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William L. Byrne and David Samuel

September 1966

ABSTRACT

Intraperitoneal injection of a naive rat with a brain extract prepared from trained donor rats (two) leads to an enhancement of learning. The enhancement is reproducible and achieves statistical significance with small groups of animals.

Several recent reports have indicated that the injection of brain derived fractions prepared from trained donors resulted in significant behavioral changes in the injected animals (1,2,3,4,5). In particular, the unreinforced performance in response to a specific stimulus following the injection of an RNA-containing extract (4) attracted widespread interest; however, attempts to achieve comparable results in other laboratories with the same or similar methodology have been unsuccessful (6). In contrast to the inconsistent results with the extraction procedures designed to contain nucleic acid-like materials, those efforts (7,8) which involved minimal or alternative prefractionation procedures have continued to yield evidence in support of the phenomenon of interanimal transfer of information (ITI) via brain extracts (9).

Our initial goal was to obtain reproducible data using automatic testing procedures where the behavioral modification was quantitatively large enough to allow statistical significance with small groups of animals. The initial experimental design was based on the report of Rosenblatt, Farrow and Rhine (7) which stated that operant conditioning of the donor animals would lead to behavioral modification of the recipients which was "task specific." Their results were based on unreinforced tests. The purpose of this report is to describe the results of experiments in which the rats, recipients, were tested without and with reinforcement. The tests with reinforcement appear to be a more sensitive means of detecting behavioral modification(s) which are consistent with ITI via brain extracts.

Three types of donors were used for the preparation of a brain extract and all injections were intraperitoneal. Two groups of donors were trained to bar-press for food reward, Noyes nutritive pellets, in a two-bar Skinner Box. The Skinner Box was a commercial model, Grason-Stadler E3125 B-100, with two modifications. The pellet dispenser was enclosed in a lucite and foamed-plastic sound-deadening housing and the chamber light was moved to a midline position on the wall opposite to the manipulanda. One group was trained to press the left bar and one group was trained to press the right bar. The third group of donors were naive animals.

The same protocol for training and testing was followed for experiments IV, VI M and VIII. Experiment II, the first experiment in which the learning rate of recipients was tested, is included since it represents data with an additional stock of rats, but the training and testing procedure was not identical with the other three. All rats, both donors and recipients, were first placed on a modified environmental complexity, EC, program (10) for 7 days in an attempt to facilitate the pretraining of the donors and to assure a population of recipients whose prior experience within and between experiments was as uniform as possible. The trained donors were pretrained to barpress, on either the right or the left bar, a minimum of 50 reinforcements with food reward, and then trained for 8 days, 20 minutes per day, on a fixed ratio schedule which began at 1:1 (1 press per pellet) and advanced each day of training so that the final day was 8:1 (8 presses per pellet). The training was completed 2 to 4 hours prior to decapitation and extraction. The naive animals, after completion of the modified EC program, were fed 10 to 12 g of food per day, about the same as the total received by the trained donors, but otherwise they did not receive special handling.

Food deprivation for training and testing was scheduled to reduce the animals to 85% of their starting weight. Animals were fed 3 g, 5 g, and then 8 g of food on days 1, 2 and 3, respectively, after initiating food deprivation. Supplementary food, 8 g or more if necessary of a commercial maintenance diet given at the end of the day, was used to maintain the animals at or above the 85% level during training and testing.

The animals were sacrificed by decapitation and the brain removed. The time for brain removal was approximately one minute with the exception of experiment VI M in which it was two minutes. The entire brain was removed, including the olfactory lobes and the portion of the brain stem which was left attached to the brain as a result of decapitation in the guillotine. Immediately after removal the brain was placed in a Potter-Elvehjem homogenizer tube containing 6.0 ml of ice-cold 0.9% NaCl-0.01 M Tris, pH 7.5. After the addition of a second brain, the two brains were homogenized with the aid of a mechanically powered pestle until there was complete dispersion of the tissue as judged by a visual inspection of the layer of homogenate which passed between the white Teflon pestle and the wall of the glass homogenizer tube. The complete dispersion takes approximately 40 seconds. The homogenate was poured into an 11 ml polypropylene centrifuge tube. An additional 2.0 ml of 0.9% NaCl-0.01 M Tris, pH 7.5, was added to the homogenizer tube, briefly homogenized, and also poured into the centrifuge tube. The samples were stored in crushed ice until a group of 8 was ready for centrifugation, 30 to 60 minutes. The samples were centrifuged for 30 minutes at 8000 x g in a Sorval centrifuge, head SS34, at 0°. The supernatant fluid was

