Pharmacology Biochemistry & Behavior, Vol. 19, pp. 241–244, 1983. © Ankho International Inc. Printed in the U.S.A.

Facilitation of an Operant Task in the Rat Following Injection of Whole Brain Extract

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Received 2 September 1982

MORRIS, P. E. AND J. M. BEATON. *Facilitation of an operant task in the rat following injection of whole brain extract.* PHARMACOL BIOCHEM BEHAV **19**(2) 241–244, 1983.—It has been shown that the administration of trained donor brain extract to naive rats results in facilitation of performance on the same task. In the present study a group of food deprived rats was trained to press a lever for food on a continuous schedule of reinforcement until they reached criterion. The animals were then sacrificed, their brains excised, homogenized and the small proteins (m.w.<3500) extracted. A group of untrained rats was also sacrificed and their brains extracted. Three groups of rats were used as recipients, receiving either trained donor or untrained donor brain extract or saline. The animals were tested individually for one-hour sessions at 18, 42 and 66 hours after the injection. The number of bar presses made by each rat was noted and the group mean plus or minus the standard deviation were calculated for each session. The results of a one-way analysis of variance showed that the group which received trained donor brain extract performed at a higher rate than either control group. These data suggest that some factor, (specific or non-specific), associated with the task has been transferred.

Transfer of behavior Operant task Trained donor brain

DURING the past two decades there have been numerous experiments performed examining the biochemical transfer of learned behavior in the rat. Many of these studies stemmed from Ungar's discovery and isolation of scotophobin, a small peptide which is formed in brain when rats are trained on a dark avoidance task. Ungar [15] found that when these rats were sacrificed, their brains excised and processed and the extract from these "trained" rats was injected into naive mice, the recipients exhibited the same dark-avoidance type behavior. Ungar attributed this behavior to scotophobin. The isolation and amino acid sequencing of the scotophobin peptide chain soon followed as did its synthesis. The synthetic material was shown by numerous laboratories [5,10] to have the same effect upon behavior.

Encouraged by the results from the scotophobin experiments, many investigators set out to study other "learning proteins" which could be formed when various tasks are learned. For example, Babich *et al.*[2] successfully transferred a food cup approach task in naive rats using brain RNA from trained donor rats. Braud and Braud [4] successfully transferred relational learning using crude whole brain homogenate, and Ungar *et al.* [14] transferred sound habituation. However, reported along with these positive findings were also a great number of negative results. For example, Gordon *et al.* [8] found that extracted brain RNA from trained donor rats did not have any behavioral effects upon recipient rats. Also, Gross and Carey [9] reported that they were unable to replicate the Babich *et al.* [1] experiment. One reason for these conflicting reports may be traced to the different methodologies used by the various investigators. For example, some investigators have reported positive transfer using purified liver and brain RNA, while others report positive transfer only when using crude whole brain homogenate. There are many other possible explanations for such failures. Three of the major reasons for a failure to observe a transfer effect may be (1) experimental criteria are lacking, i.e., there are too many procedural differences between studies, (2) the task being transferred is inappropriate, and (3) the method of brain extraction denatures the transfer factor. Other discrepancies which are found between investigators are the number of training days for the donor rats (which Ungar *et al.*[15] have shown to be a pertinent factor) and the amount of brain extract administered.

We have previously shown in a pilot study [12] that a food reinforced bar pressing task can be transferred using whole brain homogenate in a 2:1 ratio, i.e., two trained donor brains per naive rat. A simple bar press learning paradigm was chosen because it had been reported that tasks which require little effort, especially a positive, reinforced task, is the most readily transferable [13]. Also, a schedule of positive reinforcement has been shown to be a more sensitive means of detecting transfer behavior using whole brain homogenate extractions [6]. It was proposed in this present study to examine the transfer of this bar pressing task in a larger group of subjects.

