Title
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Publication Date
1977-09-01

Submitted to Brain Research

LBL-6192
Preprint

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September 1977

Prepared for the U. S. Energy Research and
Development Administration under Contract W-7405-ENG-48

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MAZE TRAINING ALTERS BRAIN ANATOMY AND CORTICAL RNA/DNA

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SUMMARY

In order to test whether training leads to anatomical and chemical changes in the brain, individual rats were given self-paced trials in mazes, traversing the maze in order to get from a food station to a water station. In 30 days of this training, during which they had no social interaction, the rats developed significant increases in weight and RNA/DNA of standard samples of cerebral cortex, as compared with littermate rats in either of two control conditions: (a) rats confined to small individual cages (N=76 per condition); (b) rats that traversed the empty maze box with no maze barriers present (N=29 per condition). The cerebral effects of maze experience versus control conditions were similar in pattern but were smaller in magnitude than effects of experience in a social group in a multisensory complex environment. This clear evidence of cerebral changes as consequences of maze training adds further support to the indications that similar cerebral changes resulting from enriched experience are due to learning rather than to other factors. The changes that follow training or enriched experience can be linked with other evidence concerning the roles of RNA and of protein synthesis in the formation of long-term memory traces.

INTRODUCTION

A major approach to studying neural processes in learning and memory has been to give animal subjects differential experience or training and then to look for resultant effects in aspects of brain anatomy and/or brain biochemistry[1,2,15,19,20,23,32,34,37]. In earlier research of this sort, we have found significant cerebral effects of experience in differential environments--enriched, standard colony, or impoverished conditions[2,29]. We have also demonstrated that these effects could not be attributed to other factors in the experiments such as stress, handling, locomotion, earlier maturation, or social stimulation[25,26]. In spite of the clear cerebral effects it produced, the enriched condition (EC) has posed problems for interpretation because of the variety and complexity of stimulation it includes: Rats in EC live in a same-sex group of 10-12 in a large cage, and about 6 varied objects ("toys") are placed in the cage daily from a pool of about 25 objects. In order to test whether clear cerebral effects could be obtained in a simpler and more readily definable situation, we devised the condition that was termed the "Group Maze". In this treatment, a plastic box containing maze barriers is inserted inside an EC cage, and animals must go up and down through the plastic box in order to get food (on the ground floor of the EC cage) and water (located above the ceiling of the inserted plastic box). This procedure yielded clear cerebral effects (as reported

in a preliminary way by Bennett[2] and in detail by Rosenzweig et al.[30], but it still included the complexities of social interaction. In the experiments to be reported here, we therefore took the further step of assigning rats individually to the plastic maze condition to test whether giving maze experience to isolated rats would bring about cerebral differences from littermates housed individually in colony cages; we also compared the magnitude of cerebral effects caused by maze training with those caused by the enriched condition. Two kinds of cerebral measures were used in these experiments because they have been among the most consistent and reliable indices of differential experience in our previous work--tissue weights and the RNA/DNA ratios of brain samples. When positive effects of maze training were found, we then varied the complexity of the maze experience, and we also employed a control condition in which rats traversed an empty box, with no barriers, to get from food to water.

METHODS

Because of limitation of the number of large cages, only six sets of littermate rats were assigned among experimental conditions at a time. In all, 13 experiments were run with six rats per condition (total Ns per experiment of 18, 24, or 30). The experiments can be divided into three sets according to the environmental conditions included, as described below. The behavioral phase of the 13 experiments was conducted from September 1975 through April 1977.

Subjects

Subjects were male rats of the Berkeley S_1 line bred in the Department of Psychology colony. In most experiments they were assigned to the conditions at either about 30 or about 60 days of age, but in one case age at assignment was 90 days. Depending upon the number of conditions included in an experiment--3, 4, or 5--we chose litters with at least 3, 4, or 5 male animals. Runts were excluded, and, as a further restriction on variability, we took only sets of littermates in which the range of body weights within littermates did not exceed 15% at the time of assignment to conditions. The littermates were then assigned randomly to conditions so that each animal of a litter went to a different experimental condition.

