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# MEMORY TRANSFER THROUGH CANNIBALISM IN PLANARIANS

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The research that I am going to outline today had its start several years ago, and I trust you will allow me to give you a few of the pertinent background details, if only to convince you that our work is more serious than it sometimes sounds, and of sufficient scope at least to approach respectability.† It was in 1953, when I was a graduate student at the University of Texas, that a fellow student, Robert Thompson, suggested to me that we attempt to condition a planarian, or common flatworm. Having avoided the rigors of introductory Zoology up to that point, my only prior experience with worms had been at the business end of a fishing pole. I soon discovered, however, that fishing worms are round, while planarians are flat. Planarians are also usually less than an inch in length, and rather interesting in their own right.

Flatworms occupy a unique niche on the phylogenetic scale, being the lowest organisms to possess bilateral symmetry, a rude form of encephalization, and a human synaptic-type nervous system. According to some psychological theories—the ones that postulate that learning is a matter of reshuffling connections among neurons—the planarian should be the lowest organism to be able to demonstrate "true" learning. As far as we knew in 1953, no one had ever demonstrated unequivocally that these organisms could indeed be trained. Since then, of course, we have discovered the usual obscure reference that antedates our work by 30 years—it appears in Dutch and was published in a little-read European journal[1]—but I am not at all sure that even this knowledge would have deterred us. At any rate, Thompson and I set out in 1953 to attempt classical conditioning in planarians.

Imagine a trough gouged out of plastic, 12 inches in length, semi-circular in cross-section, and filled with pond water. At either end are brass electrodes attached to a power source. Above the trough are two electric light bulbs. Back and forth in the trough crawls a single flatworm, and in front of the apparatus sits the experimenter, his eye on the worm, his hands on two switches. When the worm is gliding smoothly in a straight line on the bottom of the trough, the experimenter turns on the lights for 3 seconds. After the light has been on for two of the three seconds, the experimenter adds one second of electric shock, which passes through the water and causes the worm to contract. The experimenter records the behavior of the worm during the two-second period after the light has come on but before the shock has started. If the animal gives a noticeable turning movement or a contraction prior to the onset of the shock, this is scored as a "correct" or "conditioned" response.[2]

From this brief description of the experimental paradigm, many of you will

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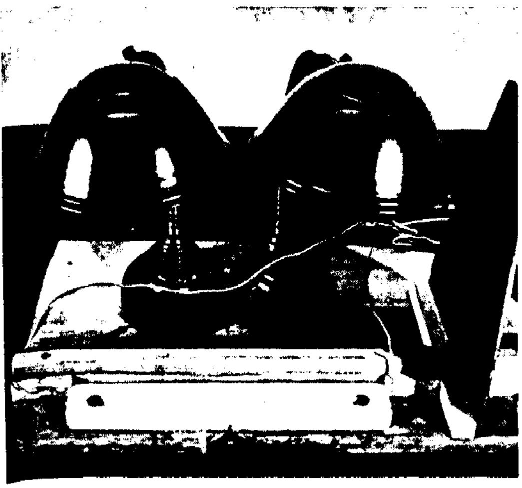
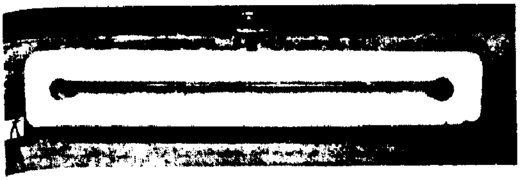
† For an excellent survey of the history of worm running, see the forthcoming paper by Allan L. Jacobson.[15]





### TABLE I

MEAN TURNS, CONTRACTIONS, AND COMBINED RESPONSES ON THE FIRST 50 AND LAST 50 TRIALS FOR GROUPS E (EXPERIMENTAL) AND LC (LIGHT CONTROL)

| Group | Response | First 50 Trials | Last 50 Trials | Diff. | p |
|---|---|---|---|---|---|
| E | Turns | 12.6 | 16.6 | 4.0 | .01 |
| | Contractions | 1.2 | 5.0 | 3.8 | .01 |
| | Combined | 13.8 | 21.6 | 7.8 | .01 |
| LC | Turns | 11.7 | 7.6 | -4.1 | .01 |
| | Contractions | 0.6 | 2.1 | 1.5 | |
| | Combined | 12.3 | 9.7 | -2.6 | |

