JOURNAL OF NEUROBIOLOGY, VOL. 7, NO. 1, PP. 75–85

Effects of Environment on Morphology of Rat Cerebral Cortex and Hippocampus

MARIAN C. DIAMOND, CAROL A. INGHAM, RUTH E. JOHNSON, EDWARD L. BENNETT, and MARK R. ROSENZWEIG

Department of Physiology–Anatomy, Laboratory of Chemical Biodynamics, and Department of Psychology, University of California, Berkeley, California 94720

INTRODUCTION

Previous reports have shown that the depth of the cerebral cortex can be modified if rats are placed in their respective environments for 30 or 80 days with varied starting ages (Diamond, Rosenzweig, Bennett, Lindner, and Lyon, 1972). For example, some rats entered their environments at weaning (at 25 days of age) and remained until 55 or 105 days of age; others entered at 60 days and remained until 90 days, and some others entered at 105 days and remained until 185 days. The depth of the cortex was altered significantly in some or in all regions in the above experiments. We have now extended this research to ask the following question: Is it possible to bring about a morphological change in the cortex in less than a 30 day period? In order to answer this question, the first part of the present report examines the cortical depth from rats in their different environments for periods shorter than 30 days.

Both the cortex and hippocampus are involved in learning, so having made cortical measures, it is now pertinent to look at the hippocampus. If the hy-

pothesis is offered that during exposure to an enriched environment, some type of learning is taking place, and if one utilizes the learning hypothesis offered by Glassman and Wilson (1972), indicating possible circuitry through the hippocampus, then morphological changes might occur in the hippocampus as well as in the cerebral cortex as a consequence of enriching the environment. In fact, a report by Walsh, Budtz-Olsen, Penny, and Cummins (1969) indicated, in eight pairs of rats, a 6% ($p < .05$) difference in hippocampal depth between animals exposed to an enriched environment compared to an impoverished environment. With this encouraging preliminary report, a more extensive study of hippocampal depth measures was undertaken in the present investigation, examining the hippocampus in 31 pairs in the same age group as Walsh et al. (1969) and 193 from other groups, giving a total of 224 pairs of rats.

and the occipital or visual (V) cortex, section 9, were cut, utilizing subcortical landmarks to insure uniform sampling. (For specific landmarks see Diamond et al., 1972, sections 2, 5, and 9.) A modified thionin stain was used (Windle, Rhines, and Rankin, 1943). The outlines of the brain sections were traced from a microslide projected image as previously reported (Diamond, Law, Rhodes, Lindner, Rosenzweig, Krech, and Bennett, 1966; Diamond, 1967). Much care was taken to demarcate the boundaries of the cerebral cortex, the hippocampus, and the diencephalon. Figure 1 designates the position of the lines measured to determine the dimensions of the cortex (Diamond et al., 1966) and the hippocampus. Cortical depth measures were taken on both the right and left hemispheres and the results were reported in two ways: as the mean of the segments from both hemispheres and separately for each hemisphere. The hippocampal depths were read from sections at the level of the occipital cortical section (V), and again a single value was obtained from the means of both the right and left hippocampus, even though each was measured separately. Only when all measures were completed were the environmental conditions made known to the anatomists.

Cortical depths were studied on brains from rats in their environments for 15 days, from 25 to 40 days; for seven days, from 25 to 32 days; for four days from 26 to 30 days, and from 60 to 64 days; for one day from 60 to 61 days. In Table 1, cortical depth measures are presented for a total of 266 pairs of rats. The hippocampal depth studies were made on tissues from the 113 pairs used in short-term cortical depth studies as well as those from 30 day experiments from 25 to 55 days and from 60 to 90 days; from 80 day experiments from 25 to 105 and 105 to 185 days;

TABLE I
Percent Differences in Cortical Depth between Enriched and Impoverished Animals