poured off with no attempt to avoid a minor contamination with the pellet fraction. This supernatant fraction was used for injection, and was designated as a right, left or naive extract based on the type of donor. Each recipient received the extract derived from the brains of two donors, each pair separately processed. The volume of the supernatant fraction was approximately 6 ml out of a total volume of 11 ml, and it contained approximately 65 mg of protein (11). No attempt has been made to characterize the multiple components present in the extract; the protein determinations were used only to evaluate the reproducibility of the homogenization and centrifugation.

Potential recipients, after completion of the modified EC program, were placed on a food deprivation schedule 48 hours prior to injection and assigned to a test chamber. They were given two exposures to the test chamber prior to injection; the first exposure, approximately 24 hours before injection, was a group session, 3 rats per chamber, for 15 minutes with 10 pellets in the cup, and the second exposure, a few hours prior to injection, was an individual session, one rat per chamber, for 10 minutes with 5 pellets in the cup. At the end of the 10-minute individual session, left and right presses were noted. This pre-injection bar-pressing rate (without reinforcement) was used to select a uniform group of recipients. Injections were made in the evening and testing was initiated the next morning. All test sessions began with 5 pellets in the cup. This avoided the problem of trying to remove food odor and dust during the unreinforced test sessions. On day 1 each rat was tested for two 10-minute sessions without reinforcement. The tests

were separated by 5 hours, and the first test was started 12 to 14 hours after injection. In the afternoon of days 2, 3 and 4, each rat was tested with reinforcement, a 20 minute session; a press on either the left or the right bar would operate the pellet dispenser. The bars in a given box were matched in terms of the weight required, approximately 25 g, to close the microswitch. The left and right presses were recorded from a digital counter and an event recorder. All experiments were coded with either a double or a triple blind code. For experiments IV and VIII the donor animals were coded prior to sacrifice. For experiments II and VI M the samples were coded immediately prior to injection.

Graphs of bar-pressing versus time for the reinforced sessions, learning curves, indicated that a cumulative total of 10 reinforcements on one bar was an adequate criterion of the time required to learn, TTL_{10}. TTL_{10}, unless otherwise specified, began with the first reinforced press and terminated with the tenth on one bar. The results were evaluated by the Mann-Whitney U test (12,13).

The data in Figure 1 include results from a typical experiment, experiment IV, in which right, left and naive extracts were injected. A histogram of the pre-injection bar-pressing is shown in Figure 1a. The animals below 3 and above 11 presses per 10 minutes were rejected. Since the recipients were injected with coded samples, it should be noted that the unavoidable random assignment of samples for injection yielded closely-matched groups; the average pre-injection bar-pressing rate for the right, left and naive injected animals was 4.7, 5.9 and 5.5. A histogram of post-injection bar-pressing rate for the sum of the two unreinforced tests is given in Figure 1b. The type of injection did

not significantly influence the behavior of the recipients as judged by the unreinforced testing. With reinforcement, however, the combined data for trained as compared to naive injections show an enhanced rate of learning, Figure 1C ($P = 0.06$). A closer examination of the individual results shows that the right trained donor yields the extract which significantly lowers the TTL_{10} ($P = 0.014$). The injections from left trained donors in this experiment were not significantly different from a naive injection ($P = 0.3$).

A TTL_{10} without correction for the time prior to the first press may also be used as a measurement of the enhancement of learning, Figure 1d. The enhancement by a right injection is significant, $P = 0.036$, but the first press with reinforcement results in a definite solenoid noise, even though the sound and vibration of the pellet dispenser has been decreased by a sound-proofing housing. This solenoid-noise, together with the delivery of the food pellet, is a potential specific stimulus to the test animal, and, therefore, the time to learn following this first, potentially specific stimulus should be a more selective means of detecting an enhancement of learning. A comparison of uncorrected and corrected TTL_{10}'s for experiments IV and VI M indicated that statistical significance of the enhancement of learning by a right injection ($P = 0.036$ vs. $P = 0.014$, and $P = 0.026$ vs. $P = 0.002$, respectively) was improved by correcting the TTL_{10} for the time prior to the first press. This suggests that the first reinforced press may be a meaningful specific stimulus to the right injected recipient and the TTL_{10} data summarized in Table 1, with the exception of experiment II, are based on a corrected TTL_{10}.