### TABLE II

MEAN TURNS, CONTRACTIONS, AND COMBINED RESPONSES ON THE FIRST 15 AND LAST 15 TEST TRIALS FOR GROUP SC (SHOCK CONTROL)

| Response | First 15 Test Trials | Last 15 Test Trials | Diff.* |
|---|---|---|---|
| Turns | 5.4 | 4.2 | -1.2 |
| Contractions | 0.2 | 0.4 | 0.2 |
| Combined | 5.6 | 4.6 | -1.0 |

* None of the differences is significant at the .05 level of confidence.

recognize that Thompson and I were attempting to establish a form of Pavlovian conditioning in our experimental animals (Group E), and according to our data, we were successful. Planarians occasionally give a mild and presumably innate response to the onset of the light even when it has not been previously paired with shock, so we ran a control group that received just trials of photic (light) stimulation (Group LC); we also ran a control group that received just shock, occasionally interspersing a test trial of light alone (Group SC). All animals were given 150 trials. Over that period of time, as Tables I and II show, the experimental animals, which received light paired with shock, showed a significant increase in responsivity, while the control groups showed either no change at all or a significant decline.

Hence Thompson and I concluded that we had accomplished what we set out to accomplish—namely, we had proven that worms could be conditioned.[3]

Those of you who have ever chopped up a planarian in a Zoology course will know that these animals have enormous powers of regeneration. A large specimen may be cut into perhaps 50 pieces, each of which will eventually regenerate into a complete organism. It was while we were running that first experiment that Thompson and I wondered aloud, feeling rather foolish as we did so, what would happen if we conditioned a flatworm, then cut it in two and let both halves regenerate. Which half would retain the memory? As it happened, we never got around to performing that experiment at Texas, for Thompson received his doctorate soon after we finished our first study and went on to Louisiana State University and bigger and better things—namely, rats. When I went to the University of Michigan in 1956, however, I was faced with the difficult problem that in the academic world, one must publish or perish. The only thing I knew much about was flatworms, so I talked two bright young students, Allan Jacobson and Daniel Kimble, into performing the obvious experiment on learning and regeneration.

Kimble, Jacobson and I did the following. We took our experimental animals and trained them to a criterion of 23 responses out of any block of 25 trials. When they had reached this criterion, we assumed that they were properly conditioned and immediately cut them in half across the middle. Head and tail sections were then put in individual bowls and allowed about 4 weeks to regenerate. At the end of this period, these experimental animals (Group E) were re-trained to the same criterion and savings scores calculated. We also ran a group of worms which were cut, allowed to regenerate, and then were conditioned for the first time—this to tell us if cutting and subsequent regeneration in any way sensitized the animals to conditioning (Group RC). Another control group was conditioned, then allowed to rest uncut for a month before being retested (Group TC)—this to tell us how much forgetting we could expect in our experimental animals had we not cut them in half.

In all honesty I must admit that we did not obtain the results we had expected. We had assumed that the regenerated heads would show fairly complete retention of the response for, after all, the head section retained the primitive brain and "everybody knows" that the brain is where memories are located. And, as Tables III, IV, and V indicate, the heads did show just as much retention as did the uncut control animals. We had also hoped, in our heart of hearts, that perhaps the tails would show a slight but perhaps significant retention of some kind, merely because we thought this would be an interesting finding. We were astounded, then, to discover that the tails not only showed as much retention as did the heads, but in many cases did much better than the heads and showed absolutely no forgetting whatsoever. Obviously memory, in the flatworm, was being stored throughout the animal's body, and as additional proof of this we found that if we cut the worm into three or even more pieces, each section typically showed clear-cut retention of the conditioned response.[4]