Environmental conditions

All 13 experiments included the following three conditions: (a) Enriched Condition (EC). In EC, 12 male animals are housed in a relatively large cage (75 x 75 x 45 cm) which is furnished with about 6 varied stimulus objects. Several EC cages are set up adjacent to each other, and each day the animals are moved from one cage to another; after several days the stimulus objects are changed in all cages so that the animals will be exposed to new objects and new combinations of objects. Since the six litters for an experiment would furnish only six animals for this condition, another six

animals from other litters were used as "fillers"; these extra animals were not used for the brain analyses. (b) Impoverished Condition (IC). This, like EC, is a standard condition used in many previous experiments. The IC rats live in individual cages (32 x 20 x 20 cm) in a separate isolation room. (c) Individual in Complex Maze (I-CM). This condition employed a plastic box inserted to provide two additional floor levels in an EC cage. The maze box was made of clear Plexiglas and measured 10 cm high x 7¼ cm wide x 7¼ cm deep; it was placed within an EC cage on flanges that supported it 15 cm above the cage floor. Holes 7 cm in diameter were placed at the four corners of the bottom and top of the plastic box, so that the rats could crawl into and out of it; any of these holes could be closed with a plastic door when desired. Plastic barriers could be placed within the box to provide a variety of maze patterns. Food pellets were made available, as in EC, on the floor of the cage, but the water bottle was placed above the plastic box so that to get from food to water the rat had to climb into the plastic box at an open corner in the bottom, traverse the box to an open corner at the top, climb out of the box and stand on its top to reach the spout of the water bottle. The following pretraining schedule was established: On day 1, the rat was placed into the cage without the plastic box present, and both food and water were available on the floor. On day 2, the top of the plastic maze (that is, a plastic sheet with holes at the corners) was placed on the brackets, and

the water bottle was placed above it, so that in order to reach the water the rat had to climb through any of the four corner holes and stand on the sheet of plastic. On day 3, the maze box was put into place with all bottom and top holes open; the maze contained a simple pattern of barriers. On day 4, only one bottom and one top hole were left open. For the next 29 days, the pattern of barriers was changed daily. Six I-CM cages were set up adjacent to each other with six different maze patterns; the animals were moved from one cage to another each day, and at the end of each sixth day, all maze patterns were changed. (Examples of the maze patterns used are shown in Rosenzweig et al.[30], Figure 1.) Experiments 1-4, which included only the EC, I-CM, and IC conditions, comprised Experimental Set #1.

Experiments 5-8 (Experimental Set #2) included not only the three conditions described above but also the following condition: (d) Individual in Simple Maze (I-SM). This condition is like I-CM with two exceptions: The animal remains in the same cage throughout the experiment, and the same simple pattern of barriers that was introduced on day 3 is maintained throughout so that the animal is not exposed to a variety of maze patterns. (The maze pattern is shown in Rosenzweig et al.[30], Figure 1.)

Experiments 9-13 (Experimental Set #3) included conditions EC, I-CM, IC, and also the following two conditions: (e) The Group Condition (GC). This condition is like EC except that no stimulus

objects are placed in the cages. As in EC, the GC are moved from one cage to another each day. As in the case of EC, six additional "filler" rats were added to bring the number in GC up to 12.

(f) Individual in Empty Box (I-EB). This condition is like I-SM except that the Plexiglas box is empty, not containing any maze barriers.

Animals in all six experimental conditions have food and water available ad libitum. In Experimental Sets 2 and 3, all animals were weighed daily; this insured that animals in I-SM, I-EB, and IC were handled just as were those of the other groups that were moved from one cage to another daily.

Brain dissection

At the end of the behavioral phase of an experiment, the animals were put into a multiple-unit cart bearing code numbers that did not reveal the experimental condition of any rat. Each animal was decapitated and the brain was dissected by our standard procedures. Using a calibrated T-square, we removed standard samples of occipital and somesthetic cortex from both hemispheres. The other brain sections were the following: remaining dorsal cortex; ventral cortex, including the hippocampus and corpus callosum; cerebellum and medulla; and remaining subcortical brain, including the olfactory bulbs. As soon as each sample was removed, it was weighed to the nearest 10th of a milligram on an automatic balance. The samples were then frozen

on dry ice and stored at -30° C for subsequent chemical analysis. Measures from all of the cortical sections were combined to give Total Cortex; measures from the cerebellum and medulla and remaining subcortical brain were combined to give Rest of Brain (Subcortex).

