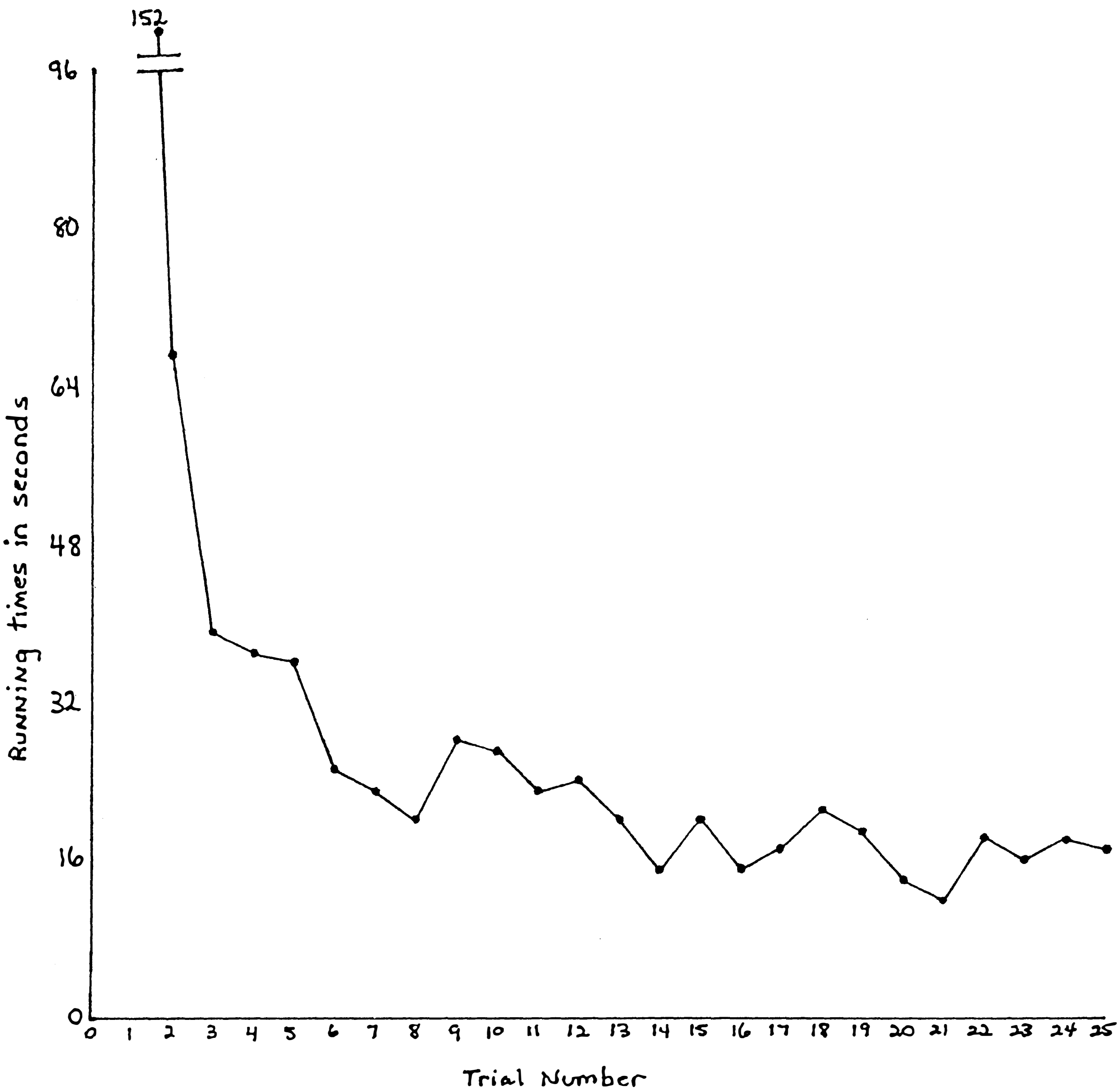

Fig 4: Mean running times for runway experiment with five Nerodia sipedon.

