BRAIN TRANSPLANTATION IN SALAMANDERS: AN APPROACH TO MEMORY TRANSFER

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INTRODUCTION

The search for a specific biological basis of memory has been a major pursuit in the behavioral sciences ever since Pavlov first began theorizing on the neurology of learning. During the past few years a potentially rich, exciting new lead has emerged in reports that memory, encoded in molecules, may be transferred from one individual to another[2,8,10,13]. Unfortunately, memory transfer has become a controversial issue, primarily because of the failure of some investigators to reproduce the findings of others[4]. It seemed to us quite unlikely that the question would be resolved barring some new approach. Transplantation may offer that, and the salamander larva appeared to be the subject of choice because of the ready transplantability of its organs and tissues[11]. While it is a relatively simple vertebrate, it nevertheless has a complex, highly integrated nervous system[7], and it can learn discrete, readily controllable tasks[7,11,12]. Conceivably, pieces of brain from a trained animal might serve as a continuous source of supply for otherwise perishable molecules that in turn would enhance learning in the host. In the present study we observed increases in performances concomitant with brain transplantations but were unable to relate this to prior learning.

EXPERIMENTS

The study involved training of animals in a light-shock escape-avoidance paradigm, transplanting brains to the coelomic cavities of other larvae and teaching the hosts to perform the same task. The coelom was selected as the host site because of: (1) reproducibility of operating conditions; (2) minimal injury to the host animal; (3) its relatively large absorption surface area; (4) the lack of alteration in external appearance of the host, an essential requirement in maintaining the code we employed.

Subjects

Animals were *Amblystoma punctatum* larvae obtained as embryos in jelly masses

from J. C. Nichols, Murphy, N. C. Larvae were reared under controlled conditions of temperature and illumination. Just before the onset of feeding each larva was given an identification number and placed in an individual bowl with spring water. Fresh food (living tubifex worms) was kept in each dish at all times to insure maximal feeding. Experiments were initiated when animals reached 31–37 mm.

All hosts throughout the study were siblings from one egg mass. Prospective hosts of the volleys to be described below were selected just before each operation; all within a volley were as alike in size, color and external appearances as judgement with the dissecting microscope would permit. Donors in 3 volleys came from the same clutch as the hosts. Size of egg mass being a limiting factor, animals of 3 donor volleys had to come from another egg mass; but these larvae were the same age as all others.

Apparatus

A 4-cm-wide circular canal was constructed by inverting an 11-cm Stendor dish in a straight walled 15-cm evaporation bowl. The canal was irrigated with spring water, raised to a depth sufficient to submerge a free-swimming larva. Platinum wire electrodes were extended around both walls of the canal. A 1-sec shock was delivered by a square wave generator at 10 V DC with a frequency of 10 c/sec and a pulse length of 10 msec. These parameters were ascertained empirically during preliminary studies and were sufficient to elicit a response without impairing performance. Photic stimulation was provided by a DC spotlight with a beam delivered at 5 V and focused to produce a halo sufficient to encompass the animal's head, gills, and pectoral appendages. All experiments were conducted in an insulated, light-tight 2.2 m $\times$ 1.4 m $\times$ 2.6 m room equipped with a thermostatically controlled refrigeration unit. Temperature was maintained at 15°C. Animals were housed in a dark compartment in the room. Manipulations were carried out under dark-room conditions of illumination. Animals came into direct contact with light only during the actual experiments or the operations; they were under narcosis during the latter time.

Behavioral procedure

Avoidance training of donors consisted of presenting an animal with light and allowing 15 sec for escape before administering a single shock. A variable intertrial interval of 10–25 sec was employed. Avoidance trainees were given 60 daily trials until the criterion of 27 out of 30 was achieved; *i.e.*, 90% escapes without shock counting the block of 30 trials from the last 3 errors.

Shock sensitization control animals received the same number of trials as avoidance trainees. The 1-sec shock was administered in the absence of light with intertrial intervals of 10–25 sec. Light sensitization control animals were confronted with light alone for 15 sec, again using the variable 10–25 sec intertrial interval and the same number of trials as the avoidance trainees. Naive animals were kept in the same room but received no treatment. Wound control animals, to be described below as leg-hosts, were selected from among prospective recipients on the day of operation.

Pretest avoidance levels were established prior to training of donors and testing of hosts by confronting each subject with light for 15 sec during 20 trials.

An additional control for pseudoconditioning was introduced by presenting to the shock sensitization control donors light alone for 10 trials at the end of every 20 trials.

Training of prospective donors and testing of hosts was carried out in volleys. Donor volleys consisted of: (a) two avoidance trainees; (b) a shock sensitization control; (c) a light sensitization control; and (d) a naive animal. Host volleys corresponded to these and included a wound control or leg-host selected by the operator.

