THE EFFECTS OF REGENERATION UPON RETENTION OF A CONDITIONED RESPONSE IN THE PLANARIAN[1]

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In a recent study, Thompson and Mc-Connell (1955) demonstrated that classical conditioning could be established in the planarian or common marine flatworm, *D. dorotocephala*. From the standpoint of evolution, the planarian is an especially significant animal. Such evolutionary advancements as true synaptic nervous transmission, definite encephalization, and bilateral symmetry, to mention only a few, appear for the first time in planaria. These characteristics alone make the flatworm an interesting animal to study. For those psychologists interested in learning theory, however, perhaps an even more striking characteristic is the planarian's great regenerative ability. A single organism can be cut into as many as six transverse sections and, under optimal conditions, each section will regenerate a complete organism. If a planarian were conditioned, then cut in half and each half were allowed to regenerate, would either section show any retention of the conditioned response (CR)? If the tail section showed any retention of the CR at all, once it had regenerated into a complete organism, would it have retained as much as the head section?

The present study is an attempt to answer the above questions. Planaria were conditioned to a set criterion of responding, then immediately cut in half and allowed to regenerate. When regeneration was complete, each half was retested by the method of savings to determine the degree of retention of the original training.

METHOD

Subjects

Fifteen planaria (*D. dorotocephala*), varying in length from 10 to 24 mm., were studied. They were obtained locally and placed in individual glass aquaria,

the dimensions of which were 8 in. by 5 in. by 5 in. The water in the aquaria was continually aerated and was maintained at room temperature, approximately 22° C.

Apparatus

The apparatus in which the Ss were conditioned and later retested consisted of a semicircular plastic trough, 12 in. long and $\frac{1}{2}$ in. in diameter. The trough was suspended between two vertical supports which were anchored to a wooden base 1 in. thick. The trough was filled to the top with aquarium water. Mounted at both ends of the trough were electrodes, which transmitted the US (weak electric shock) through the water in the trough. Current for the US was provided by storage batteries and was passed through an inductorium, which provided the means for controlling the intensity of the stimulation.

A double lamp, housing two 100-w. frosted bulbs, was placed about 6 in. above the trough, with one bulb located near each end of the trough. Light from this lamp constituted the CS. The only other source of illumination was the overhead lighting of the laboratory, which was of a much weaker intensity than the CS.

Procedure

Only one S was studied at a time. The S was transported from the aquarium to the trough in a pipette. The S was allowed to "explore" the trough for 5 min. before training was begun. After an initial period of disturbed activity, during which the animal's locomotion was ditaxic or ameboid in character, it generally began to move in a normal fashion on the bottom of the trough. This movement may be described as "gliding," accomplished by ciliary action. When S reached the end of the trough, it would turn and retrace the trough. Occasionally S would come to rest and would remain motionless. If this "sleep" state continued for more than 1 min., S was prompted to locomotion by water currents set up by a pipette. Trials were given only when S was gliding in a straight line and was oriented toward either of the electrodes.

Each trial was of 3-sec. duration. For the first 2 sec., the light (CS) alone was presented. During the third second both light and shock (US) were presented. The US consistently evoked a longitudinal contraction of the animal's entire body. All responses occurring during the initial 2-sec. period were observed and "called" by one member of the experimental team, while another recorded the responses and handled the switches for presentation of the CS and the US. A minimum of 1 min. was allowed to pass between trials. A maximum of 50 trials per day was given, with training continuing until S reached the criterion of 23 responses in 25 consecutive trials.

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The Ss were divided roughly in terms of length into three groups of five Ss each: the experimental group (Group E), the regeneration control group (Group RC), and the time control group (Group TC).

When Ss in Group E had satisfied the criterion of conditioning, they were immediately removed from the trough and cut transversely in half as closely as possible with a razor blade. The two halves were then placed in separate aquaria and allowed to regenerate. The external appearance and the behavior of the animals suggested that regeneration was usually complete within 10 to 14 days. In order to make sure that internal structures were completely regenerated, however, an additional two weeks was allowed for recovery. Retesting for savings thus began approximately four weeks after separation. Both halves were retested, and so the experimental group served partially as its own control.

Since we feared that the processes of cutting and regeneration might in some way "sensitize" the animals, and thus make them subject to a more rapid conditioning, Ss in Group RC were given no experimental training of any kind before being cut. Naive animals were cut as soon as obtained, and each half was placed in a separate aquarium and allowed approximately four weeks to regenerate. Following regeneration, each half was conditioned separately to the same criterion as used for Group E.