TABLE 1

THE MEAN NUMBER OF BAR PRESSES, PLUS OR MINUS THE STANDARD DEVIATION, MADE BY EACH GROUP OF RATS ON THE THREE TEST SESSIONS

	18 Hours	42 Hours	66 Hours
Saline Group	2.3±0.80	1.1± 0.67	43.9±27.54
Control-Brain Extract Group	2.9±1.43	17.7±10.97	80.4±45.57
Trained-Brain Extract Group	20.6±8.57	141.6±38.60	195.4±43.46

TABLE 2

t-TEST VALUES OBTAINED IN THE POST-TEST COMPARISONS

	t value	level of significance*
18 hours post-injection		
Saline vs. control brain	1.344	N.S.
Saline vs. trained brain	4.522	<0.001
Control brain vs. trained brain	3.565	<0.01
42 hours post-injection		
Saline vs. control brain	2.283	<0.05
Saline vs. trained brain	13.246	<0.001
Control brain vs. trained brain	9.534	<0.001
66 hours post-injection		
Saline vs. control brain	0.470	N.S.
Saline vs. trained brain	8.672	<0.001
Control brain vs. trained brain	3.335	<0.01

*With 18 degrees of freedom, two tailed test.

METHOD

Subjects

Seventy adult (120 day old) male Long-Evans hooded rats served as subjects. Forty rats served as donors, twenty as recipients and ten as controls. The rats were housed individually in a sound attenuated room maintained at 27±2°C with free access to food (Purina Rat Chow) and water. For fourteen days prior to the beginning of training the rats were handled and weighed daily. All the rats were then brought to 80% of their respective free feeding weights and maintained between 80–85% of their weights.

Apparatus

Three single lever operant chambers connected to BRS/Lehigh Valley Programming Equipment and digital response counters were used. The daily responses made by each rat were recorded. The chambers were also connected to cumulative recorders and recordings were made for all rats during all testing sessions. The operant chambers were situated in a dark and soundproof testing chamber illuminated only by a small light bulb located on the same wall as the lever. Fans were run during all sessions to keep the testing chambers cool and to mask outside noises.

Training

Twenty donor rats were trained on a schedule of continuous reinforcement (CRF), that is each response was reinforced with a food pellet weighing approximately 45 mg. For the first two days of training the rats were magazine trained (i.e., taught to approach the food cup at the sound of a click to receive a reinforcer) and subsequently trained to press the lever to deliver a pellet. By the third day of training all rats had started pressing the lever and were receiving reinforcers. The session terminated after the rat received 100 reinforcers.

Following the third day of shaping, the rats were tested for four subsequent days. This schedule allowed each rat to reach the criterion of 100 bar-presses per day for five consecutive days. Therefore, the level of performance of each rat was similar. On the last day of training the rats were sacrificed by decapitation immediately after their session and their brains excised, weighed, and placed in 4°C distilled water. These rats constituted the trained donor group. This trained donor group was divided into three sub-groups depending upon the chamber in which the rat had been trained. Twenty control donor rats were treated and sacrificed exactly as the trained donors, except that they received no training.

Extractions

The excised brains, in the 4°C distilled water, were homogenized and sonicated for 1.5 minutes (30 sec homogenization-sonication/30 sec cool off, × 3). Six volumes of distilled water were used for every gram of tissue. During the homogenization-sonication step crushed ice was kept packed around the centrifuge tubes to retard tissue deterioration and enzymatic action on the proteins. This mixture was then magnetically stirred for four hours at 4°C, then centrifuged at 17,000×g for two hours on a Sorvall RC 2-B refrigerated centrifuge, also at 4°C. The supernatant was dialyzed (spectrapor 3 M.W. cutoff 3500) against 12 ml of distilled water for every ml of supernatant for twenty hours. After dialysis the dialyzate was frozen in a mixture of dry ice and acetone. The frozen dialyzate was then lyophilized and the remaining powder kept frozen in a −70°C freezer until needed. This extraction method differs from that of Braud and Braud [4] in that we add sonication in the homogenization step and distilled water was substituted for physiological saline solution to avoid administration of a hypertonic solution to the recipient rats. In addition, a dialysis step was added to allow for an injection of only low molecular weight (<3500 a.m.u.) proteins and peptides. Immediately prior to injection distilled water was added to the extracts to allow each recipient rat to receive the equivalent of two donor brains in a volume of 2 ml and vortexed for 30 sec at maximum speed to allow for a homogeneous injection. All injections were administered intraperitoneally.