A code-recode system was employed. The operator knew the identity of only the leg-hosts whereas the behaviorist, when presented with newly coded hosts, knew the identity of none.

A test for experimenter bias was introduced into the study. This will be described with results.

Operations

Transplantations representing each experimental volley were performed within 2 h of each other and were paced so that all animals in the study were under narcosis for a like interval of time. Hosts and donors were rendered immobile in 0.25% MS222 (Sandoz). The latter was diluted 20-fold after 30 sec. The prospective host was positioned ventral surface up and secured in the crux of two decussating straight pins, plunged into a marble clay that lined the operating dish. Working under a dissecting microscope, a horizontal slit was made in the body wall ventral and slightly posterior to the heart and transverse septum. The donor then was brought into the operating dish and its brain dissected *in toto* working from the dorsal surfaces of the membranous neurocranium. Severed from the spinal cord at the first brachial segment, the brain then was floated over to the slit in the host and there divided into 4 pieces; the two cerebral hemispheres and regions corresponding approximately to the diencephalon, mesencephalon and rhinencephalon. Each piece was deliberately but gently contused as it was stuffed through the slit. Once inside the coelom, the pieces were manipulated posterially and positioned behind the intestines so that they would be safe from the possibility of oozing out of the slit. Also, with pieces located behind the intestines they were not visible from the surface and could not provide a clue that would break the code. The operation completed, the left hindlimb was amputated at its juncture with the body. This was done to make all animals in a volley uniform, for a 6th subject, added to the volley by the operator, received its own hindleg rather than a brain. The leg, too, was lodged behind the intestine.

Computations

Calculations were conventional and were carried out on an SDS 940 digital computer[1].

RESULTS

Learning was assessed in terms of avoidances in the initial 60 trials and by trials to the criterion stipulated in the Experimental Section. Our principal concern was how well the host groups performed both in relation to trained donor animals and to each other.

Table I contains avoidances in the initial 60 trials for trained donors and all hosts. Table II is a summary of statistical data on various interactions. Table III summarizes Table I, trials to criterion and contains significance levels on both measures. Supplemental information is contained in Tables IV and V.

Avoidances in the initial 60 trials provided the sharpest delineation of trends: all hosts out-performed the trained donors but were not statistically different from each other. In a one-way analysis of variance between the 12 donors and 12 hosts of their

TABLE I

AVOIDANCES IN THE INITIAL SIXTY TRIALS

A and B indicate donor–host relationship; *e.g.*, trained brain host B volley II received the brain from trained donor B II. Calculation with donors in the matrix showed significant differences in type interaction but not volley. Calculations for hosts alone showed no significant differences either for type or volley.

Experimental type	*Volley*					
	I	*II*	*III*	*IV*	*V*	*VI*
Trained donors A	17	15	18	24	21	22
Trained donors B	12	14	20	23	20	19
Trained brain hosts A	28	34	43	33	41	49
Trained brain hosts B	17	19	39	29	16	19
Naive brain hosts	34	51	34	21	33	28
Shock brain hosts	44	39	35	50	20	33
Light brain hosts	25	21	19	18	50	33
Leg hosts	40	23	15	57	42	48

TABLE II

ANALYSIS OF VARIANCE FOR AVOIDANCES IN INITIAL SIXTY TRIALS

Comparison	*F ratio*
Trained donor *versus* trained brain hosts*	12.19**
Trained donors *versus* hosts	5.38§
Host interaction	1.84§§
Volley interaction	0.40§§

* One-way analysis; all others were two-way analyses.
** P (0.01) = 4.46.
§ P (0.01) = 3.36.
§§ Insignificant.

TABLE III

SUMMARY OF AVOIDANCES IN INITIAL SIXTY TRIALS AND TRIALS TO CRITERION

Significance levels (*J*) were computed by the Mann–Whitney *U* test for trained donors *versus* each host group.

	Avoidances in initial 60 trials		*Trials to criterion*	
	Mean ± S.D.	*J*	*Mean ± S.D.*	*J*
Trained donors	13.7 ± 3.7	–	177 ± 25.9	–
Trained brain hosts	30.6 ± 11.1	0.005	125.2 ± 54.1	0.025
Naive brain hosts	33.5 ± 9.9	0.001	116.4 ± 61.9	*
Shock brain hosts	40.3 ± 12.1	0.005	81.8 ± 56.0	0.01
Light brain hosts	27.7 ± 12.2	0.05	153.5 ± 45.5	*
Leg hosts	37.5 ± 5.0	0.01	93.8 ± 66.4	0.05

* Not significant.

TABLE IV

PRETEST AND EXTINCTION RESPONSE LEVELS

Values are means ± S.D.