Since we had no notion of how much extinction, or forgetting, might take place spontaneously during the four-week regeneration period, Ss in Group TC were conditioned to the criterion, then placed *uncut* in their individual aquaria and allowed to "rest" for a

TABLE 1

Number of Trials to Criterion for Group E

S	Original Training	Retest Head	Retest Tail
E-1	99	50	51
E-2	191	37	24
E-3	97	48	72
E-4	83	35	44
E-5	200	30	25
M	134	40	43.2

TABLE 2

Number of Trials to Criterion for Group RC

S	Head	Tail
RC-1	134	150
RC-2	188	179
RC-3	276	85
RC-4	395	300
RC-5	250	325
M	248.6	207.8

period of approximately four weeks. Following this "rest" period, they were retrained to the same criterion.

We did not think it necessary to control for the effects of sensitization to either light or shock, since Thompson and McConnell (1955) had already demonstrated that the CR they established was not the result of repeated exposure to either of these stimuli. It did seem possible, however, that we were establishing CRs in our Os rather than in our experimental Ss. In order to determine the reliability of our observations, three Es[3] observed the conditioning of one animal, making independent observations. When the planarian had reached the criterion, and only then, the observations were compared.

RESULTS

Two types of responses were observed in the Ss during the initial stages of conditioning, with a third type appearing generally during the latter stages. The first two types were as follows: (a) a sharp turning of the cephalic region to one side or the other and (b) a longitudinal contraction of the entire body. The third type was essentially a combination of the first two, in which the cephalic region turned sharply to one side or the other while the caudal region contracted sharply. In general, as the S neared criterion, the third type of response predominated—so much so, in fact, that its first appearance almost always heralded an immediate reaching of criterion. For the purposes of this study, however, any of the three types was scored as a "response."

Table 1 presents the raw data for the experimental group in terms of the number of trials necessary to reach criterion, both for the initial conditioning and for retest of the regenerated animals. A t test for comparison of means shows that the difference between the number of trials for original training and for later retraining of both the head and the tail halves is significant at the .01 level of confidence. There was no significant difference between the head and the tail sections, in terms of the mean number of trials required for each to retrain to criterion following regeneration.

Table 2 presents the raw data for the regeneration control group in terms of the number of trials necessary to reach criterion for both the head and the tail sections. As might be guessed from a cursory inspection of the data, the head and tail sections do not differ

significantly from each other, but both groups took significantly longer to reach criterion (.01 level of confidence) than did the regenerated sections of the experimental animals. What is perhaps more surprising is the fact that the animals in Group RC also took more trials to reach criterion than did the uncut animals in Group E. While this difference is not statistically significant (.09 level of confidence), it is probably large enough for us to conclude that the processes of cutting and regeneration in no way "sensitize" the animals.

The raw data for the time control group are presented in Table 3. Again, even a rough inspection of the data indicates that the TC animals showed a significant savings of the CR (.01 level of confidence) following the four-week "rest" period. There is no significant difference between the mean trials to criterion of the TC group following the "rest" period and the mean of either the regenerated head or tail sections of Group E.

The average intertrial interval was approximately 84 sec.

The experimental animal used in the reliability study took 238 trials to reach criterion. The three sets of independent observations were then compared for per cent agreement among the Os for the entire series of 238 trials. The results are shown in the first part of Table 4. While the agreement thus obtained is fairly high for this kind of observational datum, it was apparent from the data that reliability increased as conditioning progressed. At the beginning of training, planaria often make what might be called "minimal responses," so that it is sometimes difficult to know whether the animal has actually responded or not. Toward the end of training, however, when the vigorous and

TABLE 3

Number of Trials to Criterion for Group TC

S	Original Training	Retest
TC-1	123	24
TC-2	153	25
TC-3	195	62
TC-4	131	43
TC-5	325	45
M	185.4	39.8

TABLE 4

Per Cent Agreement of Three Os Making Independent Observations During Conditioning of One Animal

Os	Agreement for 238 Trials (%)	Agreement for Final 25 Trials (%)
A-B	85	100
A-C	86	96
B-C	89	96

distinctive "third type" of response (mentioned earlier) begins to appear, the responses become much easier to judge. Hence, we also calculated per cent agreements for the final 25 (criterional) trials. As the data in Table 4 indicate, two of the three Os agreed perfectly on the final 25 trials, while the third O differed from the other two on only one response in the 25 trials.