Age and Duration, days	Cortical area	EC $\bar{x}$[a] SD	IC $\bar{x}$ SD	% Diff.[b]	EC > IC	*P*
I. 60–61	V_B	2.86 ± .13	2.88 ± .11	0.7	10/24	NS
	C	2.92 ± .13	2.91 ± .11	0.3	14/24	NS
	D	3.28 ± .14	3.23 ± .10	1.2	15/24	NS
II. 60–64	V_B	2.83 ± .10	2.75 ± .10	2.9	27/36	<.001
	C	2.84 ± .12	2.79 ± .10	1.8	26/36	<.05
	D	3.19 ± .12	3.14 ± .12	1.6	24/36	<.10
III. 26–30	M_B	4.55 ± .17	4.47 ± .18	1.8	12.5/19	NS
	C	4.54 ± .16	4.51 ± .18	0.7	11/19	NS
	S_B	4.38 ± .16	4.24 ± .18	3.5	15/19	<.01
	C	4.28 ± .19	4.22 ± .18	1.5	12/19	<.10
	D	3.78 ± .19	3.73 ± .17	1.2	11/19	NS
	V_B	2.93 ± .14	2.83 ± .08	3.7	14/19	<.001
	C	2.89 ± .15	2.77 ± .12	4.4	14/19	<.001
	D	3.28 ± .16	3.16 ± .12	3.8	13.5/19	<.01
IV. 25–32	M_B	4.68 ± .23	4.56 ± .18	2.7	17/22	<.01
	C	4.72 ± .21	4.61 ± .15	2.3	16/22	<.01
	S_B	4.50 ± .22	4.41 ± .16	2.1	18/22	<.05
	C	4.45 ± .21	4.34 ± .19	2.6	16/22	<.01
	D	3.89 ± .20	3.82 ± .16	1.7	13/22	<.05
	V_B	2.99 ± .10	2.88 ± .12	3.8	19.5/22	<.001
	C	2.91 ± .16	2.79 ± .13	4.0	17/22	<.001
	D	3.29 ± .16	3.21 ± .12	2.5	16.5/22	<.05
V. 25–40	M_B	4.44 ± .20	4.27 ± .20	3.9	17/23	<.001
	C	4.48 ± .19	4.37 ± .20	2.7	16/23	<.01
	S_B	4.22 ± .22	4.12 ± .21	2.3	15.5/23	<.05
	C	4.17 ± .22	4.10 ± .21	1.9	14.5/23	<.10
	D	3.61 ± .23	3.50 ± .23	3.1	15/23	<.05
	V_B	2.79 ± .13	2.60 ± .13	7.1	21/23	<.001
	C	2.77 ± .15	2.61 ± .15	6.2	20/23	<.001
	D	3.15 ± .17	3.01 ± .16	4.7	16/23	<.01
VI. 25–55	M_B	3.14 ± .14	3.08 ± .13	2.0	19/31	NS
	C	3.25 ± .11	3.20 ± .12	1.7	21/31	NS
	S_B	3.00 ± .10	2.01 ± .12	2.8	22/31	<.01
	C	3.03 ± .08	2.97 ± .11	2.2	22/31	<.01
	D	2.66 ± .09	2.62 ± .11	1.4	19/31	NS
	V_B	1.98 ± .10	1.86 ± .09	6.6	25/30	<.001
	C	2.04 ± .09	1.94 ± .10	5.3	24/30	<.001
	D	2.24 ± .08	2.18 ± .10	2.7	20.5/30	<.01
VII. 60–90	M_B	3.31 ± .12	3.24 ± .14	2.1	33.5/50	NS
	C	3.50 ± .15	3.46 ± .13	1.1	14.5/28	NS
	S_B	2.95 ± .08	2.82 ± .10	4.5	43.5/50	<.001
	C	3.06 ± .09	2.95 ± .09	3.5	38.5/50	<.001
	D	2.80 ± .11	2.72 ± .09	2.7	37/50	<.001
	V_B	1.94 ± .06	1.82 ± .07	6.7	45.5/50	<.001
	C	2.04 ± .06	1.92 ± .08	6.4	47/50	<.001
	D	2.18 ± .06	2.06 ± .09	5.5	39/50	<.001
VIII. 25–105	M_B	3.10 ± .12	3.01 ± .18	3.0	7/11	NS
	C	3.25 ± .12	3.17 ± .15	2.5	7.5/11	NS
	S_B	2.89 ± .11	2.86 ± .13	1.0	14/22	NS
	C	3.04 ± .09	2.97 ± .13	2.4	18.5/22	<.01
	D	2.89 ± .13	2.81 ± .14	2.8	15/22	<.1
	V_B	1.87 ± .08	1.74 ± .07	7.5	22/22	<.001
	C	1.98 ± .07	1.90 ± .09	4.2	17.5/22	<.01
	D	2.15 ± .09	2.10 ± .09	2.4	14.5/22	NS