TABLE III

Number of Trials to Criterion for Group E (Experimental)

| S | Original Training | Retest Head | Retest Tail |
|---|---|---|---|
| E1 | 99 | 50 | 51 |
| E2 | 191 | 37 | 24 |
| E3 | 97 | 48 | 72 |
| E4 | 83 | 35 | 44 |
| E5 | 200 | 30 | 25 |
| M | 134 | 40 | 43.2 |

TABLE IV

Number of Trials to Criterion for Group RC (Regeneration Control)

| S | Head | Tail |
|---|---|---|
| RC1 | 134 | 150 |
| RC2 | 188 | 179 |
| RC3 | 276 | 85 |
| RC4 | 395 | 300 |
| RC5 | 250 | 325 |
| M | 248.6 | 207.8 |

TABLE V

Number of Trials for Group TC (Time Control)

| S | Original Training | Retes |
|---|---|---|
| TC1 | 123 | 24 |
| TC2 | 153 | 25 |
| TC3 | 195 | 62 |
| TC4 | 131 | 43 |
| TC5 | 325 | 45 |
| M | 185.4 | 39.8 |

It was at this time that we first postulated our theory that conditioning caused som chemical change throughout the worm's body, and it was also about then that Reeva Jacobson came along to help us test wha seemed at the time to be rather an odd hypothesis. She took planarians, cut off their tails, and conditioned the heads before any regeneration could take place. Then she let her animals grow new tails. She next removed these new tails and let them grow new heads, ending up with apparently completely reformed organisms. These total regenerates, as we called them, were then tested for any "savings" of the original conditioning. By now we knew what to expect from planarians, and so we weren't too surprised when Reeva's regenerated flatworms showed a significant retention of what the original organism had learned. True, as Table VI suggests, these total regenerates did not demonstrate the complete retention that our original animals had shown, but they did remember enough so that our hypothesis seemed vindicated.[5]

TABLE VI

NUMBER OF TRIALS TO CRITERION FOR TOTALLY REGENERATED ANIMALS

| S | Original Training | Retest After Total Regeneration |
|---|---|---|
| 1 | 200 | 166 |
| 2 | 325 | 143 |
| 3 | 300 | 220 |
| 4 | 327 | 51 |
| 5 | 75 | 62 |
| 6 | 381 | 94 |
| mean | 268 | 122.7 |
| SD | 102 | 60 |

By now, worms were in the *Zeitgeist*. Edward Ernhart, working with Carl Sherrick at Washington University, demonstrated not only that flatworms could learn a two-unit T-maze, but also that this maze habit was retained by their animals following cutting and regeneration. Again, the tails remembered at least as much as did the heads.[6] Ernhart is perhaps most famous, however, for a more recent study of his. If one takes a flatworm and splits the head straight down the middle, time and time again, the two halves will not heal together but will each regenerate into a complete head. One ends up, then, with a two-headed worm. Ernhart compared the length of time it took two-headed animals to be conditioned with the length of time it took one-headed (or normal) animals to reach the same criterion and found that he had validated an old aphorism—two heads are indeed better than one.[7]

Roy John and William Corning, working at the University of Rochester, became quite interested in the chemical theory of learning about this time, and undertook one of the most spectacular pieces of research yet to come from any worm laboratory. John reasoned that learning in flatworms had to be mediated, at least in part, by some molecular change within the organism's cells. Since Hydén had found changes in RNA in nerve cells as a result of experience,[8] John believed that RNA might be implicated in learning and retention in planarians. So he and Corning conditioned a number of flatworms, cut them in half, and let them regenerate in a weak solution of ribonuclease, which breaks up RNA. When they compared their experimental animals with a number of controls, they found evidence that the experimental heads were relatively unaffected by the ribonuclease, while the tails showed complete forgetting. The tails could be retrained, but it took approximately as long the second time as it had the first.[9]