Analysis of RNA and DNA

Analyses of RNA and DNA were made according to procedures developed recently in our laboratories and described in detail by Morimoto et al.[21] and summarized in Rosenzweig et al.[30]. The procedure involves precipitation of the nucleic acid from a homogenate of brain tissue, separation of the RNA from the DNA and their subsequent spectrophotometric determination based on absorption at 260 and 266 nm respectively. Analyses for the larger tissue sections are routinely made in duplicate; values of duplicate assays differ on the average by 2.5% in the case of RNA and by 4.0% in the case of DNA.

RESULTS

Maze Learning

Formal measures of maze learning were not taken in Experiments 1-13, but observations of the animals during routine maintenance made it clear that they learned the maze patterns very well during the course of a day. For example, when an experimenter would open a cage to remove a rat, sometimes it would jump down from the top of the plastic box into the maze, run rapidly through the correct path, and emerge at

the bottom within a few sec of leaving the top. After completing these experiments, we made observations on a further set of 6 rats assigned to the I-CM situation. On some days these rats were removed from the large cages at the end of the afternoon and placed in small individual cages without food or water. The next morning each rat was replaced in an I-CM cage with a new pattern of barriers, and during the first 60 minutes records were taken of time of entry and emergence from the plastic box. During 60 minutes, the rats (starting out deprived of food and water) traversed the maze an average of 11 times. Time required to traverse the maze decreased over the first several trials. In the case of simpler patterns, a rat could run the maze in as little as 2 sec. With the harder patterns, rats brought the median time down from 25 sec on the first complete trial (sometimes after a few exploratory partial runs) to 8 sec by the fourth trial.

Cerebral Effects, Overall Maze Learners

The 13 experiments showed closely similar results in regard to the three main conditions--EC, I-CM, and IC--so the data from all experiments were combined for overall statistical analysis. Two animals died during the course of the experiment, so the results of their littermates were not used, and the data are based on 76 animals per condition. Some of the main results are presented in Table I in the form of percentage differences between conditions; p values are

based on analyses of variance and Duncan's multiple range test. Table II gives absolute values of tissue weight and of RNA and DNA for occipital cortex and total cortex of the same animals, and it shows the small sizes of standard deviations for these measures. (A preliminary report of these experiments was made by Bennett et al.[5].)

Column 1 of Table I shows that the percentage differences between mean values of the EC and IC littermates conformed to the pattern of results found in previous experiments (see, for example, Table II in Rosenzweig et al.[30]). That is, all of the cortical sections showed highly significant EC-IC differences in tissue weights, with the difference in the occipital cortex being larger than those of the other cortical areas. In occipital cortex, the EC rat showed greater tissue weight than its IC littermate in 61 of the 76 litters (80% of the comparisons). The Rest of Brain (Subcortex) showed very little effect, and the cortical/subcortical weight ratio was highly significant. The values of the I-CM animals also differed significantly from those of their IC littermates in all cortical regions except for somesthetic cortex. In occipital cortex, the I-CM rat exceeded its IC littermate in 56 of the 76 litters (76% of the cases). There was no difference between I-CM and IC rats in Rest of Brain, but the difference in cortical/subcortical ratios was highly significant. The weight values of the I-CM rats were consistently lower than those of their EC littermates and were significantly lower in the case of the somesthetic cortex and remaining dorsal cortex. The magnitude of differences from the IC baseline is seen to run in parallel from region to region of the brain for the I-CM and EC rats.

In weight of total brain (not shown in Table I), EC rats exceeded their IC littermates by 2.4% ($p < .001$) and I-CM rats exceeded IC rats by 1.3% ($p < .01$). In terminal body weight, IC rats were slightly greater than those in the other conditions, but only in the case of the EC condition was the difference in body weights significant (3.4%, $p < .01$); thus, the greater brain weights of rats in the EC or I-CM conditions could not be accounted for by body weight differences since their body weights were lower than those of rats in IC.