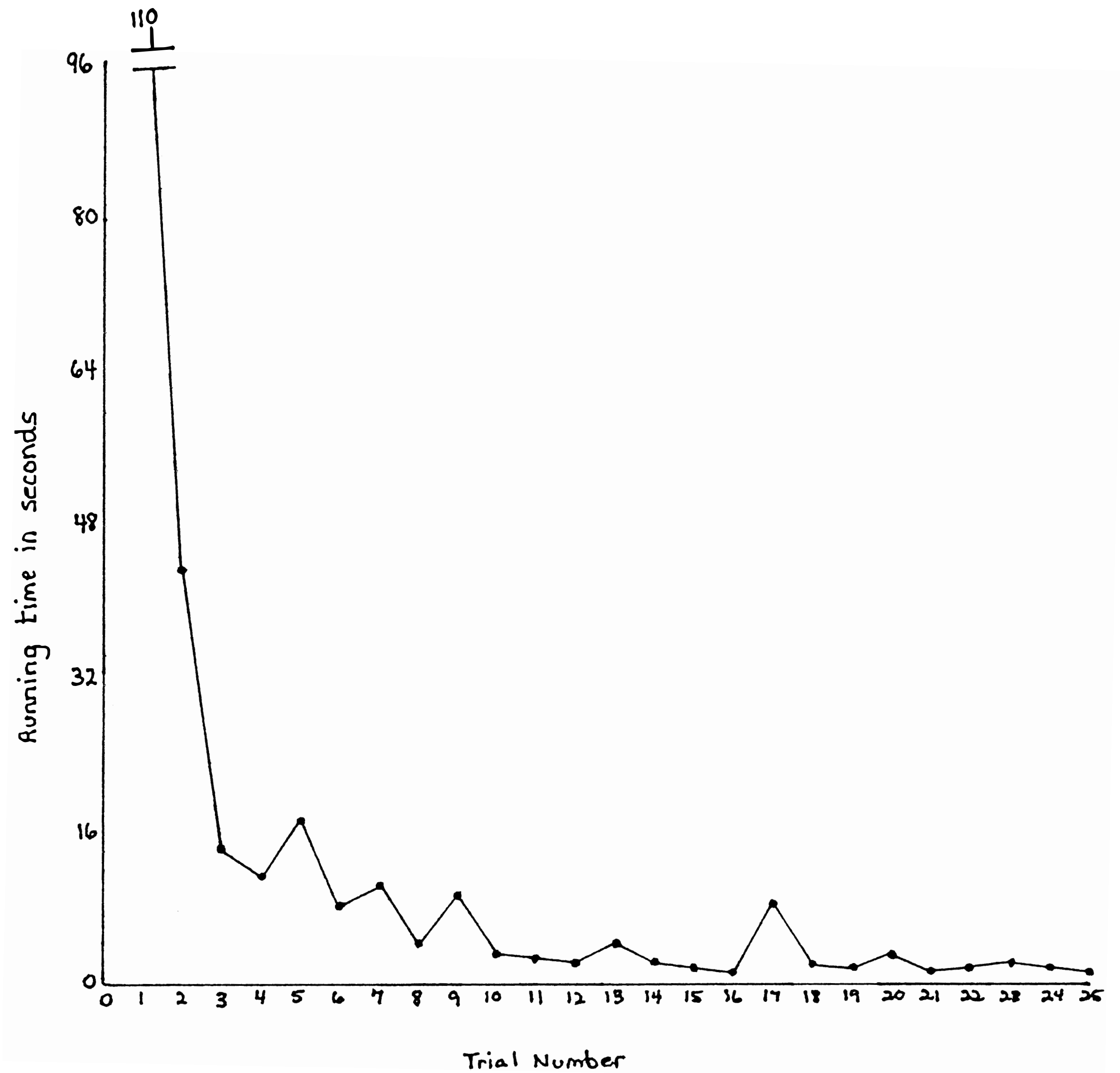

Fig 5: Mean latency times for runway experiment with four Nerodia sipedon.

