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Apparatuses for assessing maze learning in the Isopod Sp

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APPARATUSES FOR ASSESSING MAZE LEARNING IN THE ISOPOD, SP.

By

Richard Lee Kuhn

B. A. California State College at Fullerton, 1967

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[Signatures]

Chairman, Board of Examiners

Dean, Graduate School

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ACKNOWLEDGMENTS

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I. INTRODUCTION AND STATEMENT OF PROBLEM

A review of the literature indicates conflicting evidence with respect to the Isopod, which may have been the result of a failure to first determine the ecology and apparatuses most appropriate for studying the organism. The following studies both summarize research and point to the need to suit the laboratory environment to the organism.

Woodlice, an Isopod, generally live in niches which have a high moisture content, such as beneath fallen logs, piles of leaves, or crevices in the ground. Humidity has been accepted as the primary physical stimulus which controls both degree of activity and choice of ecological niche in the woodlouse (Edney, 1954, 1960; Waloff, 1941; Gunn & Kennedy, 1936). Moreover, humidity factors interact with temperature and light conditions in controlling the Isopod's behavior (Warburg, 1964).

Woodlice tend to aggregate in moist places and avoid dry places by means of orthokinesis. In other words, the woodlouse moves more rapidly in dry than moist air.
Although usually photonegative (Abbott, 1918; Banta, 1910; Blum, 1935), the woodlouse can give a positive phototropotaxis (orienting directly toward a light source), usually after a sudden rise in temperature or a period of starvation or dessication (Henke, 1930).

Orientation of the Isopod may also be telotaxic: if there are two equal sources of stimulation in the same modality, the animal can orient toward one or the other, suggesting that the influence of one of the stimuli must be inhibited (Hinde, 1966). Also, the Isopod is capable of making a light compass reaction, in which the source of light serves as a fixed point, enabling it to maintain a definite angle between the direction of motion and the light source (Buddenbrock, 1917). Further, geotactic responses can be given, and have been experimentally destroyed by extirpation of the statocyst (Langenbuch, 1928). Moreover, although evidence for form vision is not available (Franekel & Gunn, 1961), skototaxis, or the orientation toward darkness, has been demonstrated by Alverdes (1930). Finally, thigmokinesis, or slowing down or stopping when in contact with a surface, has been
demonstrated by Friedlander (1964), and is part of the
tendency of the animal to form aggregations (Allee, 1926).

Isopods show alternation in a T maze (Hughes, 1966;
alternation behavior in all animals have been offered.
Schneilere (1929) argued for "centrifugal swing," which
is the tendency of the organism to continue outward in a
circular fashion from the direction of the recently completed
turn. Dashiell and Bayroff (1931) suggested that the
animals established a "forward-going" tendency during
the start alley run, and the later turn was the correction.
Hull (1943) hypothesized reactive inhibition: once a
response is made, an inhibition is built within the organism
for another response. Tests of these hypotheses with
respect to the Isopod were undertaken by Sachs, Klopfer,
and Morrow (1965) in the absence of reinforcement. The
subjects (Ss) were forced to make a number of right turns
in succession, using square mazes with a geometrical
increase in the distance between turns. The Ss were then
run in a standard T maze, in which the hypothesis of
reactive inhibition best accounted for turn alternation
responses.
T maze turn alternation studies without reinforcement were also conducted by Iwata and Watambe (1957). By forcing one or two right turns, they increased the number of left turns made to 100%. However, a decrease in the number of left turns was noted by increasing the length of the maze stem. Alternations decreased proportionately with distance after the initial turns were made.

In another study of turn alternation in the absence of reinforcement, Kupferman (1966) ascertained that the mean turn alternation angle is a linear function of the angle of the initial forced turn, but is independent of the distance between the forced turn and the choice-point, over a range of 5-20 centimeters. He concluded that turn alternation involves a form of short-term memory.