Analyses of RNA and DNA were restricted to the following brain regions--occipital cortex, the combination of somesthetic and remaining dorsal cortex, and ventral cortex. Since the Subcortex had not shown any significant effects in RNA or DNA in previous experiments with differential experience, the Subcortex was not analyzed in these experiments. Table I shows that in the RNA/DNA ratio rats in EC and in I-CM differed significantly from littermates in IC, especially at the occipital cortex. In occipital cortex, the EC rat showed a greater RNA/DNA ratio than its IC littermate in 68 of the 76 litters (89% of the comparisons), and the I-CM rat exceeded the IC littermate on this measure in 65 cases (86% of the comparisons). These percentages for the RNA/DNA measure are somewhat greater than the comparable percentages for tissue weights. The EC versus IC effect in RNA/DNA was significantly larger than the I-CM versus IC effect in both the combination of somesthetic and remaining dorsal cortex and in total cortex.

The increases in RNA/DNA of rats in either EC or I-CM, as compared with their IC littermates, were principally due to increases to total RNA, since total DNA remained essentially identical among rats in all treatments. Table II shows values for RNA, DNA, and tissue weight for both occipital cortex and total cortex for the EC, I-CM, and IC conditions; means and standard deviations are shown, and significances of differences between EC and IC rats and between I-CM and IC littermates are given. As tissue weight increases with enriched experience (in both the EC and I-CM treatments), DNA per unit of weight shows almost proportionate declines. That is, since DNA exists in constant amounts in the nucleus of each cell, as the neurons increase in size and show growth in their extensions with enriched experience, the weight of the cortex increases but the number of neural cells does not. Thus, the number of neurons per unit of volume (or per unit of weight) decreases, and so does DNA per unit of weight. There may, however, be a small increase in the number of glial cells in the cortex as a consequence of enriched experience, as we have reported previously[9]; this may be the reason for the observed increases of total DNA in total cortex. While DNA/weight became significantly lower in the EC or I-CM conditions, RNA/weight did not decline. RNA is not fixed in amount per cell and can increase in response to functional demands. Total RNA became significantly greater in EC or in I-CM than in IC; this was found not only for occipital and total cortex, as shown in Table III, but also in the two other cortical sections analyzed that are not given in the table.

Cerebral effects as function of age

Among the 13 experiments, there were five in which the animals were assigned to differential conditions at about 30 days of age (range, 26-35 days), that is, about one week after weaning; in five other experiments the starting age was about 70 days (range, 60-77), that is, well beyond the age of sexual maturity of the rat. The cerebral effects of differential experience were analyzed separately for these two age groups. Table III reveals that significant effects of enriched experience and of maze training occurred at both ages; moreover, the magnitudes of the effects and their patterns of distribution among regions of the cortex were similar for the two ages. We have previously shown that many cerebral effects of enriched experience (EC) versus colony (SC) or restricted experience (IC) are not limited to the immediate postweaning period and, in fact, can even be found in year-old rats[2,22,31]. The cerebral effects of maze training (I-CM) are now also seen to occur as readily in postpubescent as in weanling rats.

Experimental Set #2

After completing the initial set of experiments which included only the EC, I-CM and IC treatments, we ran the four experiments of Experimental Set #2 which also included the condition of Individual in Simple Maze (I-SM). This condition with its rather simple and unvarying maze pattern was included in order to test whether a less

demanding learning situation than I-CM would cause smaller cerebral changes than I-CM. The results of four experiments, with durations ranging from 32 to 35 days and starting ages ranging from 30 to 90 days, were closely similar, so the data were combined for overall statistical analyses; one animal died during the course of an experiment, so the results are based on 23 animals per condition. Some of the main results are presented in Table IV in the form of percentage differences between mean values of IC rats and those from the other conditions; p values are based on analyses of variance and Duncan's multiple range test.

In tissue weight measures, the results for I-SM rats were found to differ significantly from those of the IC littermates in each region where I-CM versus IC showed significant differences. The I-SM values are typically smaller than the I-CM values, but results for these two conditions did not differ significantly in any of the measures of tissue weights.

On the RNA/DNA measure, the I-SM treatment was relatively less effective than in the case of tissue weights. Whereas I-CM produced highly significant differences from IC in RNA/DNA of occipital cortex, somesthetic plus remaining dorsal cortex, and total cortex ($p < .01$ for each of these), I-SM gave only indications of differences from the IC treatment ($p < .10$ for occipital cortex and for total cortex). In the case of occipital cortex, the I-CM versus IC effect was

significantly greater than the I-SM versus IC effect ($p < .05$), and in total cortex the I-CM effect tended to be larger than the I-SM effect ($p < .10$).