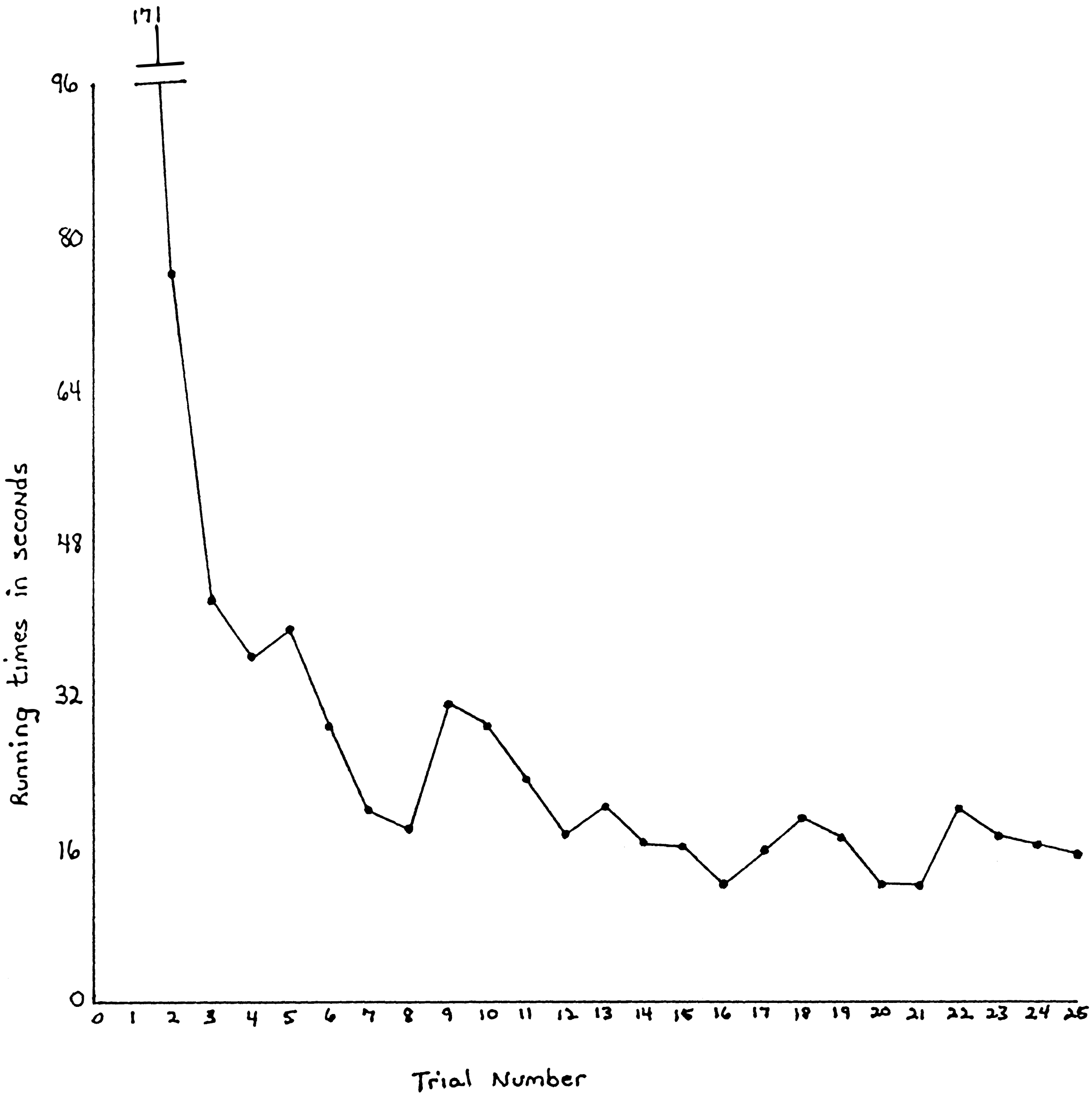

Fig 6: Mean running times for runway experiment with four Nerodia sipedon.

cage door signaled the start of a testing session. If they were facing away from the door or not otherwise attentive, they learned to respond to the rattle of the glass and became alert immediately. This was important in the decreased latency times. One subject (#3) did not appear to learn this very well and continued throughout the 25 trials to be very sporadic in its behavior. Sometimes it would respond immediately and other times it was immobile for the entire testing time or very slow to exit the home box. A possible explanation is that this subject was almost certainly older than the rest, being significantly larger than the other subjects. The effect of this greater size and age on this subject's latency of response is not clear. Once it exited the home box, it did travel the runway and its running times did improve, which shows learning took place.

The one subject which did not complete 25 trials in the runway (#6) was the subject most recently caught. It may not have had time to habituate to captivity because when it did successfully run the runway, it often failed to accept the reinforcement. When reinforcement is not taken, learning cannot occur.

TWO CHOICE DISCRIMINATION AND REVERSAL EXPERIMENT

Subjects

The four subjects (#1,2,4,5) which successfully completed the runway experiment were used in this experiment. Subject #6 did not reach criterion for the runway experiment and #3 did not perform consistently; both were therefore excluded from further study.

Apparatus

The T-maze consisted of an alley piece measuring 53cm x 18cm x 26.5cm opening into a cross piece measuring 57cm x 18cm x 26.5cm. Goal boxes measuring 27cm x 18cm x 27cm fit on each arm of the T-maze. Strips of plastic measuring 18cm x 1mm x 2cm were glued 2cm in from the edge of each goal box to prevent subjects from seeing the reinforcement before entrance into the goal box. The entire apparatus was painted black outside and light green inside and was roofed with pieces of removable glass to prevent escape of subjects during testing (Fig.7).

Procedure

One hour prior to each days testing, the newspaper flooring and water bowls were removed from all subjects' cages. At the start of the testing session, the T-maze apparatus with goal boxes attached was moved up against the subject's home box. The sliding door was vibrated and removed. The first time each snake was tested, reinforcement in the form of a freshly killed minnow was available in either goal box. The goal box that was chosen in the first trial then

