degree of sensitivity is apparent when the collimator is aimed at other areas of the head (Fig. 2). This may mean that there are other areas in the head which are also sensitive to x-ray. An equally tenable hypothesis is that there is only a single sensitive area in the olfactory brain area and that whole-head exposure is a more reliable method of stimulating the critical area. The apparent sensitivity of other head areas may be explained by inadvertent radiation of the sensitive area by scattered x-rays or by minor errors in positioning of the collimator. This question might be resolved by surgical lesion studies. Observations of behavioral arousal and electroencephalogram desynchronization in rats with the entire head shielded and the body exposed have been reported (*4*); this effect was abolished by spinal transections (*4*). The apparent contradiction may mean that radiation arousal operates by way of diverse mechanisms. At least two other mechanisms are known to be responsive to extremely low doses of radiation. One is the well-known retinal effect (*5*). In addition, radiation of the abdomen operates as an aversive stimulus to produce avoidance reactions (*6*).

J. GARCIA, N. A. BUCHWALD
B. H. FEDER, R. A. KOELLING
L. TEDROW
Veterans Administration Hospital,
Long Beach, California

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20 April 1964

Conditioned Discrimination in the Planarian

Abstract. *To demonstrate classical conditioning in the planarian in a situation uncontaminated by the possible artifacts of pseudoconditioning or sensitization 30* Phagocata gracilis *were successfully trained, by the use of directional shock as the unconditioned stimulus, to turn in one direction to a light, and in the opposite direction to vibration. Ten similarly trained planaria tested by an independent observer who did not know the previous training conditions experienced by any animal showed similar results.*

Thompson and McConnell's report of classical conditioning in the planarian (*1*) has revived interest in the learning abilities of this primitive organism. The literature has been reviewed recently by Jacobson (*2*). However, there has been concern with the possibility that much, or all, of the evidence for classical conditioning in the planarian might really be an artifact of the experimental situation. A basic source of possible artifact lies in the tendency of a response which has been repeatedly elicited to become "dominant," or more probable of occurrence (*3*). If the response is one elicited by the unconditioned stimulus (UCS), the tendency to make this same response to any other stimulus, in the absence of associative pairing, has been termed pseudoconditioning. Moreover, if the response is one directly (innately) elicited by the conditioned stimulus (CS), the increased probability of response has been called sensitization (*3*), and is of concern here because the light used as a CS by Thompson and McConnell can, innately, elicit the criterion response of contraction or turning. In addition to their classical conditioning group, Thompson and McConnell ran control groups which were exposed, respectively, only to the CS, only to the UCS (electric shock), and to no stimulation whatever. Neither the CS-only nor the UCS-only group showed ultimate response levels significantly higher than that of the no-stimulation group. On the other hand, Halas, James, and Knutson (*4*) found that a CS-only group, in a situation similar to Thompson and McConnell's, did give significantly more responses than a no-stimulation control.

We were interested in showing classical conditioning in a situation where there could be no question of artifactual "conditioning" due to sensitization or pseudoconditioning. Exploiting the planarian's marked galvanotropism, we were able to concurrently condition homologous, mutually exclusive responses to two different CS's and thus not only demonstrate conditioning but also test the planarian's ability to form a conditioned discrimination.

The subjects were 30 large *Phagocata gracilis*, obtained as needed during the study from a local stream. All were run within 24 hours of capture.

A plastic petri dish, 8.8 cm in diameter by 2.5 cm deep, filled to a depth of 2 cm with aged tap water, constituted the experimental chamber. A white base, with a reference grid to aid in evaluating the response, was glued to the underside of the chamber. This assembly was then firmly secured to the cover of a Johnson Speed-X constant-frequency buzzer, the vibration from which served as one CS. A commutator, 12.5 cm above the chamber, supported both a clear 12-watt light, which served as the second CS, and two nonpolarizing, platinum electrodes which extended down into the water on opposite sides of the chamber. By rotating the commutator, the electrodes could be oriented across any diameter of the chamber. The UCS, electric shock, was supplied by a filtered 8.5-volt d-c power supply in series with a variable resistance and a milliameter. Since the planaria showed differential sensitivity to the UCS, the current was individually adjusted during the first

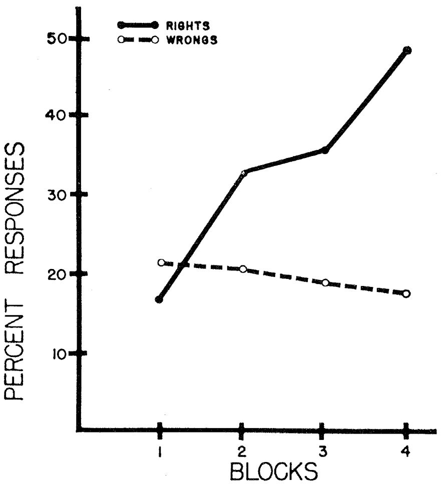

Fig. 1. Percentage of correct and incorrect test-trial responses (of amplitude greater than 22.5 degrees) in successive blocks of 50 training trials.