Experimental Set #3

Experiments 9-13 included not only conditions EC, I-CM, and IC, but also the Group Condition (GC) and the treatment of Individual in Empty Box (I-EB). GC was included in order to test the relative effectiveness of stimulation of the social group as compared with that of maze training (I-CM) or EC. Since the I-CM treatment had been found in the previous experiments to produce significant effects on brain weights and brain RNA/DNA, the I-EB treatment with no maze barriers in the Plexiglas box was included as a control condition. Four of these experiments were run with sets of five littermates assigned as described above, and the last was run with weight-matched groups that were treated as litters. In three experiments the animals were assigned to conditions at about 30 days of age, in one at 60 days, and in one at 73 days of age.

The five experiments all gave rather similar results, so that the data were combined for overall statistical analyses with an N of 29 per condition (one animal having died during the course of an experiment). Some of the main results of Experimental Set #3 are shown in Table V.

The social stimulation of housing 12 animals in a group in a large cage (GC) was found to be about as effective in producing

cerebral changes as giving individual animals maze training (I-CM). The EC treatment produced larger brain values than did the GC treatment, and some of these differences were statistically significant. Thus, the EC group exceeded the GC group in the cortical/subcortical weight ratio ($p < .01$) and in RNA/DNA of occipital cortex and of somesthetic plus remaining dorsal cortex ($p < .05$).

In contrast to the effectiveness of the other conditions, requiring rats to traverse an empty plastic box between food and water sources in the I-EB treatment was almost completely ineffective in altering brain values. The sensitive cortical/subcortical weight ratio was the only measure to show a significant difference between I-EB rats and their IC littermates ($p < .05$). The I-CM treatment produced a significantly greater effect than the I-EB treatment on the cortical/subcortical weight ratio, and indeed on most of the measures in Table V, so that the addition of maze barriers to the plastic boxes was sufficient to produce clearly significant cerebral effects in both tissue weights and RNA/DNA.

DISCUSSION

The findings of these experiments appear to offer stronger and more clearcut evidence than has been heretofore available to support the hypothesis that learning leads to measurable changes in the mammalian brain. The evidence for cerebral changes is strong in these

results because differences between rats that had to traverse maze patterns (I-CM) and littermates that passed their time in colony cages (IC) were highly significant and replicable for both cortical weights and cortical RNA/DNA. Furthermore, these experiments are based on substantial numbers of subjects (N=76 per condition for all experiments combined), and the 13 experiments each showed results closely similar to the overall results.

The evidence for cerebral changes with learning appears to be particularly clearcut because many of the factors that have caused difficulties in interpretation in other experiments were controlled or eliminated in these experiments. Thus, the cerebral differences that develop between rats in EC and in IC can be attributed in part to the social stimulation in EC, and we found that even the Group Condition leads to significant cerebral effects, although smaller than those caused by EC. But rats in I-CM, like those in IC, receive no social stimulation throughout the course of the experiment, so the social factor can play no role in determining the cerebral differences between rats in I-CM versus littermates in IC. Motivational processes have often been indicated as alternatives to an explanation in terms of learning. Thus, it might be suggested that rats that have to climb into and out of the plastic maze box might therefore eat less, which could affect their brain measures, but the overall I-CM mean in terminal body weights was found to differ from the IC mean by only 1.2% (NS). Even so, it might be claimed that the rats in I-CM might

experience some motivational effect of having to run back and forth between their sources of food and water and that this might be the cause of the observed cerebral effects. If this explanation were correct, then cerebral effects should also have been observed in rats that had to climb into and out of and traverse an empty plastic box to get from food to water--the I-EB condition; but, as we have seen, rats in I-EB did not develop significant cerebral differences from littermates in IC, although they did develop significant differences from the rats in I-CM. Ferchmin and Eterovic[11] have recently made a similar observation; they found that requiring rats to climb the side of a cage and hang upside down while they gnawed on food pellets did not alter cortical weight or cortical RNA/DNA, whereas they found that giving the EC treatment to other rats at the same time was effective in changing brain measures. Handling is another treatment that has been demonstrated to produce some physiological and behavioral effects in rodents[8], and it had been suggested as a possible source of differences in EC-IC experiments. We have shown that daily handling of rats does not produce cerebral differences on the brain measures we have taken[26,28]. In the present experiments, since rats in I-CM were moved from one cage to another daily, we also removed each rat in I-SM, I-EB, and IC daily from it cage to weigh it, so that all rats received daily handling.