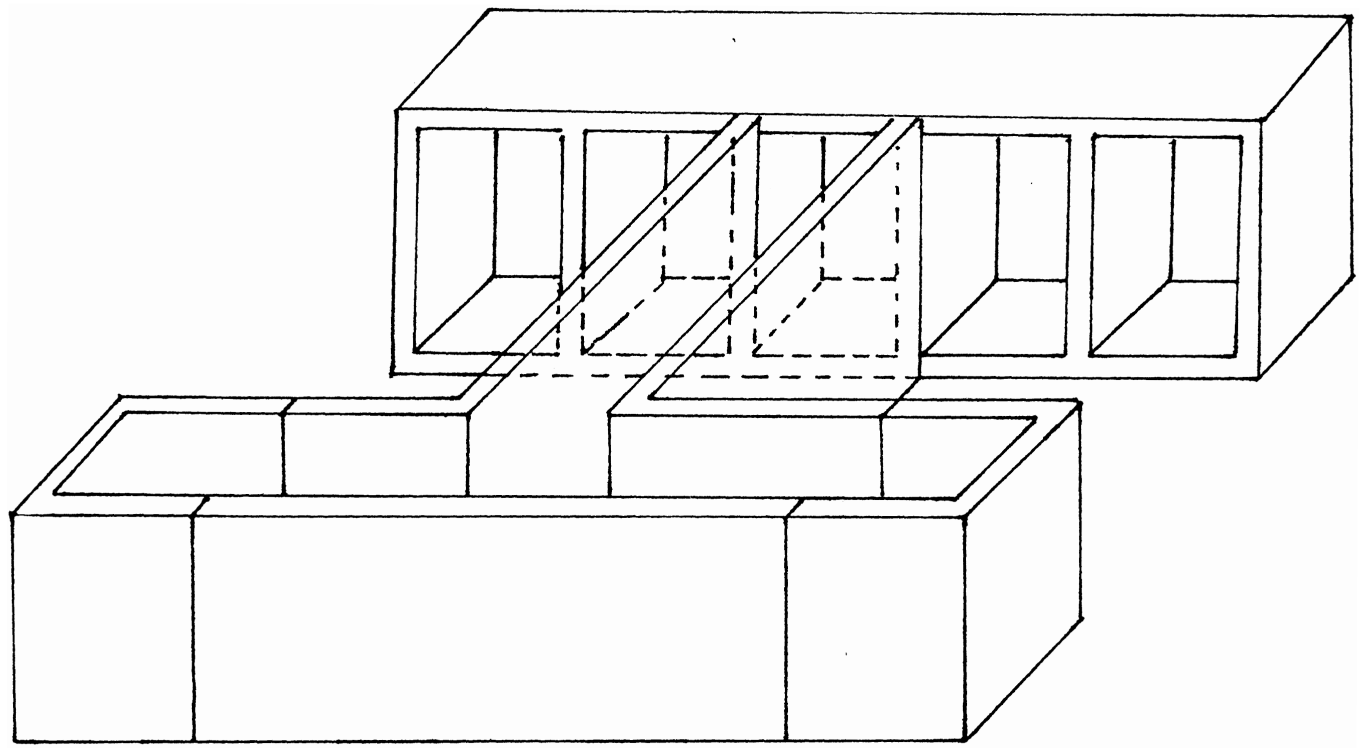

Fig 7: T-maze apparatus, in position, used for second set of experiments with *Nerodia sipedon*.

became "incorrect" for the initial discrimination experiments. After the first trial, a minnow was placed near the back of the correct goal box and "minnow juice" in the back of the incorrect box.

Data recorded for each trial included: Latency (the time it took a subject's head to cross the door of the home cage into the alley), running time (the time between entrance into the alley and entrance into a goal box), correct or incorrect choice, and the presence or absence of "wall seeking" behavior. Subjects were tested once each day. After one goal box was chosen, the testing session was ended for that day and the subject was guided back to its home box using a wooden meter stick. A time limit of ten minutes was given at each testing session for both latency and running times. If a subject had not responded in this length of time, the session was ended for that day. Criterion for mastery of the two-choice discrimination was set at eleven out of twelve correct trials with the last eight being correct. As soon as a subject reached criterion for the two-choice discrimination experiment, the correct goal box was made incorrect and the formerly incorrect one was now correct. Subsequent procedure was the same as that followed in the initial two-choice discrimination experiment as was the criterion for acquisition. Each time criterion was reached, the "correct" goal box changed positions and a new reversal was begun.

Results

One hundred seventy trials were completed by each subject in the T-maze experiment. All subjects completed the initial two-choice discrimination and at least one reversal. Two subjects completed four reversals and one subject met criterion for six reversals. The number of trials to criterion decreased for each subject from the first two-choice discrimination experiment to the last reversal (Fig.8). The number of errors to criterion also decreased from the initial discrimination to each subjects last reversal (Fig.9). The mean number of trials for the initial discrimination and each reversal for all subjects that met criterion decreased over the 170 trials (Fig.10) as did the mean errors (Fig.11).

Wall seeking behavior was observed in 97.6% of the trials. In 83.3% of the trials where wall seeking was observed, the subject turned in the same direction as the wall he crawled along. In 16.6% of the trials demonstrating wall seeking, the subject crawled along one wall, but when he got to the end of the alley, he crossed over the open space of the T to go into the opposite goal box. Of these responses, 83% were correct choices while 13% were incorrect turns; significantly better results than in trials when this crossing over did not occur. Raw data are presented in Table 1. This has been found to be significant using a Chi-square test for each subject and for all subjects combined.