(Insert Table 1 here)

The difference in learning enhancement of a right as compared to a left injection was not predicted, but it approached significance in experiment IV and VI M and was significant in experiments VIII and II ($P = 0.005$ and 0.004, respectively). Possible explanations for this difference in left bar versus right bar training include asymmetry of the rat or asymmetry of the training chamber; for example, the access door to the chamber is on the side adjacent to the right bar.

The reproducibility of the enhancement of learning by extracts from right trained donors (15) is indicated in Table 1. The experiments have involved changes in stock and sex of the rat and changes in personnel. In terms of personnel, two roles were considered important: The removal of the brain, VI M, and the individual responsible for the overall experiments, VIII, including coding, testing and compilation of the results. The individual responsible for II, IV and VI M did not participate in VIII.

The enhancement phenomenon described in this report is consistent with ITI, but the specificity and fundamental significance of this enhancement of learning by injection of brain extracts derived from trained donors remains to be determined. (16)

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Table 1

Enhancement of learning by injection of brain extracts as determined for the time to learn, TTL_{10} (14). P was determined by the Mann-Whitney U test. P is the probability, one-tailed test, of observing a value of U as large as the observed value if groups A and B are drawn from the same population. U is a statistic which depends on the relative ranking of the animals in groups A and B. If P is less than 0.5, group A has a shorter TTL_{10} than group B. The numbers in parentheses indicate the number of animals in each group.

Group A	Group B		
Experiment IV			
Trained (17)	Naive (4)	U = 17	P = 0.06
Right (8)	Naive (4)	U = 3	P = 0.014
Left (9)	Naive (4)	U = 14	P = 0.27
Right (8)	Left (9)	U = 22	P = 0.09
Experiment VI M			
Trained (11)	Naive (5)	U = 12.5	P = 0.05
Right (6)	Naive (5)	U = 0	P = 0.002
Left (5)	Naive (5)	U = 12.5	P = 0.54
Right (6)	Left (5)	U = 6	P = 0.06
Experiment VIII			
Right (8)	Naive (8)	U = 16.5	P = 0.06
Left (8)	Naive (8)	U = 35.5	P = 0.62
Right (8)	Left (8)	U = 8	P = 0.005
Experiment II			
Right (5)	Naive (4)	U = 0	P = 0.008
Left (5)	Naive (4)	U = 9	P = 0.45
Right (5)	Left (5)	U = 0	P = 0.004

FIGURE CAPTION

Figure 1.

(a) Pre-injection bar-pressing rate for potential recipients. In this experiment only, 8 potential recipients had a second pre-injection session, their average rate was used.