The fact that the presence or absence of maze barriers led to significant differences in cortical weights and RNA/DNA between rats

in I-CM or in I-EB is powerful evidence that learning leads to cerebral effects. A further test of the hypotheses could be made by seeing whether graded amounts of training lead to graded cerebral effects. For this test we exposed some rats to a simple and unvarying pattern of barriers in the I-SM condition, and they also developed significant cerebral differences from rats in IC. While the rats exposed to the varied and more complex maze patterns in I-CM tended to differ somewhat more from their IC littermates than did the rats in I-SM, the only significant differences between I-CM and I-SM rats were in RNA/DNA of occipital cortex (3.2%, $p < .05$) and of total cortex (1.0%, $p < .10$). It should also be recalled that whereas rats in I-CM were moved from one cage to the next each day, rats in I-SM remained in the same cage throughout; experience in different cages is not therefore required to produce the brain differences found between rats in I-SM and those in IC. It appears that even a rather simple maze pattern provides a challenge to the rat sufficient to cause cerebral responses. Use of even simpler maze patterns is therefore indicated in future work on this question.

The similarity of the pattern of cerebral effects induced by experience in EC with that induced by the I-CM condition, when comparison is made with baseline brain values that develop in IC, lends further support to the position that much if not all of the EC effects reflect learning, as has been argued previously from other

evidence[3,15,26,28]. If this interpretation of the EC effects can be established, it will be of benefit both because many findings with a variety of brain measures have already been made using the EC treatment and because the EC treatment with 10-12 animals housed in a cage is more economical in terms of space and experimenter time than is the I-CM treatment with individual rats occupying large cages. Thus the study of effects of the I-CM treatment may allow investigators to return with renewed confidence to using the EC treatment.

The fact that cerebral differences between rats in I-CM and rats in IC could be induced as readily in postpubescent as in weanling animals deserves comment. We have previously pointed out that the effects of differential experience (EC) on brain measures are not limited to a "sensitive period" early in the life of the animal, as are the effects of severely restricted or distorted stimulus input on sensory development reported by such investigators as Hubel and Wiesel[17,18,36] and Blakemore and Mitchell[6]. We have also suggested that the fact that cerebral effects of differential experience can be induced throughout the life span seems to make them a better model for studying neural processes of learning and memory than are the effects of sensory restriction or distortion[24,27]. Now we believe that our position is reinforced by the finding that cerebral effects of maze learning, as well as of enriched experience, appear just as clearly among rats that start their training as young adults as among those that begin as weanlings.

Biosynthetic steps in formation of long-term memory traces

What are some of the main biosynthetic steps that are involved in laying down the neural substrates for storage of long-term memories? A number of investigators have suggested that the receipt of information in neurons can lead to derepression of DNA and to the transcription of appropriate molecules of RNA[7,13,33]. While we agree that this is a likely sequence of events in many cases, we have presented evidence that this sequence is not obligatory; in cases of strong training, synthesis of proteins involved in memory storage may involve the stimulated utilization of certain already existing molecules of RNA[4,24]. Clear supporting evidence for greater synthesis of RNA is the earlier finding of significantly higher RNA/DNA ratios in enriched-experience rats than in their colony or impoverished-experience littermates[2,12] and the present finding of increase of RNA/DNA caused by maze training. Another type of evidence of difference in brain function as a consequence of experience is the finding that enriched experience appears to lead to fuller expression of the genomes; that is, rats placed in EC for 30 days show 30% greater diversity in types of RNA in brain than do littermates that were in IC for the same period of time[16]. The interpretation of these results is not yet clear; that is, experiments to date have not yet distinguished whether the changes in sequence diversity reflect an increase in the number of copies of certain RNA species in the EC rats or whether the increase in diversity reflects an increase in the number of species of RNA transcribed. In either case, the magnitude

of the observed difference in brain RNA populations suggests a significant difference in brain function between EC and IC animals.