Iwahara (1963) did a study testing three hypotheses for alternation: reactive inhibition, centrifugal swing, and "thigmotropism," which is the tendency of the organism to consistently attempt to stay in contact with one wall of the maze. Using a T maze, he found no effects of prior experience on turning responses, all alternation being due merely to chance. Then, a T maze was constructed with a
central stem divider. Tropisms were observed in all the Ss. Their orientation toward one wall was consistent for any one individual.

Bock (1943) was the first investigator to analyze the effects of reinforcement upon Isopod turning behavior in T and Y mazes. He used an aversive stimulus, in the form of a light touch upon the back of the S with a fine brush to punish errors. This negative reinforcement, along with tactile cues (a rough maze floor) produced learning.

Using electric shock as a negative reinforcer in a T maze study, Thompson (1957) attempted to reveal the presence of learning sets through the reversal of a position habit in invertebrate behavior. However, he was not successful.

Harless (1963), using Y mazes, was the first to utilize light as a negative reinforcement. Isopods in her experiment were trained to turn against preference. An error was punished by turning on the light. She was able to demonstrate clearly the acquisition of a position habit without the addition of tactile cues as used by Bock (1943).
Finally, Morrow (1966) investigated Isopod learning ability with two types of negative reinforcement. Ss were trained in a Y maze to turn opposite preference to a criterion of 8 out of 10 correct turns per day for two days. Half the Ss were reinforced by the application of the aversive stimulus, light, for turning toward preference; the remaining half of the Ss were reinforced by the withdrawal of the aversive stimulus, light, for turning against preference. No differences between reinforcement conditions were observed.

Because all of the foregoing studies used undetermined adequate and efficient single unit maze behavior of the Isopod, it was proposed that a comprehensive analysis be undertaken. Variables that affect behavior, such as intra- and inter-apparatus structural characteristics were studied. It was hypothesized that there is an optimal structural cross section for the Isopod maze. The apparatus cross section used may influence performance. Apparatuses were selected because they were used frequently in previous studies, (T and Y mazes) and on the basis of supposed increasing control potential (cross maze).

This study was divided into two experiments. The
first, an intra-apparatus study using straight mazes with four different cross sections: square, circle, triangle, and semi-circle. The second, an interapparatus study using T, Y, and cross mazes attempted to determine which type of maze was most appropriate for testing the Isopod's learning potential. The findings of the intra-apparatus study were incorporated into the inter-apparatus study.
II. METHOD

Subjects. The Ss were 70 Isopods gathered locally and unsystematically selected from a man-made "environment" consisting of moist sponges, rotting leaves, old wood, and abundant soil and vegetation. Ss were housed in three separate 10-gallon terrariums. Forty Ss were used in the intra-apparatus study; 30 Ss were used in the inter-apparatus study. Husbandry conditions were recorded and held as constant as possible. In order to identify Ss during the time they were used in an experiment, each was housed in an individual container. The containers consisted of commercial paper cups about 4-in. high with about 2-in. of damp mulch in the bottom. No attempt was made to separate Ss on the basis of sex, but larger Ss were used because their size made them easier to handle.

Apparatus. The intra-apparatus study used four straight mazes constructed from .125-in. thick plexiglass. Each maze allowed .06-in. medial space on both sides of S. The first had a circular cross section with an inside
diameter of .25 in.; the second had a square cross section of .50-in. per inside; the third had an equilateral triangle cross section of .75-in. per inside; the fourth had a semicircular cross section with a base .50-in. across the inside. These apparatuses were each used in a flat cardboard container 14x9x2-in. high.

The inter-apparatus study began with the simplest of choice mazes, the T, and progressed by increasing degrees of control potential to the Y maze, which allows for arm/stem rotation and provides a means of controlling for possible scent trails as demonstrated in the earthworm by Ressler, Cialdini, Ghoca, and Kleist (1968). The cross maze allows for manipulation without experimenter (E) contact, for arm/stem rotation, and for cancellation of scent trail cues.

The T and Y mazes used a square cross section, determined most appropriate for the organism by the straight maze study. Each arm/stem was 1.25-in. long and wide enough only for forward movement. Because the structure was made of transparent plastic, E was able to see S at all times and shine a light on S when appropriate. Blockage of S in an arm or stem during periods of reinforcement was
by hand-operated plugs. After leaving the arm, S was manually replaced in the stem between each trial. With the Y maze, the right arm served as the stem for a trial by clock-wise rotation of the maze after each trial.