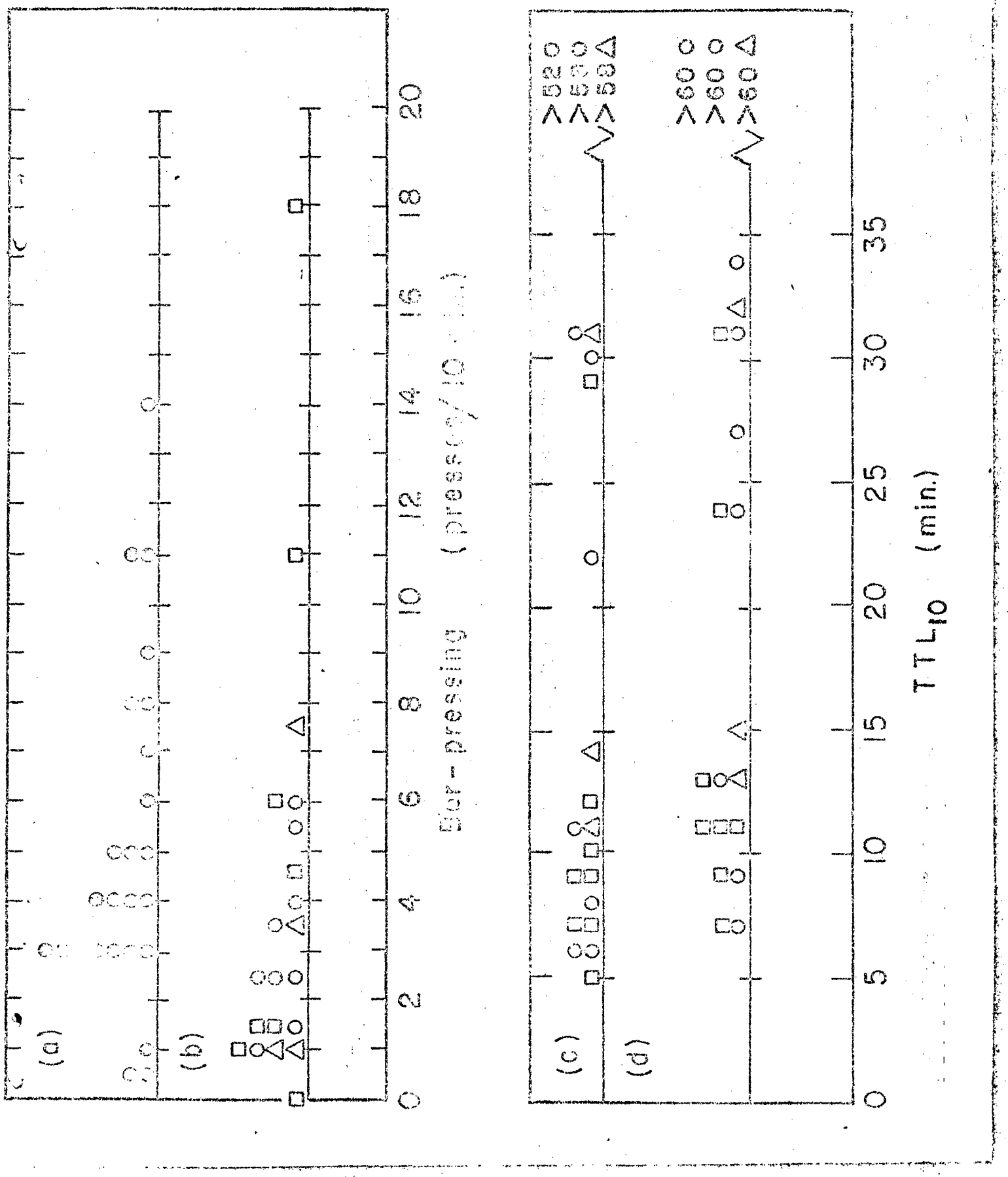
(b) Unreinforced bar-pressing, average for two sessions, following injection;

(c) Time to learn, TTL_{10}, based on reinforced tests, with a correction for the time prior to the first reinforced press.

(d) TTL_{10} for the same animals, without correction.

● prior to injection; o left injection; □ right injection;

Δ naive injection.


(a)
(b)
(c)
(d)
Blood-pressing (pressure / 10 mm Hg)
TTL10 (min.)
0
2
4
6
8
10
12
14
16
18
20
5
15
25
30
35
52
55
58
60
60
60

REFERENCES AND FOOTNOTES

1. E. J. Fjerdingstad, T. Nissen, H. H. Roigaard-Petersen, Scand. J. Psychol., 6, 1 (1965); T. Nissen, H. H., Roigaard-Petersen, E. J. Fjerdingstad, ibid., 6, 265 (1965).

2. S. Reinis, Activitas Nervosa Superior, 7, 2 (1965).

3. G. Ungar, Nature, 207, 301 (1965).

4. F. R. Babich, A. L. Jacobson, S. Bubash, A. Jacobson, Science, 149, 656 (1965); A. L. Jacobson, F. R. Babich, S. Bubash, A. Jacobson, ibid., 150, 636 (1965); F. R. Babich, A. L. Jacobson, S. Bubash, Proc. Natl. Acad. Sci., 54, 1299 (1965).

