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EFFECTS OF RIBONUCLEASE UPON THE
REGENERATING TISSUE OF CONDITIONED PLANARIA

by

Harland E. Noll

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Harland E. Noll

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The fresh water planarian is one of the lowest organisms that possesses bilateral symmetry and a basic synaptic-type nervous system. The planarian is an invertebrate, has a blind-ended gut, and no circulatory system(Fig. 1, p. 5). Because of the planarian's rudimentary nervous system, the planarian may be the lowest organism that is able to be classically conditioned.

The first attempt at a controlled conditioning experiment was made by Thompson and McConnell(1955). A classical conditioning design using shock as the UCS(unconditioned stimulus) and a light as the CS(conditioned stimulus) was employed. The light was presented two seconds before a mild electric shock and was terminated with the shock, which lasted for one second. The mild shock normally caused a contraction of the entire body of the planarian and the behavior of the planarian was recorded during the CS interval. If the organism gave a noticeable turning movement or contraction prior to shock, then this was recorded as a CR(conditioned response). McConnell and Thompson found a significant increase in CRs and concluded that the planarian was conditioned when the CR was elicited in 23 out of 25 successive trials.

It is important that it be established that conditioning actually occurs rather than pseudo-conditioning or an effect of sensitization. Baxter and Kemmel (1963) compared paired and unpaired CS-UCS in planaria at two intensity levels each of the CS and UCS. Their results showed a significantly greater number of CRs for the paired group than the unpaired and they concluded that this difference was due to the contiguous CS-UCS relationship and not to sensitization.

In a later study by McConnell et al. (1959a) planaria were conditioned to a criterion of 23 CRs out of any block of 25 trials. The worms were then cut in half across the middle and the head and tail sections were placed in individual containers and allowed to regenerate for four weeks. After regeneration, the head and tail pieces were tested for savings. It was found that the tail pieces showed as much savings as the head pieces; regenerated head sections or tail sections of a planarian are thus capable of memory storage. From this regeneration study McConnell and his colleagues postulated that conditioning caused some chemical change throughout the planarian's body. To test this hypothesis (McConnell et al., 1959b), planaria were cut in half before conditioning and the heads were con-

ditioned before regeneration. The head sections were then allowed to grow new tails and these new tails were then removed and allowed to grow new heads. Total regenerates were then tested for savings of the original conditioning and showed a significant retention of the original learning.

The question arises as to what chemical change occurs and how this "memory" is stored? It has usually been assumed that the brain is responsible for storing memory, yet these studies indicate that memory may be stored elsewhere in the body, at least in the planarian. It is well established that genetic information is stored in deoxyribonucleic acid (DNA). Further investigations (Hyden, 1961) have indicated that acquired information may possibly be stored in giant protein molecules of nerve cells known as ribonucleic acid (RNA).

In a test of the RNA hypothesis, John and Corning (1961) conditioned planaria by the McConnell et al. method. The conditioned planaria were then cut in half and allowed to regenerate in a solution of ribonuclease (RNase), an enzyme that breaks down RNA. The concentration of RNase in pond water ranged from 0.07 to 0.1 mg/ml. After regeneration was complete, head and tail sections were tested for savings and it was found that the retention of head sections was relatively unaffected,

but the tail sections showed no savings. An uncut conditioned planarian placed in RNase also showed no forgetting. Why was memory destroyed in the tail sections but not in the head sections? John and Corning believed the RNase apparently did not affect intact tissue, but the primary effect occurred only at the regenerating surface. This and other studies by McConnell(1962) and Jacobson, Fried and Horowitz(1966) are suggestive evidence that memory is mediated by some biochemical change and that this change may very well be changes in RNA.

To further explore the RNA hypothesis of memory transfer, this study was concerned with testing Corning and John's suggestion that ribonuclease acts only on regenerating surfaces of planaria placed in RNase. If memory is mediated by RNA, the destruction of RNA at the regenerating surface of conditioned tail sections would prevent memory transfer to the regenerated head. Conditioned tails exposed to RNase solution were subjected to a second cutting, removing regenerating surface tissue exposed to RNase solution, and tested for savings upon regeneration of head ends.