	*Pretest**	*Extinction***	
		4 days	*8 days*
Trained donors	2.17 ± 1.4	–	–
Shock (sensitized) donors	2.17 ± 1.1	–	–
Light (sensitized) donors	1.67 ± 0.9	–	–
Trained brain hosts	5.75 ± 5.0	12.8 ± 7.7	8.9 ± 7.7
Shock brain hosts	4.5 ± 3.0	19.8 ± 11.2	9.2 ± 7.1
Light brain hosts	5.7 ± 5.3	9 0 + 7.2	7.2 ± 3.4
Naive brain hosts	4.3 ± 4.1	15.0 ± 8.8	12.8 ± 6.7
Leg hosts	5.7 ± 3.1	16.0 ± 7.5	7.2 ± 3.6

* Twenty trials with light alone at the outset.
** Thirty trials with light alone at the days indicated following the end of training.

TABLE V

REGRESSION ANALYSIS TO TEST THE RELATIONSHIP BETWEEN PERFORMANCES OF TRAINED DONORS AND THE HOSTS OF THEIR BRAINS

Postulated function	*Coefficient*	
	Determination	*Correlation*
Linear	0.38	0.62
Exponential	0.45	0.67
Power	0.51	0.72

brains, an F ratio of 12.2 was obtained. The probability of this ratio arising purely by chance is 0.003. The data in Table I are presented in the array used to calculate a two-way analysis of variance. In this computation the interactions measured were experimental type and volley. With donor rows in the matrix, type interactions were significantly different whereas those arising from volley were not. In another computation each host group was compared separately with trained donors by establishing significance levels for their differences (Table III). Each host group, individually, performed better than the trained donors. However, as the training progressed to trials to criteria the significance levels diminished.

Donors were omitted from the matrix in order to assess differences among the various host groups. Two-way analysis of variance again revealed that volley (and variables that were a subset thereof) did not contribute significantly to differences among the groups. In contrast with calculations made with donor rows in the matrix, type interactions among hosts were not significantly different.

The same trends were evident in examining trials to criterion (*cf.*, Table III). In this measure, as in avoidances in the initial 60 trials, individual variances were unusually large in comparison with those of trained donors.

Table IV contains pretest avoidance levels; *i.e.*, escapes from light during 20 test trials administered prior to initiating the light-shock paradigm. Especially noteworthy among these data are the sizable differences in individual variances, host *versus* donor groups.

Extinction tests were carried out on the host animals in volleys I–V, 4 and 8 days after criterion had been reached (Table IV). Extinction occurred in all host groups but there were no significant differences among them. This is further evidence that the rate of learning was similar among host groups.

There might have been a quantitative relationship between hosts and donors submerged in the large variances just mentioned. This possibility was tested by defining host performances as a dependent variable of donor values and carrying out regression analyses for linear, exponential and power function relationships. Two sets of host-donor values do not fit the apparent trend (*cf.*, rows 1–4, Table I). These were omitted from computation. The functions in the order stated generated the following coefficients of correlation: 0.62, 0.67, and 0.72. These values are too far removed from unity to permit the inference that the relationships between the 2 sets of data are functional. Fig. 1 is presented to illustrate this point.

A test of experimenter bias was introduced into the study during the course of testing the host animals of volley III. All of the training and testing had been conducted by one investigator (A). On the 2nd day for testing the volley in question, just prior to the session and without advance warning, investigator B suggested that he go into the room alone, assign each animal a provisional code and place it in the training alley. This done, investigator A would enter the room and carry out the 60 trials. The suggested procedure was carried out with 5 animals of the volley. The 2 sessions then were compared with each other on the basis of differences between the first and second sets of 30 trials.

Let α be the mean differences between the first and second 30 trials in the first