Some of the ways in which the altered synthesis of protein is expressed probably include chemical and anatomical changes such as increased branching of dendrites[15], increased numbers of dendritic spines[14], and enlarged synaptic receptor areas[10,35]. This is by no means an exhaustive listing; changes suggested by other investigators--such as alteration in glycoproteins--may well fit into the larger picture of cerebral modifications that store memories.

Now that self-paced maze trials have been found to lead to measurable cerebral effects that are presumably related to learning and memory storage, a number of questions should clearly be taken up in further research: What is the time course of these cerebral changes? What is their more detailed distribution in the brain? What effects will be found in more detailed anatomical measures such as dendritic branching, dendritic spine counts, synaptic size and number?

ACKNOWLEDGMENTS

This research was partially supported by ADAMHA grant R01MH26704 and it received support from the Division of Biomedical and Environmental Research of the U.S. Energy Research and Development Administration through the Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory. We wish to thank animal technicians Donald Dryden and Kenneth Chin for their careful work with the rats in the differential environments, Marie Hebert for the skillful dissection of the rat brains and for compiling and analyzing statistically much of the data, and Hiromi Morimoto for performing the analyses of RNA and DNA.

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Table I

Percentage Differences in Weight and RNA/DNA of Brain Samples Among Rats in 3 Conditions (N = 76 per condition)

	Tissue Weights			RNA/DNA		
	EC vs. IC	I-CM vs. IC	EC vs. I-CM	EC vs. IC	I-CM vs. IC	EC vs. I-CM
Cortex						
Occipital	6.0****	4.6****	1.4	6.4****	5.3****	1.1*
Somesthetic	3.6****	0.4	3.2****	--	--	--
Rem. Dorsal	4.8****	2.0***	2.7****	--	--	--
Somesthetic + Rem. Dorsal	4.6****	1.8***	2.8****	3.8****	1.8****	1.9****
Ventral	4.7****	3.9****	0.7	0.4	0.9*	-0.5
Total	4.8****	2.9****	1.8****	2.8****	1.8****	0.9***
Rest of Brain	0.7	0.1	0.6	--	--	--
Cortex / Rest of Brain	4.1****	2.9****	1.2****	--	--	--
Terminal Body Weight	-3.4***	-1.2	-2.2*	--	--	--

*$p < .10$, **$p < .05$, ***$p < .01$, ****$p < .001$.

Table II

Mean of Cortical Weights, RNA and DNA for Rats in EC, I-CM, and IC, and Significance of Differences from IC Values

(N = 76 per condition)

		Wt(mg)	RNA/wt[a]	DNA/wt[a]	Total RNA(μg)	Total DNA(μg)	RNA/DNA
A. Occipital Cortex							
EC	$\bar{X}$	77.6****	158.9**	104.6****	123.3****	81.2	1.522****
	SD	±5.1	±5.7	±5.4	±9.5	±7.0	±.078
I-CM	$\bar{X}$	76.5****	158.7**	105.6****	121.4****	80.9	1.506****
	SD	±4.8	±6.4	±4.7	±8.2	±6.6	±.070
IC	$\bar{X}$	73.2	157.2	110.1	115.1	80.7	1.430
	SD	±4.1	±6.5	±5.6	±8.7	±6.9	±.062
B. Total Cortex							
EC	$\bar{X}$	709.3****	151.4	100.7***	1074****	714****	1.508****
	SD	±29.1	±3.8	±2.9	±46	±34	±.042
I-CM	$\bar{X}$	696.6****	151.2	101.5****	1053****	707**	1.494****
	SD	±27.3	±3.2	±2.9	±42	±35	±.040
IC	$\bar{X}$	677.0	150.8	103.1	1021	698	1.468
	SD	±25.2	±2.9	±2.8	±41	±34	±.035

[a] μg/100mg

* $p < .10$, ** $p < .05$, *** $p < .01$, **** $p < .001$

Table III

Comparison of Effects of Differential Experience Begun at 30 or at 70 Days of Age; Percentage Differences

	30-64 day experiments (N = 29 per condition)			70-104 day experiments (N = 30 per condition)		
	EC vs. IC	I-CM vs. IC	EC vs. I-CM	EC vs. IC	I-CM vs. IC	EC vs. I-CM
A. Tissue Weight						
Occipital Cortex	5.5**	3.9*	1.5	6.3***	4.8**	1.5
Total Cortex	6.0***	3.5***	2.4**	4.1***	2.6***	1.5*
Cortex/Rest of Brain	5.0***	3.5***	1.4**	4.1***	2.8***	1.3*
B. RNA/DNA						
Occipital Cortex	5.6***	5.6***	0.1	8.1***	5.4***	2.6***
Total Cortex	2.6***	1.6**	1.0*	3.0***	2.4***	0.6