Ralph Gerard, the noted neurophysiologist, interprets the data as follows: There are probably two distinct but related physiological mechanisms for learning in planarians. The first such mechanism is the familiar one of neural interconnections which are reshuffled in the brain due to the animal's experiences—the so-called circuit-diagram model, if I may be permitted the analogy. Structural changes in the neural pathways in the brain would presumably not be altered by ribonuclease, which accounts for the fact that the Rochester head-regenerates showed no real forgetting. The second type of memory mechanism, however, involves a change in the coding of the RNA molecules in the cells throughout the worm's body. Presumably whenever the animal learns, the RNA is altered appropriately so that when regeneration takes place, the altered RNA builds the memory into the regenerated animal right from the start. If the RNA were destroyed by the ribonuclease, it is likely that the DNA in the cells would replace the lost RNA, but this replacement RNA would not carry the changed code since the DNA was presumably unaffected by the learning.[10]

If all of this sounds rather complex, you must forgive me. I am not at all sure that at this early date we have more than the vaguest notion just how learning could affect RNA nor how, much less why, this altered RNA might build the memory into the regenerating tissue. The important thing to remember is that John's hunch that RNA might be involved in memory seems to have been substantiated.

Before further discussing RNA and memory, I should like to detail, briefly, some other research that Roy John and Bill Corning, at Rochester, and my own group of worm runners at the University of Michigan and at the Britannica Center in Palo Alto, have been pursuing jointly. In 1957, when we got our first results on retention of learning following regeneration, and came up with our chemical hypothesis, it seemed to us that we might be able to transfer a memory from a trained animal to an untrained animal if we could somehow get the right chemicals out of the first worm and into the second. We spent several years trying to test this admittedly wild notion without much success. First we tried grafting the head of a trained animal onto the tail of an untrained planarian, but this never worked very well. If one reads introductory zoology texts, one often gets the notion that this little operation is most easy to perform. Sadly enough, the best average on record is three successes out of 150 attempts[11] and we simply did not have 150 trained heads to waste. We tried grinding the trained worms up and injecting the pieces into untrained animals, but we never could master the injection techniques. It was only some time after we began this work that it occurred to us that we could let the animals do the transferring for us. For, under the proper conditions, one worm will eat another. And since planarians have but the most rudimentary of digestive tracts, there seemed an excellent chance that the tissue from the food worm would pass into the body of the cannibal relatively unchanged.

So, with Barbara Humphries as our chief experimenter, we conditioned a number of worms, chopped them into small pieces and hand-fed the pieces to untrained cannibals. We also took the precaution of feeding a number of untrained worms to untrained cannibals for a control or comparison group. Our first pilot study gave us such unbelievable results that we immediately instituted several changes in our procedure and repeated the study not once, but four times. And each time the results were quite significant—and still rather unbelievable. I should mention before going any further that the chief procedural change we made was the institution of a "blind" running technique which helped guard against experimenter bias. Under this blind procedure, the person actually training the worms never knows anything about the animals he runs—we follow an elaborate coding system in which each animal's code letter is changed daily. The experimenter then doesn't know which animal is in which group, nor even which animal is which from day to day. Thus, as far as we could tell, we could not have unconsciously tampered with the data.

The results of this work, as Table VII shows, were somewhat startling. In all five studies, it was clear that the cannibals which had fed on trained worms gave approximately half again as many conditioned responses during the first days of training in did the cannibals which had fed on untrained worms. In our studies, the differences between the two groups tended to disappear after the first few days as the control animals approached criterion. The experimental animals were presumably so close to criterion right from the start that the slope of their learning curve was much less than that of the controls.[12, 13]

TABLE VII

Number of Responses in First 25 Training Trials for Cannibals Fed Conditioned Planarians (Experimentals) and for Cannibals Fed Unconditioned Planarians (Controls)