DISCUSSION

Perhaps the one most startling result of this study is the fact that the tail sections in the experimental group showed such a great savings of the initial conditioning. The results of Group RC indicate that this phenomenon is not due to any "sensitization" due to cutting and regeneration. Had the tails showed only some slight savings, it might have passed with little notice. That the tails showed at least as much savings as did the heads, and that both heads and tails showed as much retention as did uncut animals, calls for considerable cogitation and perhaps even some reorganization of our thoughts concerning the mechanisms of learning and retention in such organisms as the planarian.

A brief consideration of the nervous system of the planarian might well be of service here. As can be seen in Figure 1, "The nerve cell bodies are contained in two masses of nervous tissue, the cerebral ganglia, commonly referred to as the brain. From this concentrated point two longitudinal nerve cords pass posteriorly and two short nerves extended anteriorly to connect with the eyes. Along the two longitudinal cords are many transverse nerves, which are distributed to the internal structures of the body. . . ." Thus we find in the planarian "the beginnings of a definite central nervous system" (Elliott, 1952, pp. 164–165).
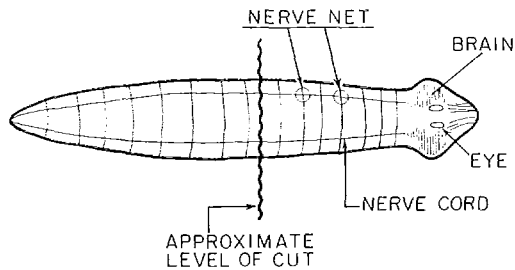
FIG. 1. The nervous system of the planarian (magnified many times). Adapted from Maier and Schneirla (1935, p. 80).

This has important implications for explanations of the behavior of the animal. The cephalic ganglion receives impulses from the sensitive elements of the snout and head, i.e., the eyespots, which are sensitive to changes of illumination but have no lenses and consequently form no image, and the auricles on the sides of the head region, which "may be chemoreceptors of 'taste' or 'smell'" (Storer, 1943, p. 328). This means, then, that "the head end leads in behavior, and through its nerve-ganglion impulses originating in the head receptors are conducted in two principal nerve strands and in their branches to other parts of the body" (Maier & Schneirla, 1935, p. 79).

This short description makes apparent the importance of the cephalic ganglia for the organization and direction of behavior in the planarian. This would lead one to believe that the ganglia should be crucial for establishment of learning in this animal. Hovey, who established a type of "negative adaptation" in these animals, reports that "following extirpation of the cerebral ganglia, worms did not learn to move less in the light, and repeated hindrance to such movement had no effect. Evidently the brain is involved in associative learning in polyclads" (Hovey, 1929, p. 332).

Although it may be dangerous to generalize too freely from Hovey's data, we are presently of the opinion that the cephalic ganglia may be necessary for *acquisition* but not for *retention* of a conditioned response in the planarian. This conclusion leaves us several interesting problems to face. To begin with, how does the tail section retain anything? We must conclude that if structural changes are to

account for the learning, these changes must occur throughout the nervous system, not solely in the cerebral ganglia. An even more intriguing question is this: Since the tail section must grow a new anterior half, including eyespots and cephalic ganglia, how does the relearning become established so quickly? It seems apparent that these cephalic centers must be involved in the rather complicated response that eventually becomes the CR. And yet two of the tail sections in Group E gave this highly complex response in their very first trials! Did the tail sections then regenerate new ganglia with the CR already "built in"?

A concerted program of experimentation is now underway in an effort to answer these and other questions.

SUMMARY

The purpose of the present experiment was to study the effects of regeneration upon retention of a conditioned response in the planarian (*D. dorotocephala*). A classical conditioning situation was employed in which light was the CS and shock the US. Fifteen animals were divided into three equal groups, an experimental group and two control groups. The experimental animals were first conditioned to a criterion of 23 responses in 25 consecutive trials. They were then cut in half transversely and allowed to regenerate. Following regeneration, both head and tail sections were retrained to the original criterion.

It was found that both head and tail sections showed significant retention of the CR and to the same degree. That these findings were not the result of sensitization due to the processes of cutting and regeneration was indicated by the performance of one of the control groups. That the small amount of "forgetting" of the CR which occurred in the experimental animals was probably due merely to the passage of time was indicated by the performance of the other control group.

It was concluded that in planaria the rudimentary brain is necessary for learning to take place but not for retention of the learned response. Two intriguing questions posed by these findings are: How does the tail retain

anything? and When the tail section regenerates a new brain, is the CR "built into" the cerebral ganglia?

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