Discussion

A great deal of intra-subject variation was found in the number of trials and errors to criterion for the initial discrimination and subsequent reversals (Figs.8 & 9). It is clear that each subject's performance improved from the initial discrimination to the last reversal completed. When averaged, these numbers take on a relatively smooth decreasing curve (Figs.10 & 11).

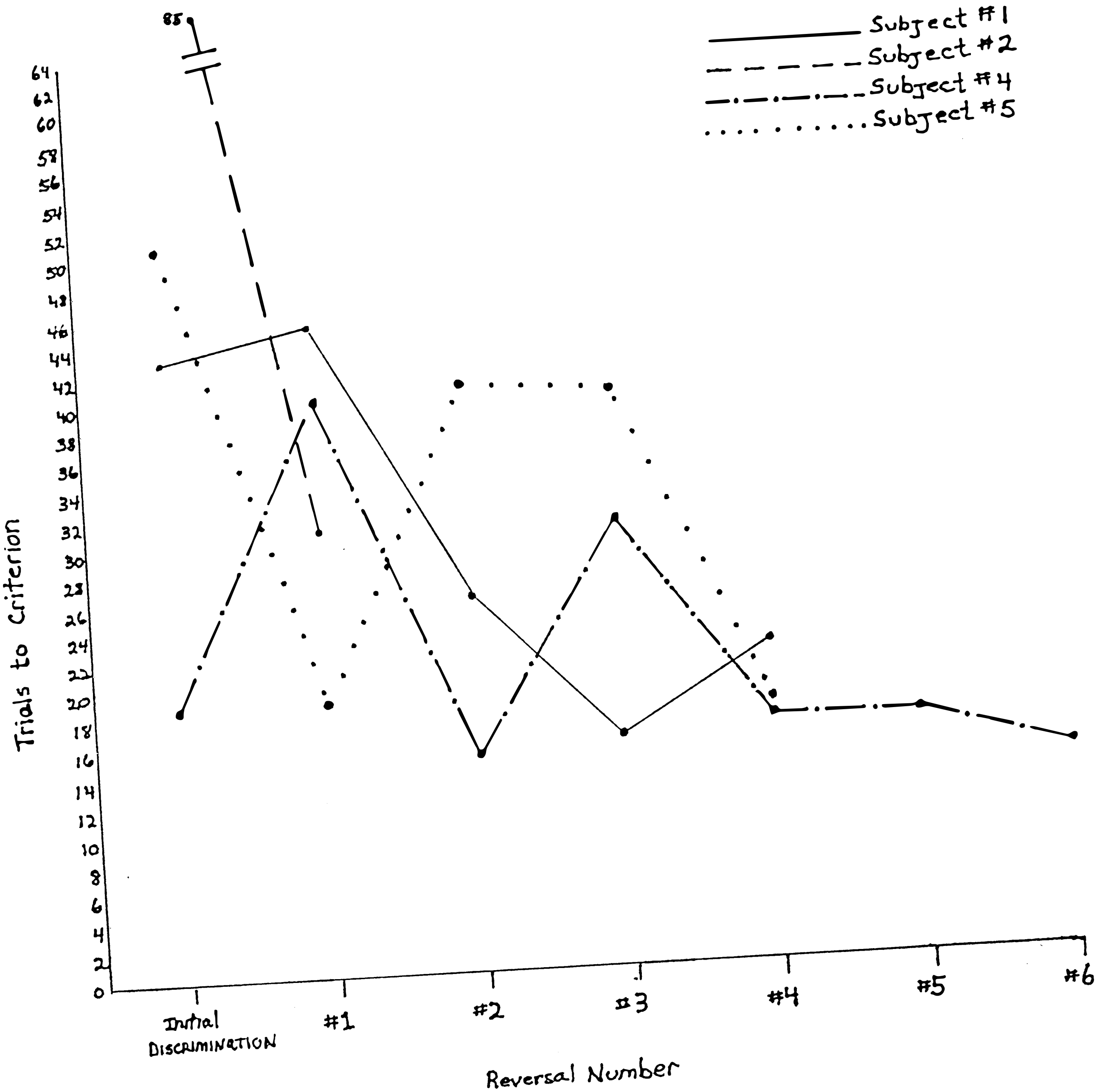

Fig 8: Trials to criterion for two-choice discrimination and reversal training using four subjects of <u>Nerodia sipedon</u>.

