Ignotum per Ignotius

James V. McConnell, editor

THE WORM RUNNER'S DIGEST

an Informal Journal of Comparative Psychology, Published Irregularly by

PLANARIA RESEARCH GROUP • THE UNIVERSITY OF MICHIGAN
MENTAL HEALTH RESEARCH INSTITUTE • NO 3-1531, ext. 7513
P.O. Box 644, Ann Arbor, Michigan 48107

Volume VII, No. 1
$1.50 per copy

TABLE OF CONTENTS

Worms...	James V. McConnell	1
Learning In Plants?	Eric Holmes Gail Gruenberg	9
Grafting Techniques For Planarians	Stuart J. Coward	13
Genesis - Or Gaolbreak...?	Peter Lorden	18
Diurnal Cycles and Maze Learning In Planarians	Joel E. Cohen	20
Accident And Direction In Evolution	Thomas Morrill	25
Influence Of RNA And Victim Training On Maze Learning By Cannibal Planarians	J.B.E. Pickett, III L.B. Jennings P.H. Wells	30
Book Review (The Act of Creation, by Arthur Koestler)	Karl H. Pribram	39
The Annotated Bibliography of Research On Planarians, Part VI.	Ursula C. Mosler Margaret L. Clay	43

(Continued on Page ii)

TITLE: DIURNAL CYCLES AND MAZE LEARNING IN PLANARIANS

AUTHOR: Joel E. Cohen
Adams House, C-45
Harvard University
Cambridge, Massachusetts

Abstract. Ten planarians (Dugesia dorotocephala) were trained to enter the white arm of a T-maze, the other arm of which was painted black. The five planarians trained in the morning learned the correct response more quickly than the five trained at night, although factors other than time of day were held constant during training.

* * * * * *

Human experimenters have long observed in themselves a diurnal cycle in their willingness to learn. Not surprisingly, they have discovered similar proclivities in their animal subjects, even worms.

Arbit, for example, found that earthworms (Lumbricus terrestris) trained in a T-maze at night reached a criterion of seven successive correct trials significantly sooner than did a group trained in the morning. He explained the difference as a result of the worms' diurnal cycle of activity.

Similarly, Best found that planarians (Dugesia tigrina) deprived of food would cannibalize a related species, Cura foremani, at a rate depending on the time of day. Although D. tigrina were kept at constant illumination and temperature, they attacked C. foremani significantly more often at night (6 p.m. - 6 a.m.) than during the day.

These studies (1) suggest that planarians should show a variation in rate of learning comparable to the earthworm's -- faster by night, slower by day--and directly opposite to the human experimenter's. To test this suggestion, the following experiment was performed.

Planarians were trained in a single-unit Plexiglas T-maze assembled with Duco cement. The stem of the maze was 1.5 cm long; each arm had an inside length of 2.3 cm. The channels were cut into the plastic by means of a 0.32 cm (1/8") end mill cutter, designed to produce a U-shaped groove(2). The channels were 0.32 cm wide and 0.16 cm deep. The left arm was painted with black lacquer, the right arm with white. The stem was left transparent. The only source of light during training was a 40 watt frosted lightbulb located approximately 15 cm above the choice point of the maze.

For two weeks prior to training, a stock of planarians (Dugesia dorotocephala) maintained in water from a local (California) lake at room temperature (22-24° C.) was exposed to diffuse natural light during the day and kept dark at night.

The ten planarians selected for the experiment were placed in individual cereal bowls full of lake water. They were kept in an air-conditioned room, removed from the main activity of the laboratory; during the experiment, the room was used only for training these worms; Once selected, the worms remained in total darkness at all times except during feeding, changing of bowls (which occurred every five days), and the actual training.

In one afternoon, the day after selection, each of the ten worms was given five spaced preference trials. In a preference trial, the worm was transported on the tip of a soft camel's-hair brush from its bowl to the stem of the maze, which was filled with lake water. The animal was allowed to crawl along the stem alley until it entered either the black or white arm, i.e., until its tail had completely entered a painted part of the maze. Regardless of its choice, the worm was then immediately returned to its home bowl by picking up and tilting the maze and letting the water run out with the worm.

Only three worms entered the white arm at all during preference trials (Table 1). It was arbitrarily decided to put the worm (A4) that went white twice in one group and the two (P2, P3) that went white once each in the other (3). The remaining worms were randomly distributed to give the morning (A) group and the evening (P) group five worms each.

Because the animals displayed a clear initial preference for the black arm, they were all trained to go white, beginning the day after their preferences were determined. When a worm entered the black arm, it was picked up on the tip of the brush and returned to the stem of the maze. If the worm then proceeded directly to go white, it was allowed to complete the choice and was immediately returned to the home bowl and covered. The response was recorded as "B/W." If, after being returned to the stem, the worm tried to go to black again, it was gently forced to go to white with the tip of the brush. The response was recorded as "B." When a worm chose the white arm initially, it was immediately returned to the home bowl and darkness. The response was recorded as "W."

After each trial, the maze was emptied and dried with a cloth, and fresh lake water was put in. Training trials were given cyclically so that, for example, all five worms in a group received their first trial for the day before any animal in the group received its second.

The worms received 30 days of training, 6 trials per day. There were one day interruptions in training after days 8, 24, and 28. Trials were spaced at least 5 minutes apart. Group A received training between 10 a.m. and 1 p.m.; group P received training between 10 p.m. and 1 a.m.