Testing

Prior to injection, the operant chambers were partially

TABLE 3

THE MEAN NUMBER OF ACTIVITY COUNTS, PLUS OR MINUS THE STANDARD DEVIATION, FOR EACH GROUP OF RATS ON THE THREE TEST SESSIONS

	18 hours	42 hours	66 hours
Saline group	1,105.2±418.7	1,451.2±457.0	963.9±535.1
Control-brain extract group	1,644.5±552.5	1,295.1±573.6	1,346.6±647.8

disassembled and thoroughly cleaned. This cleaning procedure assured that the recipient rats would not be attracted to the bar or food cup by residual pellet odor or particles, during testing.

The remaining thirty food deprived rats were divided into three equal groups. Group I received the trained donor extracts and Group II received the untrained donor extracts. Group III received an injection of 2 ml saline. Each group was tested at 18, 42 and 66 hours after injection.

RESULTS

The daily number of bar presses made by each rat was recorded and Table 1 shows the mean number, plus or minus the standard deviation, for each group on each of the three test sessions. It can be seen from this table that there was an increase in bar pressing by all groups over the three days. However, the group of rats which received the extract from the brains of trained rats have a much higher mean number of bar presses for all sessions.

A one-way analysis of variance was carried out on the data for each test time. Significant differences were found at all three testing sessions. At 18 hours, $F(27,2)=3.99, p<0.005$; at 42 hours, $F(27,2)=10.98$, $p<0.001$ and at 66 hours, $F(27,2)=3.97$, $p<0.05$. A series of t-tests was then carried out to examine the source of the significance. The results of these t-tests showed that the group of rats which received trained donor brain extract performed significantly better than either control group on all test sessions. The t values can be seen in Table 2.

One interesting finding was the significant difference found between the saline and control brain extract groups at 42 hours after injection. This was the only significant difference found between these groups but we felt that it merited further investigation.

To address this point forty rats were added to the study. Twenty of these rats were food deprived and sacrificed under the same conditions as the control group in the first study, and their brains were prepared for injection as in the original study. The other twenty rats were food deprived and maintained at 80% of their free-feeding weights for ten days. They were then randomly assigned to two groups, (1) saline injection, (2) control brain extract group. These rats were then placed in a BRS/LVE Open-Field Apparatus (Model PAC 001) for 15 minutes, 18, 42 and 66 hours after injection. The total number of counts were recorded for each animal during each session. A two-way ANOVA for repeated measures showed no significant differences between either group at any time after injection. Table 3 shows the mean plus or minus the standard deviation of the mean for these data. The large standard deviations may in part account for the lack of any significant difference between the treatments.

DISCUSSION

The present study was carried out to examine the behavioral effects of small proteins and peptides extracted from the brains of trained rats on naive rats. The results showed that the brain extract from trained donor rats did facilitate learning in the operant chambers. These results support the theory that learning and memory are encoded in specific proteins or peptides which can be transferred between organisms. However, it cannot be concluded that the increase in performance is due totally to a specific behavior-inducing compound, because the increased performance may be due to some non-specific agent which is extracted and injected, or to a non-specific increase in activity. Because of the significant increase in lever pressing by the control-brain extract group at 42 hours after injection, a brief experiment was carried out to determine whether or not this difference was due to an increase in general activity. Using an open-field test we found no significant differences between the saline and control-brain extract treatment. These data show that there was no increase in activity induced by the brain extract, indicating that the findings of the first study cannot be attributed to behavioral activation.

The effect in the main study may be due to a non-specific agent, for example, a more careful study of scotophobin may show that it is an anxiety-inducing substance which causes the rat to "freeze" when placed in the testing chamber, thus avoiding the black compartment and giving a positive score for the rat. This may be taken inadvertently as an indication of learning. On the other hand, anxiolytic agents may be found in the brains of rats trained on an appetitive task, which may reduce the stress placed on the rat in the new situation and thereby stimulate new behaviors.