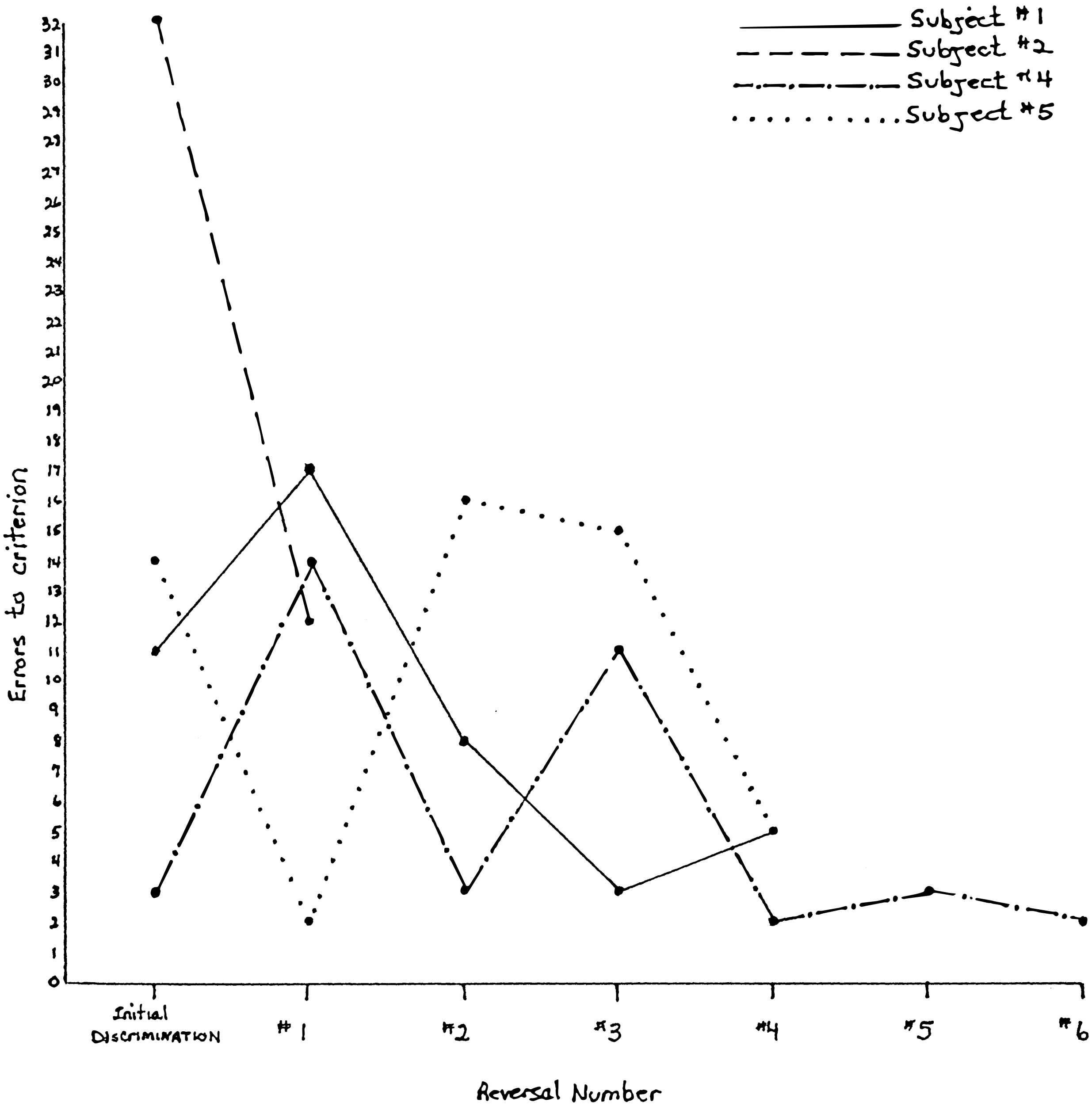

Fig 9: Errors to criterion for two-choice discrimination and reversal training using four subjects of Nerodia sipedon.