5. D. J. Albert, Neuropsychologia, 4, 79 (1966).

6. C. G. Gross and F. M. Carey, Science, 150, 1749 (1965); M. Luttges, T. Johnson, C. Buck, J. Holland, J. McGaugh, ibid., 151, 834 (1966); M. W. Gordon, G. G. Deanin, H. L. Leonhardt, R. H. Gwinn, Amer. J. Psychiat., 122, 1174 (1966); W. L. Byrne, D. Samuel, E. L. Bennett, M. R. Rosenzweig, E. Wasserman, A. R. Wagner, F. Gardner, R. Galambos, B. D. Berger, D. L. Margules, R. L. Fenichel, L. Stein, J. A. Corson, H. E. Enesco, S. L. Chorover, C. E. Holt III, P. H. Schiller, L. Chiappetta, M. E. Jarvik, R. C. Leaf, J. D. Dutcher, Z. P. Horovitz, P. L. Carlson, Science, 153, 658 (1966).

7. F. Rosenblatt, J. T. Farrow, S. Rhine, Proc. Natl. Acad. Sci., 55, 548, 787 (1966); F. Rosenblatt, J. T. Farrow, and W. F. Herblin, Nature, 209, 46 (1966). We would like to acknowledge the extensive cooperation of Frank Rosenblatt. The initial training, extraction and testing (without reinforcement) procedures/were based on his published and unpublished results..

8. G. Ungar, Fed. Proc., 25, 207 (1966).

9. The term "information" does not imply a specific mechanism, and it should be considered as a term which would describe both specific and nonspecific transfer phenomena.

10. D. Krech, M. R. Rosenzweig, E. L. Bennett, J. Comp. and Physiol. Psychol. 55, 801 (1962).

11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, J. Biol. Chem., 193, 265 (1951).

12. H. B. Mann, D. R. Whitney, Ann. Math. Statist., 18, 52 (1947); S. Siegel, Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill, New York (1956), p. 116.

13. Even though the animals receive reinforcement on both bars, a typical animal establishes and retains a bar preference soon after he learns to bar-press. Since the trained donors were either left or right trained, the early bar preference, at TTL_{10}, and the final preferences were recorded. The final preference criterion was 90% or more on the same bar in sequential test sessions, but the great majority of the animals established a greater than 95% preference. The bar preference will be the subject of a future communication; it did not show statistical significance in the individual experiments. For the recipients who developed a final bar preference the trend was in the predicted direction in experiments II, IV and VIII, but the trend was in the nonpredicted direction in VI M. In experiment VI M the brain removal time was approximately 2 minutes instead of the normal 1 minute.

14. For experiment IV the rats were Holtzman females, 150 g, approximately 5 weeks old. In experiment VI M and VIII the rats were Holtzman males, 150 g, approximately 5 weeks old. The Holtzman rats are a Sprague-Dawley derived stock. In experiment II the trained donors and recipients were Berkeley S_1 males, 220 to 300 g, approximately 12 weeks old, but the naive donors were Buffalo males. The TTL_{10} data used to calculate the U and P values in Table 1 are as follows: Experiment IV - right injected 5, 7, 7, 9, 9, 10, 12 and 29; left injected 6, 6, 8, 11, 22, 30, 32, >58 and >52; naive injected 11, 14, 32, and >58; Experiment VI M - right injected 5, 5, 6, 6, 7, and 8; left injected 5, 8, 34, 40 and >40; naive injected 13, 15, 18, 22, and >60; Experiment VIII - right injected 4, 8, 9, 10, 13, 13, 16 and 20; left injected 4, 20, 21, 26, 37, >0, >17 and >57; naive injected 3, 4, 18, 29, 46, >49, >49 and >57; Experiment II - right injected 6, 9, 9, 12 and 13; left injected 15, 16, 26, 35 and >45; naive injected 14, 21, >45 and >45. The TTL_{10} values with the exception of experiment II are corrected for the time prior to the first reinforced press. The animals which did not reach the TTL_{10} criterion are listed as having TTL_{10}s greater than the testing time which followed the first reinforced press, but for the calculation of U all of the animals which did not learn were given the same ranking.

15. In retrospect, it is interesting to note that if the initial experiments had been attempted with animals trained on the left bar only, the extracts would not have shown an enhancement of learning. Current

experiments with a modified Skinner Box, a "mirror image" design, indicate that the design of the chamber might modify the enhancement potential of the brain extract prepared from a left or a right trained donor.

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