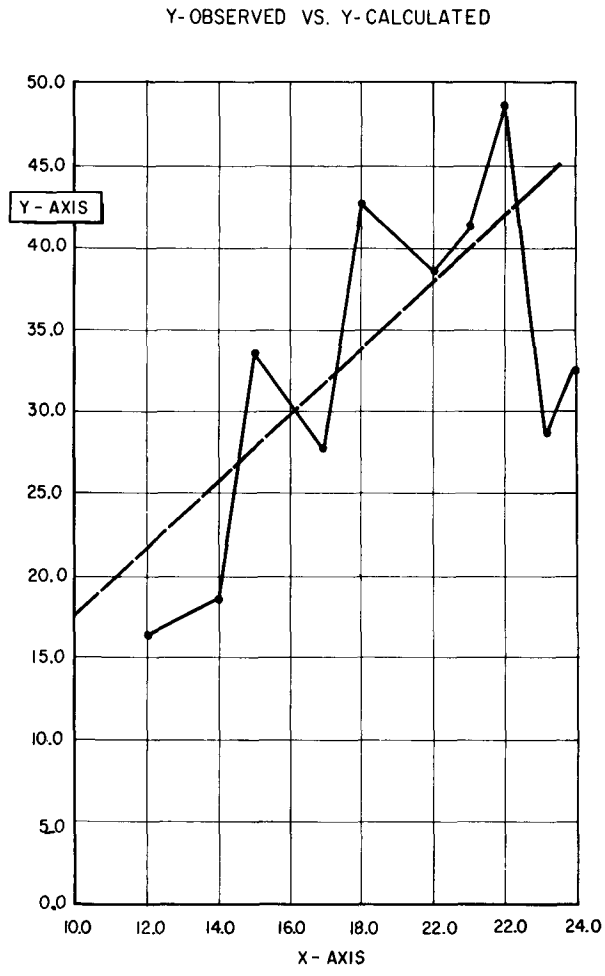

Fig. 1. Test of the hypothesis that performance (avoidance in the initial 60 trials) of the host animal of a trained brain (Y) is a dependent variable of performance of the donor (X). The solid line represents observed values. The dashed line represents values calculated from the function that generated the correlation coefficient closest to unity (*cf.*, Table V) ($y = 1.4X^{1.1}$). This plot suggests that the hypothesis is invalid.

session and β the same value for the second session. Then, attributing all differences to experimenter bias, bias $\approx (\alpha \pm \text{S.D.}) - (\beta \pm \text{S.D.})$; $[(6 \pm 2) - (6 \pm 4)]$ was observed for the 2 sets. Now, the observed values relate only to the 5 sets in each session. To generalize, population must be taken into account. Variations for each block of 60 trials, therefore, were used to compute the theoretical maximum mean values for population. Population was assumed to be infinitely large; the maximum mean for the first session (at 99.999% confidence limits) was 10.417; for the second, 14.944. The difference between these two values, 4.5 trials, might be influenced by experimenter bias — assuming that all fluctuations relate solely to bias of the experimenter. A useful way to phrase this is that the maximum theoretical contribution of bias to differences

among individuals in a block of 60 trials would be some 7.5%; or, the most pessimistic estimate of the experimenter's objectivity yields a value well within the range of anticipated individual variances. In short, there was no objective evidence that experimenter bias influenced the results. Germane to this point is the fact that when investigator A was carrying out the earlier of the 2 sessions of trials he did not know that on the following day he would be required to test the same animals without knowledge of their code assignments.

Finally, concerning pseudoconditioning: the shock sensitization donors that were given 10 trials with light alone after every 20 shocks made a mean avoidance of 1.7 during the first 30 light presentations and 1.5 during the last 30. In that these values are below operant avoidance levels it seems quite unlikely that pseudoconditioning was a factor in the study.

DISCUSSION

We observed increased performances after transplanting brains to the coelomic cavity of salamander larvae, but enhancement was independent of training and the nature of the transplanted tissues as well. Ironically, the best single performance of any animal in the study came from one of the leg-hosts, while the worst came from a recipient of a trained brain.

Our findings resemble those of Hartry *et al.* with planaria[6], Brown working with pigeons[3], and Halas *et al.* who employed rats as their subjects[5]. These studies as well as our own suggest that factors arising from experimental conditions, but unrelated to learning, operated in a non-specific way to lower sensitivity to stimulation.

Obviously, we found no evidence for memory transfer in our studies. Indeed, the criteria we employed did not satisfy what seems, in retrospect, to be a minimal condition for judging memory, namely a paradigm where hosts would have required no training. To introduce training, which we know can be influenced by non-specific variables in the experiment, in effect, begs the question.

'Sensitization' might be a convenient and simple way of dismissing the entire issue at hand. But, memory transfer is too important, potentially, to be discounted so lightly. It is well known that macromolecules can alter the physiology of cells, as for example in viral infections and in embryonic induction, and these phenomena entail information transfer. Memory may be associated with a labile molecular architecture and the transfer of it may depend upon concentrations that fall within narrow limits, as pointed out by Rashevsky[9]. Achieving the appropriate limits while preserving the integrity of chemical mediators might be exceedingly fortuitous under biochemical conditions and, therefore, difficult to reproduce. With transplantation it might be possible to overcome such obstacles.

SUMMARY

Brain transplantation was employed as an approach to the question of memory transfer. *Amblystoma* larvae were trained in a light-shock escape-avoidance paradigm.

Their brains were transplanted to the coelomic cavities of untrained animals. Transplantations also were made of legs and the brains of naive and stimulus-sensitized animals. Hosts then were trained to perform the same task. All hosts performed significantly better than the donor animals, but there were no differences in the various host groups.

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