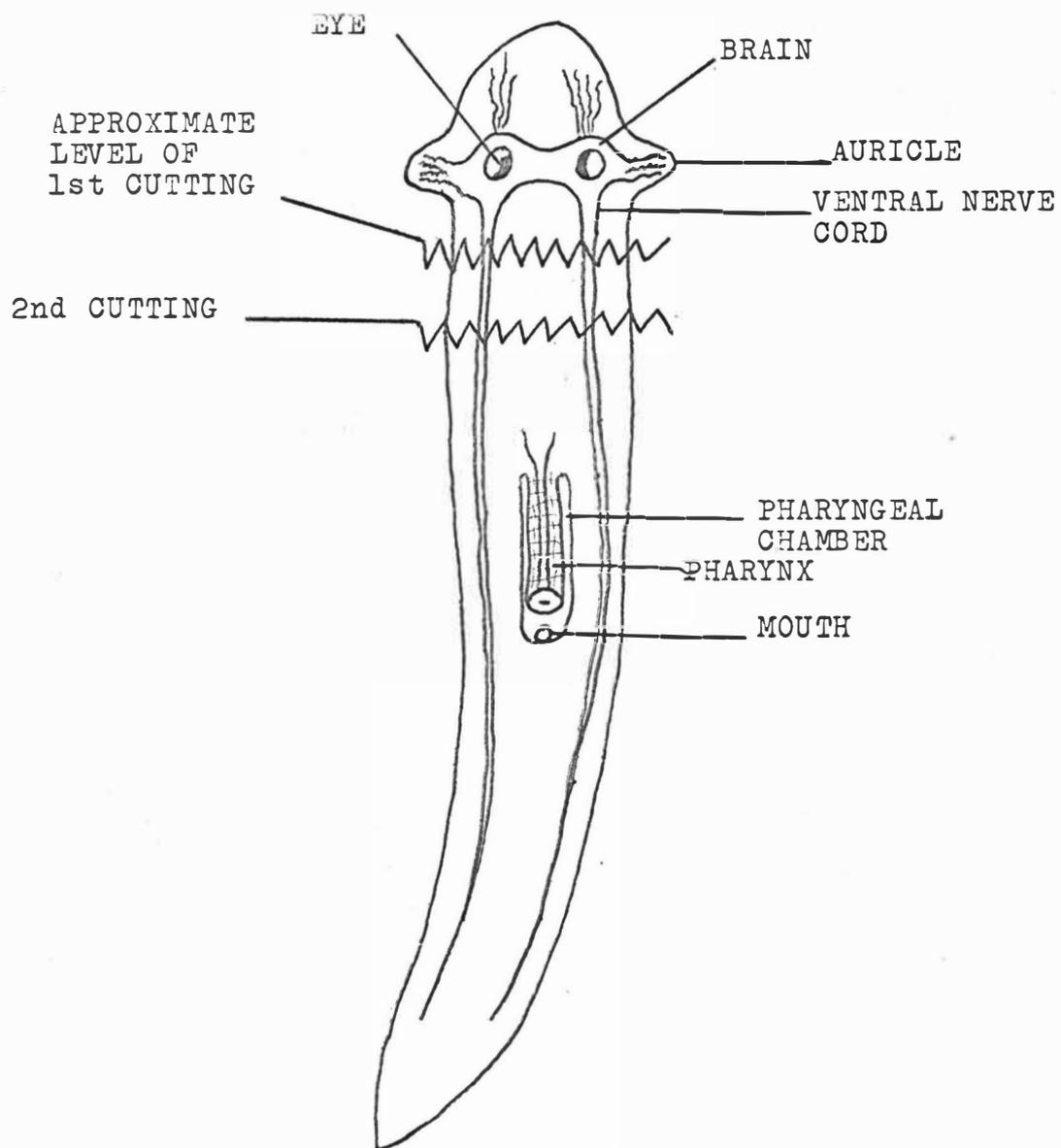


Fig. 1. The nervous and digestive system of the planarian with references to approximate level of cuts during the experiment.

METHOD

Subjects

Dugesia dorotocephala were obtained from the Biology Department at Western Michigan University. Only large planaria, approximately 1 cm length, were used in order to insure a good protein supply for regeneration. The Ss were maintained at room temperature and not fed during the course of the experiment.

Apparatus

The apparatus in which the planaria were conditioned consisted of a plastic trough, semi-circular in shape, 12 in. long and 1/2 in. in diameter. The trough was filled to the top with pond water. Mounted at both ends of the trough were electrodes, which transmitted a weak electric shock (UCS) through the water in the trough. DC current for the shock was provided by a 12 volt storage battery (28 ma at terminals). Two 100-w bulbs placed about 6 in. above the trough constituted the CS and room light was held to a minimum necessary for observation. The light and shock presentations were controlled by two Hunter Interval Timers.

Procedure

One planarian was placed in the trough at a time and was allowed to adapt to the trough for five minutes prior to training trials. A trial was of three seconds duration and was given when the planarian was gliding in a straight line toward either electrode. For the first 2 seconds, the CS was presented and during the third second the shock was presented without the light. The shock was of an intensity to cause a longitudinal contraction of the S's entire body. All CRs, either a contraction or a head turning, occurring during the CS period were recorded. There was a 20 second interval between trials. A maximum of 50 trials a day were given each planarian, with training continuing until the S reached a criterion of 20 CRs in 25 successive trials.

The subjects were divided into four groups of four subjects each. Group I was trained to criterion, cut in half, and the tail sections placed in RNase (0.1 mg/ml) for three days. The tails were then taken out of the RNase and another 3mm of body length were removed. The tails were then placed in PW (pond water) to regenerate, and after regeneration they were given 25 conditioning trials and again the number of CRs were recorded.

Group II was treated identically to Group I with

the single exception of not receiving the second cutting; this group was a control for lack of retention of CRs in planaria tails exposed to RNase as demonstrated by Corning and John (1961).