A cross maze was constructed with .125-in. plexiglass walls mounted on a flat 10x10x.25-in. thick plexiglass frame. As with the T and Y mazes, a square cross section was used. Each maze arm was 1.25-in. long. The width of the arms was such that S could easily move forward but could not turn around. Each arm could be turned around 360 degrees by means of a pivot. This eliminated handling S while at the same time pointing S back toward the choice-point. The structure was transparent for reasons previously discussed. The arm ends were closed by four fixed end plugs, against which the arms were slid, and an E inserted plug that closed the gap left near the choice-point. This maze was designed to control for scent trails and/or other chemical factors that the organism might leave that may represent sources of error which cannot be controlled in a simple T maze.

A 60-watt lamp suspended 4-in. above the choice-point of all the apparatuses provided the reinforcement.
The lamp was controlled by a floor-operated switch which left E's hands free. A 5-watt lamp provided indirect dim lighting and remained on during and between trials, enabling E to observe the experimental proceedings while hopefully being sufficiently nonaversive to S, at least compared to the 60-watt lamp. A stopwatch was used to measure latencies, stimulus durations, and ITI's. A humidifier (Ward's catalogue No. X69A6038RT) was used continuously during both studies. Humidity was measured daily by a wet bulb hygrometer. Further, a Minneapolis-Honeywell Regulator Co. constant recording humidity and temperature guage (No. 1571T) was used continuously during the intra- and three days during the inter-apparatus study. The pH was measured daily. All S's used in the experiment were killed by individually storing in 95% ethanol for later accurate species identification.

Procedure. For the intra-apparatus study the 40 S's were randomly divided into four groups of 10 S's each. Each group ran only one of the four types of straight alleys. Each S received 100 trials at the rate of 10 per day. ITI was about 30-sec., the time required to manually transport S from exit to entry. Reinforcement
was administered in the form of the aversive light stimulus, which was switched off when Ss ran and left the alley, or after 300-sec. The measure was Ss' latency from onset of the light to the end of the maze.

For the inter-apparatus study Ss were randomly divided into three groups of 10 Ss each, one for each apparatus. Each group was given 20 preference trials (10 per day), and then trained to turn against their individual preference. Criterion for preference was 11 or more turns in one direction in the 20 trials. ITI during the preference phase was about 4-min. per S, which was the time required to run the rest of the group. In all groups, Ss were returned to their individual containers between trials. Reinforcement consisted of withdrawal of light, contingent on the direction of the turn. The light reinforcement was switched on simultaneously with Ss' entry into the maze stem. The assumption was that this procedure tended to facilitate rapid locomotion to the choice-point besides punishing an error in choice. Upon entering an arm of the maze, S was immediately blocked for an arbitrary duration of 15-sec. If the arm selected was correct in terms of the training procedure, the negatively reinforcing
light was immediately turned off. If the arm selected was incorrect, the light was continued for 15-sec. There were 10 trials per session per day. The ITI during training was about 30-sec.--the time required to transport manually from arm exit to stem entry. Each session was run 100 trials.
III. RESULTS AND DISCUSSION

Humidity, temperature, and pH content data are summarized in Table 1. Individual containers were watered twice daily by pressing a Windex spray bottle three times per container; colonies were watered daily by splashing four cups of water on the surface of each terrarium. Soil water was judged by noting soil color for darkness and by feeling for moisture. The attempt was to avoid puddles as well as dryness in the soil such that a constant damp soil state was maintained.

Results from the intra-apparatus study analysis of variance are summarized in Table 2. Further analysis of the data using Duncan's New Multiple Range test (Edwards, 1960) with a protection level of .97 yielded: the square cross section significantly different \( (p < .01) \) from circle, semi-circle, and triangle cross sections; the triangle cross section significantly different \( (p < .01) \) from the square, circle, and semi-circle cross sections; the circle and semi-circle cross sections not significantly different from each other, but both significantly different \( (p < .01) \)
from the square and triangle cross sections. The order of cross sections in terms of shortest to the longest latency was: square, circle, semi-circle, triangle. Latency scores are presented graphically in Figure 1.