Number of Responses in First 25 Trials

| Experimentals | Controls |
|---|---|
| 4 | 1 |
| 6 | 1 |
| 7 | 3 |
| 8 | 4 |
| 8 | 4 |
| 8 | 4 |
| 9 | 5 |
| 10 | 5 |
| 10 | 5 |
| 10 | 6 |
| 11 | 6 |
| 12 | 6 |
| 13 | 6 |
| 14 | 7 |
| 14 | 7 |
| 15 | 10 |
| 15 | 10 |
| 15 | 11 |
| 15 | 11 |
| 17 | 16 |
| 18 | 22 |
| 19 | |
| ean 11.73 | 7.14 |

I would also like to mention a couple of ortunate mistakes we made which do not rove anything but which are interesting idence in their own right. One time our laborate coding system broke down and a ntrol animal was fed a piece of condition- worm. For several days prior to this eding, the control animal had been responding at an average of 2 or 3 times out of any 25 trials. Immediately following this inadvertent meal of conditioned tissue, the animal performed at criterion level, giving 23 responses out of the next 25 trials. Then there was one group of cannibals which we accidentally fed animals that had been given a number of conditioning trials, but which were not even close to criterion when we cut them up. The cannibals which ate these trained but not-yet-conditioned worms showed absolutely no transfer effect at all.

Now, if we had been the only ones to have obtained such results, our findings might be dismissed as the achievement of crackpots. Luckily for us, Corning, Karpick, and John instituted their own program of cannibalism shortly after we did and so far have run two large and very well controlled studies, both using the blind technique, and have obtained results which are essentially identical to ours.[14]

And, as if this were not enough, our work has just been replicated by a high school student. Let me quote briefly from the Washington *Post* of 25 March, 1962. "A 17-year-old girl's rather startling answer to a rather startling question—'Is Knowledge Edible?'—brought her one of the two top prizes in a Northern Virginia Science Fair yesterday. Tentatively, Ruth Ann Ziegler's answer is 'yes.'

"What Miss Ziegler found was that a worm who eats an educated worm learns things twice as fast as his brother who eats an uneducated worm. Hence her title, 'Is Knowledge Edible?'

"By electrical shocks she taught flatworms to respond to light. An ordinary flatworm needs about 260 shocks before he responds without one. He is then 'conditioned.'

"Experiments taught Miss Ziegler that a worm fed the head of an unconditioned worm needs an average of 264 shocks. A worm fed an unconditioned tail needs 269.

"But a worm fed a conditioned tail takes only 168 shocks and a worm fed a conditioned head a mere 140 shocks.

"This experiment was part of Miss Ziegler's effort to see if conditioned learning is affected by chemicals and, if it is, if it can be passed on through regeneration and ingestion. It's apparently 'yes' all the way."

Frankly, we are not quite sure where all of this work leaves us—except that we are most definitely out on a limb of some kind. At the moment, a number of laboratories around the country are starting investigations into the biochemistry of learning, using planarians as their tools. Specifically, several of us are attempting to extract RNA, DNA and other biochemicals from conditioned worms to feed to untrained cannibals. If we can show, for example, that RNA and only RNA causes the memory transfer, we can surely hope to determine the subtle molecular differences between "trained" and "untrained" RNA. If this could be done, we would be one step closer to cracking the problem of the molecular properties of memory—perhaps a giant step closer at that, particularly if it turns out that teaching the animals different sorts of habits causes different sorts of changes in the RNA molecules. But perhaps that is too much to hope for at the present.

Now, in conclusion, let me attempt to tie all of this research together. We have shown that planarians are capable of learning, that this learning survives cutting and regeneration, that the memory storage mechanism has a biochemical component (probably RNA) which is widely distributed throughout the animal's body, and that learning seems to be transferrable from one animal to another via cannibalistic ingestion. If memory in higher organisms is also mediated via biochemical changes, and if these changes are specific to the habits learned, we might eventually discover a substance (probably RNA with a deliberately modified structure) which would facilitate learning if it were incorporated into animal or human bodies. If so, the research we have been doing with our lowly flatworms may have practical consequences we never dreamed of when we began our work some nine years ago.

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