(continued)

Table 1 (*continued*)

Percent Differences in Cortical Depth between Enriched and Impoverished Animals

Age and Duration, days	Cortical area	EC $\bar{x}$[a] SD	IC $\bar{x}$ SD	% Diff.[b]	EC > IC	*P*
IX. 105–185	M_B	2.86 ± .12	2.83 ± .20	0.8	11.5/18	NS
	C	3.04 ± .09	3.05 ± .14	0.6	10/18	NS
	S_B	2.74 ± .08	2.68 ± .16	2.1	12/18	NS
	C	2.86 ± .10	2.86 ± .12	0.1	9/18	NS
	D	2.67 ± .13	2.72 ± .10	−1.5	7.5/18	NS
	V_B	1.79 ± .08	1.70 ± .10	5.0	13/18	<.05
	C	1.90 ± .06	1.84 ± .10	3.5	13/18	<.10
	D	2.01 ± .08	1.98 ± .10	1.4	9.5/18	NS
X. 25–185	M_B	4.00 ± .29	4.01 ± .23	−0.2	9/22	NS
	C	4.09 ± .29	4.07 ± .22	0.5	11/22	NS
	S_B	3.58 ± .25	3.65 ± .22	−1.9	6.5/22	NS
	C	3.72 ± .25	3.69 ± .22	0.8	12/22	NS
	D	3.23 ± .26	3.15 ± .25	2.5	14/21	NS
	V_B	2.36 ± .14	2.29 ± .15	3.0	16/22	<.05
	C	2.38 ± .12	2.38 ± .16	0	11/22	NS
	D	2.61 ± .20	2.67 ± .20	−2.2	7.5/22	NS

[a] Data expressed in microscopic units. To obtain micra multiply by 440.
[b] EC–IC/IC × 100 = % difference calculated from sums.

and from 160 day experiments from 25 to 185 days. Hippocampal depths were recorded on 224 pairs of animals.

RESULTS

Table 1 presents the percentage differences between the cortical depth of various regions of the brain from enriched and impoverished rats. For some of the short durations only data from the occipital cortex are presented, because no changes were seen in the motor or somesthetic cortex. In the longer durations, data are given from the three cortical sections studied: the motor, somesthetic, and occipital. For each section the cortical depth differences between the enriched and impoverished groups are shown for the B (medial), the C (dorsal), and D (lateral) segments (see Fig. 1).

In Table 1 (part I) no cortical depth differences are seen for the one day experiments, from 60 to 61 days of age. However, by four days, the 60–64 day groups, (Table 1, part II) a 3% ($p < .001$) difference occurs in the B segment of the occipital cortical section. Less significant differences occur in the C and D segments. In the younger four day group, from 26 to 30 days of age (Table 1, part III), significant cortical depth differences have developed in many segments of the somesthetic and occipital cortex, with 4% ($p < .001$) differences in both the B and C segments of the occipital cortex.

By extending the period in the two environments to seven days, from 25 to 32 days (Table 1, part IV), the entire cortex shows significant differences between the brains from animals in enriched and impoverished environments. The maximum differences for the seven day group are 4% ($p < 0.001$). If the animals remain in the conditions for 15 days, 25 to 40 day group (Table 1, part V), the occipital cortical differences increase and reach a 7% difference