Table 1. Summary of analysis of variance for responses of 30 planaria to two conditioned stimuli (light and vibration).

Source	*df*	Sum of squares	Mean square	*F*	*p*
Subjects (Ss)	29	85.84			
Blocks of trials (B)	3	26.19	8.73	11.60	< .01
Right-wrong (RW)	1	57.41	57.41	35.22	< .01
Light-vibration (LV)	1	1.41	1.41	1.32	> .05
B × RW	3	49.87	16.62	12.49	< .01
B × LV	3	1.97	0.66	1.08	> .05
RW × LV	1	7.50	7.50	3.15	> .05
B × RW × LV	3	9.15	3.05	2.44	> .05
B × Ss	87	65.44	0.752		
RW × Ss	29	47.22	1.63		
LV × Ss	29	31.22	1.07		
B × RW × Ss	87	116.00	1.33		
B × LV × Ss	87	54.90	0.63		
RW × LV × Ss	29	68.87	2.37		
B × RW × LV × Ss	87	108.98	1.25		

few trials to the minimum level required to evoke a vigorous response. Most subjects required between 1.7 and 2.2 milliamperes.

All the planaria were trained and tested individually, receiving 200 training trials divided into four blocks of 50 training trials each. Ten test trials were given per block, five for each CS. The sequence both of training and of test CS presentation was randomly, though separately, determined. Test trials were given after each five training trials. The average intertrial interval was about 18 seconds, and rest periods of 1 to 2 hours were given between blocks of trials. During rest periods, the planaria were kept in individual containers in a dark room.

Each training trial consisted of a 3-second presentation of one or the other CS, with the UCS starting approximately 0.5 second after the onset of the CS and terminating with the termination of the CS. A test trial consisted of a 3-second presentation of one or the other CS alone.

By rotating the commutator so that the anodal electrode was in a position about 25 to 30 degrees to one or the other side of the subject's line of travel, either a right- or a left-turning unconditioned response could be elicited, the direction of the turn being away from the anode. As the animal started to turn at the onset of shock, the electrodes were rotated, "tracking" the animal and forcing a turn of more than 90 degrees. Half the subjects were run with the light paired with right turning and the buzzer with left turning, the other half with the reverse arrangement.

Responses were recorded in terms of both direction and amplitude of turn. No test trial was given until the animal was gliding in a straight line. In cases where it turned first in one direction, then in the other, only the initial direction and magnitude were recorded. A score of 0 was entered on trials where the animal did not turn or where the turn was judged as being less than 22.5 degrees, since planaria frequently make small turning motions of the head during normal locomotion. The measure for each subject, then, consisted of the number of correct and incorrect responses judged to be greater than 22.5 degrees. These data were used as the cell entries in a 30 (subjects) × 4 (blocks of trials) × 2 (right-wrong × 2 (light-vibration) design. By means of the analysis of variance, tests of significance were made for each of the main effects and for each of the possible interactions. A summary of this analysis appears in Table 1. Both the blocks main effect (B) and the right-wrong (RW) main effect were significant at the .001 level. Most important is the B × RW interaction, which was also significant at the .001 level. Fig. 1 shows that this interaction was due to an increasing number of correct responses and a decreasing number of incorrect responses from one block of trials to the next. This is, of course, our indication of learning.

While an additional test indicated individual differences in right-turning and left-turning tendencies, a separate analysis showed that the probability of a correct response on a test trial was not significantly affected by whether the preceding trial had been with the same CS as that used in the test trial or with the alternate.

As a check on possible experimenter bias in recording responses, 10 additional planaria were run under the same conditions as the original 30 except that all test-trial responses were scored by an independent observer, who observed only the test trials and was unaware of what constituted a correct or incorrect response for any subject. Group curves for these subjects were virtually identical to those shown in Fig. 1, and similar, statistically significant evidence of learning was obtained.

We believe that these data give less equivocal evidence of classical conditioning in the planarian than have any previously reported. In earlier studies, the only measure taken was whether the subject did or did not respond on a given test trial. By conditioning two distinct and homologous responses to the two different CS's, we are in a position to measure learning in terms of both correct and incorrect responses, rather than simply measure the total number of responses. Though it is possible that the subjects of this study became somewhat sensitized or pseudoconditioned to the experimental stimuli, neither sensitization nor pseudoconditioning can account for the increasing divergence in number of the correct and the incorrect responses, since there is no way these can differentially affect the responses to the two CS's, and since an equal number of unconditioned turns had been made to each side.

To the extent that other species of planaria have similar learning capacities, it seems likely that Thompson and McConnell's subjects did develop a simple conditioned response. The development of a conditioned discrimination by the subjects of this study suggests the need for further research concerning the limits of learning in this primitive organism.

C. D. GRIFFARD
American Institute for Research,
Pittsburgh 32, Pennsylvania
J. TREVOR PEIRCE
Department of Psychology,
University of Pittsburgh,
Pittsburgh 13, Pennsylvania

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14 February 1964