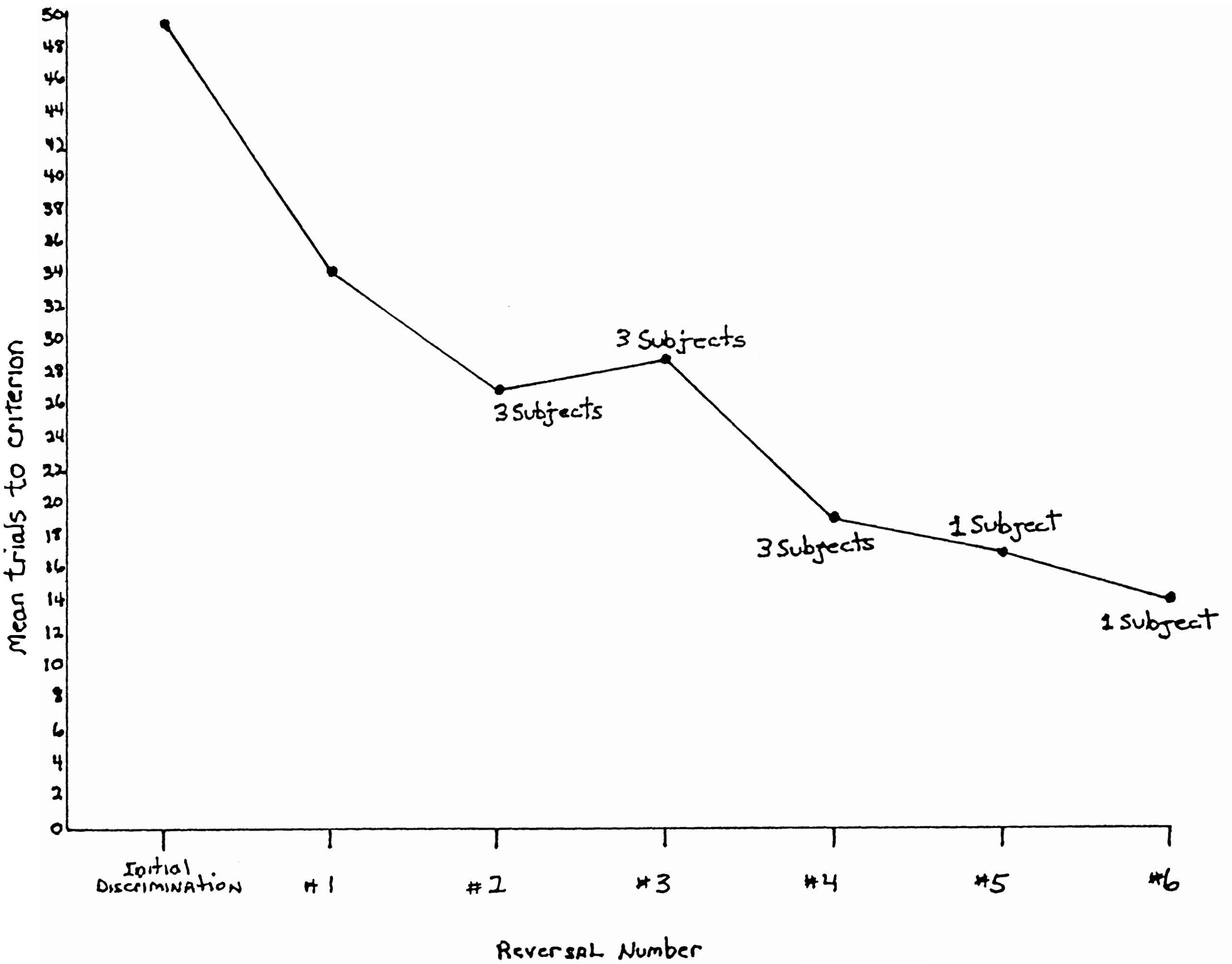

Fig 10: Mean trials to criterion for two-choice discrimination and reversal training using four subjects of Nerodia sipedon.