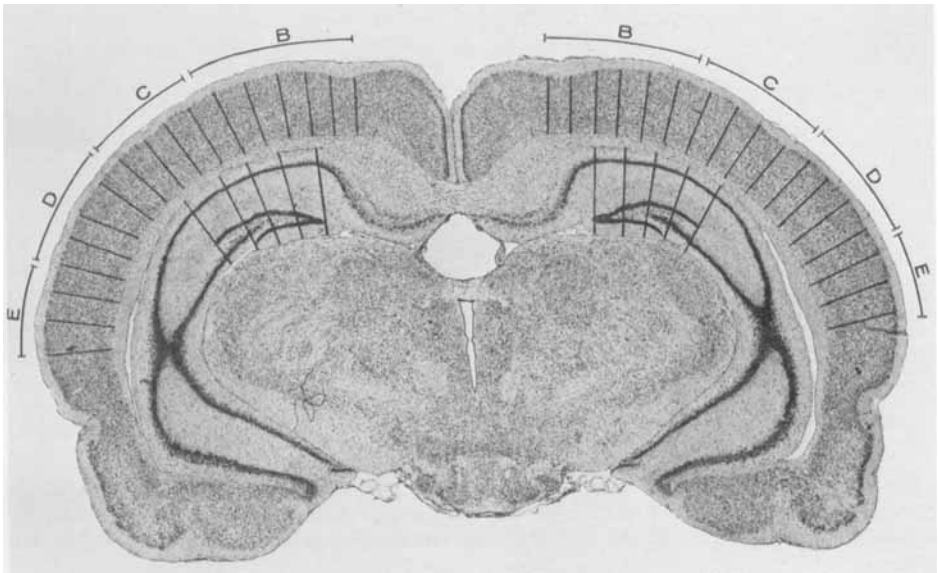

Fig. 1. A transverse section of the rat brain at the level of the occipital cortex showing the position of the lines measured to determine the dimensions of the cortex and the hippocampus.

($p < 0.001$). By 15 days in the experimental conditions, all of the segments except one show greater cortical depth changes than the seven day group.

The next group of animals were kept in their environments for 30 days, from 25 to 55 days (Table 1, part VI), and again the most significant differences were seen in the occipital cortex with 7% ($p < 0.001$) in the B segment. No group beyond the 15 day group demonstrated significant experimental differences in the motor cortex. The later 30 day group, from 60 to 90 days (Table 1, part VII) showed even more significant changes than did the younger 30 day group. The greatest experimental difference for the 60–90 day group was 7% ($p < 0.001$) at the B segment in the occipital cortex, also seen in the 25 to 55 day group.

When these enriched and impoverished experiments were first designed, it was not clear how long it would take to develop brain changes so an arbitrary 80 day period was chosen. We can see by examining Table 1 (Part VIII) that animals in their environments from 25 to 105 days of age develop experimental differences that are no greater than those in the 60 to 90 day group. In fact, several areas in the latter group are even more subject to change than in the 80 day group. The B segment from the occipital cortex from the 25–105 day group does show the greatest change, an 8% ($p < 0.001$), seen in any of the segments compared in any of the groups.

If we examine the older 80 day group, 105 to 185 days (Table 1, part IX), smaller experimental differences are noted than in the younger 80 day group. Only the occipital cortex indicates changes due to the environmental conditions and these changes are smaller than those seen in the younger 80 day group.

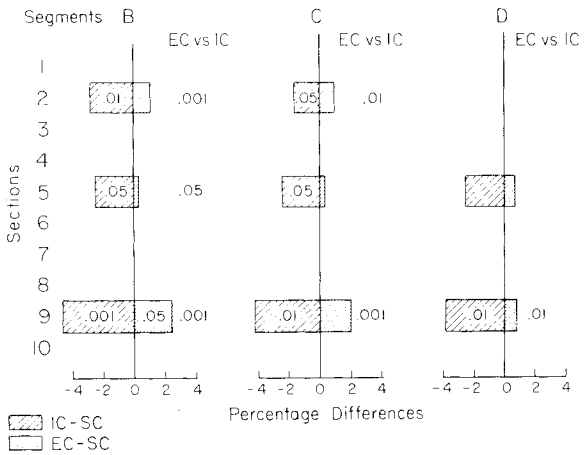

Fig. 2. The percentage differences in cortical depth are shown from 23 sets of EC–SC–IC rats in their environments from 25 to 40 days. The total length of the bar represents the EC–IC differences, the diagonal lines the SC–IC differences, and the dotted bars represent the EC–SC differences.