* $p < .05$, ** $p < .01$, *** $p < .001$

Table IV

Percentage Differences in Weight and RNA/DNA of Brain Samples Among Rats in Four Conditions, Experimental Set #2

(N = 23 per condition)

	Tissue Weights			RNA/DNA		
	Occipital Cortex			Occipital Cortex		
	I-CM	I-SM	IC	I-CM	I-SM	IC
EC	2.6**	3.6**	8.5****	0.4	3.6***	6.1****
I-CM		1.0	5.8****		3.2**	5.7****
I-SM			4.8***			2.4*
	Somesthetic Cortex					
EC	4.1**	3.8**	3.9**			
I-CM		-0.3	-0.2			
I-SM			0.1			
	Remaining Dorsal Cortex			Somesthetic + Remaining Dorsal Cortex		
EC	3.9***	4.2***	7.8****	1.2	2.2**	3.6***
I-CM		0.2	3.7**		1.0	2.4***
I-SM			3.5**			1.3
	Ventral Cortex			Ventral Cortex		
EC	0.6	0.9	4.8***	0.7	1.1	1.6
I-CM		0.3	4.2**		0.4	0.9
I-SM			3.9**			0.5

Table IV (continued)

	I-CM	I-SM	IC	I-CM	I-SM	IC
		Total Cortex			Total Cortex	
EC	2.5**	2.8***	6.4****	1.0*	2.0***	3.2****
I-CM		0.3	3.8****		1.0*	2.2****
I-SM			3.5***			1.2*
		Rest of Brain				
EC	1.2	0.9	2.0*			
I-CM		-0.2	0.8			
I-SM			1.1			
		Cortex/Rest of Brain				
EC	1.2*	1.8**	4.3****			
I-CM		0.6	3.1****			
I-SM			2.5***			

* $p < .10$, ** $p < .05$, *** $p < .01$, **** $p < .001$

Table V

Percentage Differences in Weight and RNA/DNA of Brain Samples Among Rats in Five Conditions

(N = 29 per condition)

	Tissue Weights				RNA/DNA			
	I-CM	GC	I-EB	IC	I-CM	GC	I-EB	IC
			Occipital Cortex				Occipital Cortex	
EC	1.6	1.4	6.2****	6.2****	1.0	2.4**	4.5****	6.1****
I-CM		-0.2	4.6***	4.5***		1.4	3.5***	5.0****
GC			4.8***	4.7***			2.0*	3.6***
I-EB				-0.1				1.5
			Somesthetic + Remaining Dorsal Cortex				Somesthetic + Remaining Dorsal Cortex	
EC	2.5**	0.3	4.1****	4.3****	2.0***	1.4**	3.7****	3.7****
I-CM		-2.2**	1.6	1.8		-0.6	1.6**	1.6**
GC			3.8****	4.0****			2.2***	2.2***
I-EB				0.2				0.0
			Ventral Cortex				Ventral Cortex	
EC	1.3	3.4**	3.5**	5.3****	-1.5*	-0.5	0.5	0.5
I-CM		2.1	2.2	3.9***		1.0	2.1**	2.1**
GC			0.0	1.8			1.1	1.0
I-EB				1.7				0.0

Table V (continued)

	I-CM	GC	I-EB	IC	I-CM	GC	I-EB	IC
			Total Cortex				Cortal Cortex	
EC	1.9**	1.6*	4.1****	4.9****	0.5	0.7	2.5****	2.6****
I-CM		-0.4	2.1**	2.9***		0.2	2.0****	2.1****
GC			2.5***	3.2****			1.9****	2.0****
I-EB				0.7				0.1
			Cortex/Rest of Brain					
EC	1.1**	1.7***	3.0****	4.3****				
I-CM		0.6	1.9***	3.2****				
GC			1.2**	2.5***				
I-EB				1.3**				

* $\underline{p} < .10$, ** $\underline{p} < .05$, *** $\underline{p} < .01$, **** $\underline{p} < .001$

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