Preference data preceding the inter-apparatus study are summarized in Table 1. Results of the analysis of variance of the inter-apparatus study are summarized in Table 2. The significant \( (p \leq .001) \) ApparatusXTrials interaction suggests differential learning rates for each apparatus. The trials effect in the analysis indicates that a highly significant \( (p \leq .001) \) increase in performance occurred across trial blocks. However, as this is the data summed across all three apparatuses, an analysis of variance for trials for each apparatus was done in order to determine individual learning significance levels. The data is summarized in Table 3.

Further analysis of the data using Duncan's New Multiple Range test (Edwards, 1960) with a protection level of .98 showed that the Y maze produced significantly \( (p \leq .01) \) lower performance than the T and cross mazes. Figure 2 shows the daily mean correct responses for each group.
Colony size for each "environment" was roughly 300. During almost a year of continuous observation conditions appeared to favor either an increase in the birth rate or a decrease in the death rate. Both were probably the case, inasmuch as while many new Isopods appeared, dead S's were relatively rare. At the end of a year there was an increase in colony size of about 25%. Room lighting conditions consisted mostly of darkness. However, during training, there were several hours daily of dim (5-watt) light interrupted by frequent 1 to 300-sec. flashes (60-watt).

Handling S's appeared to be greatly facilitated by the use of "natural" surfaces. Thus, in selecting and removing S's from containers a thin piece of wood was most effective. It was also noted that S's tendency to roll into a ball and remain immobile could be overcome without apparent ill effect by an abrupt contact with a hard surface. Flicking S against a piece of cardboard about 2-in. away or dropping about 1-in. onto a hard surface worked well to uncurl him. Further, and most important to laboratory handling techniques, it was observed that if S was running unaided when entering the maze stem or straight
alley, the probability of further locomotion was greatly increased. Thus, the tendency to stop and remain in the apparatus for long intervals was all but overcome if care was taken to have $S$ running rapidly with minimal $E$ interference prior to maze entry.

It was observed that the triangular maze cross section allowed immediate overhead contact of $S's$ antennae, a condition not present in other maze cross sections. On the basis of this group's performance it is speculated that dorsal thigmotaxis may exert a negative influence on forward running.
IV. SUMMARY

This study was designed to test the differences between maze cross sections and mazes in order to determine the apparatus most appropriate for testing Isopod learning. The study consisted of two parts, an intra-apparatus and an inter-apparatus section.

In the intra-apparatus study, straight alley latency data were recorded for square, semi-circle, circle, and triangle cross sections. Ss ran significantly faster in a square cross section and significantly slower in a triangular cross section, with circle and semi-circle cross sections intermediate. In the inter-apparatus study, correct responses made to negative light reinforcement were recorded for T, Y, and cross mazes. Results indicated that learning at the .001 level of significance occurred in all apparatuses. However, the T and cross mazes produced significantly higher performance levels than the Y maze. Detailed observations on husbandry and ecological conditions were reported.
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Schmeilera, T. C. Learning and orientation in ants. Comp. Psychol. Monog., 1929, 6, 1-142.


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<td>in the inter-apparatus study</td>
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TABLE 1.—Mean, standard deviation, and range for humidity, temperature, pH, and trials to preference criterion.

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<td>.59</td>
<td>15 to 20</td>
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<tr>
<td>Y MAZE</td>
<td>18.1</td>
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<td>CROSS MAZE</td>
<td>18.2</td>
<td>.52</td>
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TABLE 2.--Analysis of variance for intra- and inter-apparatus studies, respectively.

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* p .05
** p .001

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* p .001

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TABLE 3.--Analysis of variance for T, Y, and Cross mazes, respectively.

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* p .001

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* p .001

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* p .001
Figure 1. Mean latency scores for each group in the intra-apparatus study.
Figure 2.--Mean daily correct responses for each Group in the inter-apparatus study.