With respect to this hypothesis, there is an increasing amount of evidence that the brain contains a variety of endogenous compounds which are capable of producing behavioral effects, e.g., the opiate-like endorphins, the hallucinogen N,N-dimethyltryptamine (DMT), adrenocorticotropic hormone (ACTH) and at least two separate classes of endogenous tranquilizers [2]. It may be that in a stressful situation, such as with the scotophobin studies, the brains of the donor subjects have increased levels of an agent which is capable of inducing stress in the recipients, thereby modifying their behavior. The agent could very well be an endorphin-like substance since it has been shown that endorphin levels increase in stressed animals [11]. The hallucinogen DMT has also been shown to be elevated four to twenty-fold in rats given repeated shocks [3]. Similarly, in this present study a tranquilizing-type agent may have been transferred which was specific for a bar-pressing task. DeWied *et al.* have shown that ACTH and peptides related to ACTH can in-

crease the motivational value of environmental stimuli and this increases the probability of stimulus-specific behavioral responses [7].

CONCLUSION

The present study was carried out to examine the effects of small proteins, extracted from the brains of rats trained to press a lever for food reinforcement, on the performance of untrained rats. The results of the data analysis showed that the rats which received the trained donor extracts acquired and performed the task significantly better than the control groups. Therefore, it can be concluded that some form of transfer factor exists for this learning paradigm. However, this study cannot answer the question of the specificity versus the non-specificity of the transfer factor.

REFERENCES

1. Babich, F. R., A. L. Jacobson, S. Bubash and A. Jacobson. Transfer of a response to naive rats by injection of ribonucleic acid extracted from trained rats. *Science* **149:** 656–657, 1965.
2. Barker, S. A., J. M. Beaton, G. B. Brown, S. T. Christian, J. R. Smythies and L. C. Tolbert. Recent advances in the neuropsychopharmacology of behavior: Endogenous hallucinogens and tranquilizers. In: *Psychoneuroendocrinology in Reproduction: An Interdisciplinary Approach*, vol 5, edited by L. Zichella and P. Pancheri. New York: Elsevier/North-Holland Biomedical Press, 1979, pp. 225–234.
3. Beaton, J. M., R. E. Harrison and S. T. Christian. The effects of electrical shock-stress on brain indolealkylamine levels in whole brain synaptosomes from different strains of rat, using gas liquid chromotography-mass spectrometry. 11th Congress C.I.N.P., Vienna, Abstracts, 180, 1978.
4. Braud, L. W. and W. G. Braud. Biochemical transfer of relational responding (transposition). *Science* **176:** 942–944, 1976.
5. Bryant, R. C., W. L. Byrne and N. N. Santos. Synthetic scotophobin in goldfish: specificity and effect on learning. *Science* **177:** 635–636, 1972.
6. Byrne, W. L. and D. Samuel. Behavioral modification by injection of brain extract prepared from a trained donor. *Science* **154:** 418, 1966.
7. DeWied, D. Hormonal influences on motivation, learning, memory, and psychosis. In: *Neuroendocrinology*, vol. 1, edited by D. T. Krieger and J. C. Hughes. New York: HP Publishing Co., 1980, pp. 194–204.
8. Gordon, M. W., G. G. Deanin, H. L. Leonhardt and R. H. Gwynn. RNA and memory: A negative experiment. *Am J Psychiatry* **126:** 1174–1178, 1966.
9. Gross, C. G. and F. M. Carey. Transfer of learned response by RNA injection: Failure of attempts to replicate. *Science* **150:** 1749, 1965.
10. Gutman, N. N., G. Matruyshyn and G. Wariner. Synthetic scotophobin-mediated passive-transfer of dark avoidance. *Nature* **235:** 26–27, 1972.
11. Jacquet, Y. F., W. A. Klee, K. C. Rice, I. Injima and J. Minanikawa. Stereospecific and nonstereospecific effects of (+)- and (−)-morphine: Evidence for a new class of receptors. *Science* **198:** 842–845, 1977.
12. Morris, P. E. and J. M. Beaton. The biochemical transfer via whole brain homogenate injection of an operant task in the rat. *J Ala Acad Sci* **51:** 236, 1980.
13. Rosenblatt, F., and R. G. Miller. Behavioral assay procedures for transfer of learned behavior by brain extracts. *Proc Nat Acad Sci* **56:** 1683, 1966.
14. Ungar, G. Molecular mechanisms in learning. *Perspect Biol Med* **11:** 217–232, 1968.
15. Ungar, G. and C. Ocequera-Navarro. Transfer of habituation by material extracted from brain. *Nature* **207:** 301–302, 1965.