Out of 1800 trials, only 54, or 3%, were B/W. The B/W trials appeared to be fairly evenly distributed across worms and trials, so B/W trials were simply considered, along with choices of black, as incorrect.

Table 1 presents the number of white choices in the 5 preference trials and the number of white choices in blocks of thirty training trials. The probability that the frequency of white in the first 90 trials equals the frequency of white in the last 90 trials, using a X^2 with 1 degree of freedom, is less than 0.001 for every worm except P1, and for P1 the probability is less than 0.01. All the behavior changes are in the same direction. Thus the frequency of white has been significantly increased.

These results are similar to those of investigators who have concluded that worms can learn. Their results and the issue of behavioral change vs. learning are reviewed by Jacobson (4).

Training changed the worms' behavior in other ways as well. Initially, once the worms began crawling in a straight line, they approached the choice point and turned into the crossbar without hesitation. Very frequently, during the last 90 trials, however, a worm would poke its head into one arm, withdraw, poke it into the other, and continue to alternate back and forth until it chose one arm (usually black) or returned down the stem to start all over again. This is similar to "conflict behavior" in higher animals.

A decline in correct choices is apparent in several of the worms in group P. Although Table 1 does not show it, the decline also appeared in the behavior of several A worms. For example, A1 went to white 9 out of 10 times between trials 62 and 71; in the next 30 trials it went to white only once.

Both "conflict" behavior and a drop in correct choices have been observed previously in planarians, and attempts have been made to explain the phenomena (5).

The "repeated measurements" design (6) gives an F value (14.288) with 1 and 8 degrees of freedom for the difference between groups A and P which has a probability less than 0.01; thus the morning group went to white significantly more often than did the evening group.

The darkness and relative isolation of the experimental room ruled out the possibility that differential light-dark or vibration cues could account for the morning-evening differences. Since the same experimenter ran both morning and evening groups, and worms were not prodded to make them move once they were in the maze (although they were guided if they tried to repeat a choice of black), there would seem to be no difference in the handling of the two groups.

Although the experimental room was air-conditioned, temperature occasionally fluctuated because of equipment breakdown. Group P was trained at a slightly higher temperature and slightly lower humidity than was group A (Table 2). But it seems unlikely that the difference in temperature suffices to account for the difference in acquisition (7). Some informal qualitative observations at the University of Michigan suggest that high humidity facilitates planarians' acquisition of a conditioned response, even though the worms are kept under water. If the same is true of maze learning, the effect might help explain why group A went to white more. But again the size of the effect is unknown.

In the absence of secure information about the effects of temperature and humidity, it appears that a diurnal cycle plays an important role in maze learning in planarians (8).

References and Notes

1. J. Arbit, Science 126, 654 (1957); F. Baldwin, J. Anim. Behav. 7, 187 (1917); J. Best, Science 131, 1884 (1960).

2. A. Jacobson, "An attempt to demonstrate transfer of a maze habit by ingestion in planarians," doctoral dissertation, Univ. of Michigan, 1962.

3. Whatever non-randomness this procedure introduced is negligible since the selection was based on two observations in one case and on one in the remaining two.

4. A. Jacobson, Psychol. Bull. 60, 74 (1963).

5. J. Best and I. Rubinstein, J. Comp. Physiol. Psychol. 55, 560 (1962). They comment (p. 563): "The abrupt decline in performance following the learning was an unexpected finding. . . . The precipitous decline under conditions of continuing reinforcement, and the fact that performance breaks to values significantly lower than. . . the naive base, indicates the decline derives from some active process rather than simple forgetting. It appears, in certain respects, to resemble the 'reactive inhibition' processes in higher animals..."

6. A. Edwards, Experimental Design in Psychological Research (Rinehart, New York, 1950).

7. C. Prosser, J. Comp. Neurol. 59, 61 (1934). He found that in the range 10-30° C. low temperatures correlate with a decrease in earthworms' negativity to light, high temperatures with an increase. On the other hand, Best (1) found a diurnal effect on attacking behavior in D. tigrina under constant temperature and illumination.

8. This research was supported by grants to J.V. McConnell from the National Institute of Mental Health and the Atomic Energy Commission. I thank Drs. McConnell, A. L. Jacobson, and C.J. Burke for their help.

Table 1. Number of choices of white arm during preference and training trials.

Worm	Pref.	Training (per block of 30 trials) I	II	III	IV	V	VI
A1	0	3	4	10	11	18	19
A2	0	1	3	1	7	12	9
A3	0	1	1	6	10	13	21
A4	2	0	1	0	10	13	11
A5	0	3	4	7	11	16	13
Total	2	8	13	24	49	72	73
P1	0	2	0	0	2	8	2
P2	1	3	0	1	3	17	3
P3	1	4	0	1	3	15	6
P4	0	0	0	0	5	9	2
P5	0	1	0	3	4	12	5
Total	2	10	0	5	17	61	18

Table 2. Temperature and humidity during training.

	Temp. (°C.) Mean	S.D.	Humid. (%) Mean	S.D.
Group A				
Days 1-15	17.9	1.6	61.6	3.3
Days 16-30	16.9	0.9	66.4	1.7
Group P				
Days 1-15	19.8	1.8	60.6	3.3
Days 16-30	18.7	1.2	64.8	2.4