Groups III and IV were used to control for the effects of ribonuclease on learning ability. Neither Group III nor Group IV was conditioned. Planaria in Group III were cut in half, placed in RNase for 3 days and then placed in pond water to regenerate. After regeneration the planaria were checked for the number of CRs elicited in 25 training trials. Group IV was also cut and placed in RNase. The planaria were then placed in PW to regenerate and after regeneration they were given 25 trials and the number of CRs were recorded.

Table I presents a summary of the treatments under which the different groups were exposed.

TABLE I
OUTLINE OF TREATMENTS OF
EXPERIMENTAL AND CONTROL GROUPS

Treatment	Group			
	I	II	III	IV
Conditioned	Yes	Yes	No	No
Sectioned	Yes	Yes	Yes	Yes
Exposed RNase	Yes	Yes	Yes	Yes
Surface cut	Yes	No	Yes	No
Regenerate PW	Yes	Yes	Yes	Yes
Savings	Yes	Yes	Yes	Yes

RESULTS

A CR was defined as a sharp head turning or a contraction of the planarian. Twenty CRs out of 25 successive trials was the criterion for conditioning. As a means of testing for savings after exposure to RNase and regeneration, each group was given an additional 25 trials. T-tests were used to compare the mean number of CRs for each group with every other group. Table II presents a summary of the CRs for each group and the t-test values.

The mean difference between Group I and Group II is significant at the .001 level. The same is true for the difference between Group I and Group III and between Group I and Group IV. There are no significant differences between Groups II and III, II and IV, and III and IV.

TABLE 2
 MEAN CRs DURING RETENTION TRIALS OF EACH GROUP(N=4)
 AND t VALUES FOR ALL GROUP COMPARISONS

Group (mean CR)	t	p	df
I(16.25) vs. II(6.75)	7.85	.001	6
I(16.25) vs. III(4.75)	7.56	.001	6
I(16.25) vs. IV(4.5)	11.8	.001	6
II(6.75) vs. III(4.75)	1.09	NS	6
II(6.75) vs. IV(4.5)	1.60	NS	6
III(4.75) vs. II(4.5)	0.15	NS	6

DISCUSSION

The results support the hypothesis that RNase acts only on the regenerating surface. Planaria in Group I, which were exposed to RNase for three days and then had their regenerating surface area cut, showed a significant savings of the learned response. Group II, which was also exposed to RNase for three days but did not have the regenerating surface area cut again, showed no savings. Mean number of CRs for Group II, which had been conditioned, did not differ significantly in mean CRs from III and IV which had no previous conditioning trials.

Results obtained are in accord with the Corning and John data (1961) in that tail sections regenerated in an RNase solution showed no significant savings. The enzyme ribonuclease apparently destroyed the RNA at the cut surface, and even though the memory is present in the tail it is unable to transfer to the new head. This means the RNase has acted to "block" memory transfer to the head. Since the head end leads in behavior (Maier and Schneirla, 1935, p. 79) no apparent savings is detected although memory still resides in the tail. If the surface which has been exposed to RNase is cut off, then memory transfer can take place and savings can be detected.

In previous studies with conditioned tail sections unexposed to RNase significant savings were demonstrated and assumed transfer of memory from the tails to the regenerated heads did occur. This study found significant savings in conditioned tail sections that were exposed to RNase but then had the exposed area cut off. No savings was detected when the exposed area was left on. It is assumed that memory transfer to the regenerated head takes place after the second cutting only; RNase is destroying RNA at the cut surface only and "blocking" memory transfer. A conditioned tail that has been exposed to RNase will regenerate a naive head, and since the head end controls behavior no savings will be detected even though the tail retains memory.

Further investigation of the effects of RNase and memory transfer may investigate an extension of the present study by conditioning planaria, transecting, and placing tails in RNase for three days and then allowing regeneration in PW. These regenerated planaria should show no savings. If the heads were then removed at a point caudal to the original exposed surface and new heads were allowed to regenerate, then we should find savings or "memory" of the original conditioning.

SUMMARY

The purpose of this study was to examine the effects of ribonuclease on the regenerating tissue of tail sections of conditioned planaria. It was hypothesized that RNase acts only upon the cut surface of a sectioned planaria and thus acts to "block" memory transfer to a regenerated head. A classical conditioning procedure was employed in which light was the CS and shock the UCS. Sixteen planaria were divided into 4 equal groups, an experimental group and three control groups. The experimental group and one control group were conditioned to a criterion of 20 responses in 25 consecutive trials. They were then cut in half and placed in RNase for three days. The experimental group were then cut again to remove the exposed surface. The control group was not cut again. Both groups were then placed in pond water and regenerated new heads. They were then given 25 trials each and the number of CRs in the experimental group was compared with the CRs in the control group.

It was found that the experimental group showed a significantly greater number of CRs than control groups. This difference indicated that RNase only acts on the cut surface of tail sections and thus explains the absence of savings in regenerated tail sections exposed to RNase and the presence of savings in regenerated head sections as found in previous research.

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