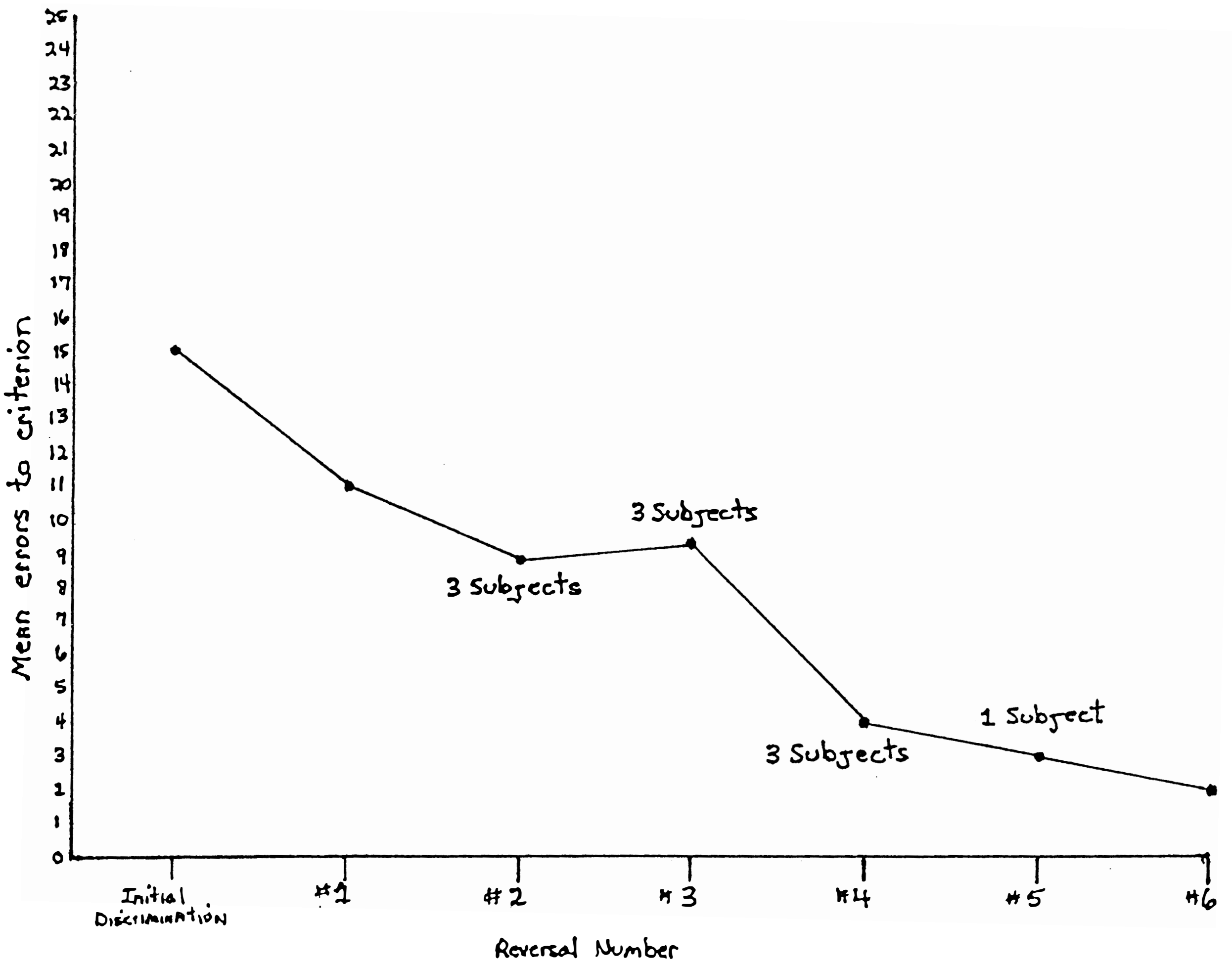

Fig 11: Mean errors to criterion for two-choice discrimination and reversal training using four subjects of Nerodia sipedon.

Wall seeking behavior was observed in all snakes more than 90% of the time (Table 1). This means that the snake generally chose a wall to crawl against immediately after exiting its home box. In 83% of the trials, the animal continued to turn in the same direction as the wall it started against. This indicates that a snake usually makes a decision determining which goal box it will enter at the beginning of the alley, not at the beginning of the T. This lengthens the time between the response (choosing a wall) and reinforcement (getting the minnow). Reinforcement is maximized when the reinforcer is presented immediately after a subject's response and decreases the longer reinforcement is delayed. This may explain the poor performance of subject #2, whose running times were generally slower than times for the other subjects. Nevertheless this problem was overcome, as the association between spatial orientation and reinforcement did occur.

In 17% of the responses in which the subjects moved along a wall, they subsequently crossed over to the opposite goal box. The indication is that wall seeking is a strong response in these snakes and that they do not frequently deviate from it unless they have learned the correct side for reinforcement and must cross over to obtain it. Subject #4 completed the most reversals over the 170 trials and had the fastest average running times. The interval between response and reinforcement was less, thus potentially increasing the association between the two.

Table 1: Wall seeking response in four *Nerodia sipedon* with numbers of correct and incorrect responses for turns in the direction of the initial response compared to cross-over responses.

	Wall seeking response/total trials	Turns in direction first chosen		Cross-over responses	
		correct	incorrect	correct	incorrect
Subject # 1	169/170	98	39	24	8
Subject # 2	169/170	81	57	24	7
Subject # 4	161/170	103	36	20	1
Subject # 5	166/170	90	50	23	3
Total	665/680	372	182	91	19

CONCLUSIONS

This study demonstates that subjects of *Nerodia sipedon* can be conditioned to run a runway and solve two-choice discrimination and reversal problems. Results for the runway experiment and the two-choice discrimination experiment are equal or superior to those obtained by Schmitz and Goodrich (1977) using *Thamnophis sirtalis*. In the two-choice discrimination experiment of the prior study, four of the nine subjects failed to reach criterion and two subjects reached criterion only after well over 100 trials.

Positive reinforcement as a technique is much preferable to aversive conditioning, as noted by the poor results obtained by numerous workers using negative reinforcement: (Kellog and Pomeroy, 1936; Wolfle and Brown, 1940; Crawford and Holmes, 1966; Fuenzalida and Ulrich, 1975). Using positive reinforcement has a drawback in that only one trial or very few trials per day can be run on each subject. Schmitz and Goodrich (1977) obtained poor results when attempting to run two consecutive trials during the same testing period, possibly related to stress.

Consecutive reversal solving is the simplest way to test learning set formation that can be demonstrated. Up to the time of this study, learning set formation has not been demonstrated for the Suborder Serpentes. This study shows a significant decrease in number of trials to criterion over the series of reversals, but enough reversals were not performed to conclude if a learning set could be formed. The formation of a conditioned response and reversal learning in these subjects is learning comparable to other non-human vertebrates as reported by Voronin (1962).

It is possible that different species of snakes have different learning capacities, which might account for the variations obtained in other snake studies. Further studies should address this issue by testing different species in a similar apparatus and comparing the results obtained. Obviously, this and prior studies have demonstrated that snakes can and do learn. The limits of the learning capacity have yet to be discovered.

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