In the last age group examined, 25 to 185 days (Table 1, part X), only one section of the cortex, the B segment from the occipital cortex, indicates a difference, 3% ($p < 0.05$), in cortical depth between the enriched and impoverished animals. This small difference is similar in magnitude to those differences seen between experimental animals in their environments for short periods of time, namely four and seven days.

Table 1 presented the percentage differences in cortical depth between the enriched and impoverished rats, but did not indicate which condition was responsible for these differences. Figures 2 through 5 are offered to compare the cortical depth differences between the enriched and the standard colony animal, and the standard colony with the impoverished animal. Figure 2 shows the percentage differences in cortical depth from 23 sets of enriched (EC), standard colony (SC) and impoverished (IC) triplets in their environments from 25 to 40 days of age. In a previous report (Diamond et al., 1972), nine transverse sections were taken through the brain, but in the present experiments, only three sections were cut, numbers 2, 5, and 9 as designated on Figure 2. All of the section numbers are presented in the figure to compare with previous results, if desired.

The results indicate that if animals enter EC–SC–IC at weaning and remain in their environments for 15 days, the cortical depth differences occur primarily between SC and IC. In only one segment, B, in one section, 9, is a significant difference observed between EC and SC.

In Figure 3 cortical depth readings are presented from 22 pairs of animals in EC and IC from 25 to 32 days. No SC animals were included in this group because not enough animals were available at the time these experiments were planned. For this seven day experiment significant EC–IC differences are seen in every section and in every segment. Whether they are due to the

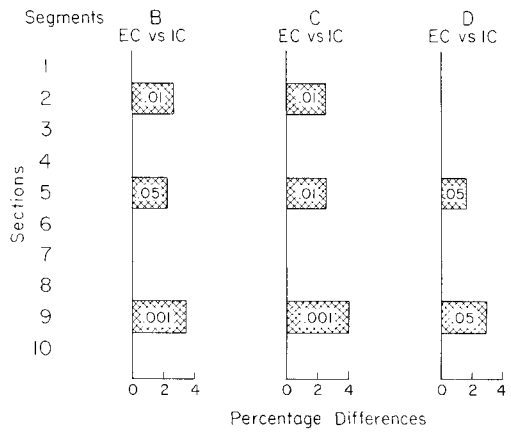

Fig. 3. Cortical depth readings are presented from 22 pairs of animals in EC and IC from 25 to 32 days of age. No SC animals are included in this group. See Fig. 2 for details.

EC or the IC is not clear, but at least the data indicate that EC–IC induce morphological differences after only seven days.

Figure 4 presents the EC–SC–IC differences from 19 sets of triplets in their environments for four days, 26 to 30 days. Once again cortical changes are evident, but they are solely due to the IC. All segments from section 9 are affected by the IC as early as four days.

Figure 5 shows results from 24 sets of adult rats in EC–SC–IC, placed in their conditions from 60 to 64 days to compare with the four day group, from 26 to 30 days. In this figure in only one area, section 9, segment B, is there a significant difference. It is due to the EC. In the younger rats only the ef-

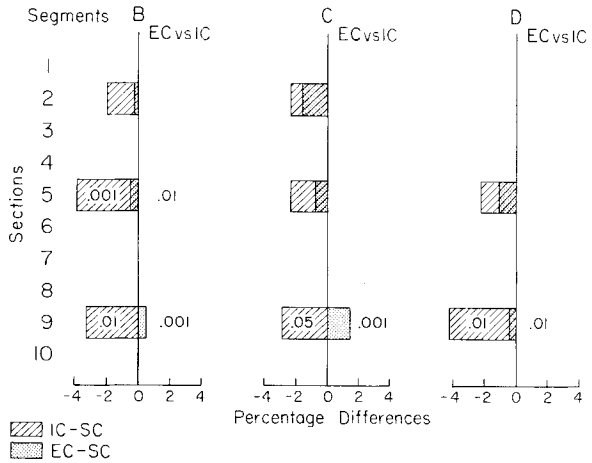

Fig. 4. EC–SC–IC differences are shown from 19 sets of triplets in their environments from 26 to 30 days of age. In some cases the SC is larger than the EC so the dotted bar is placed to the left of zero or SC. See Fig. 2 for details.

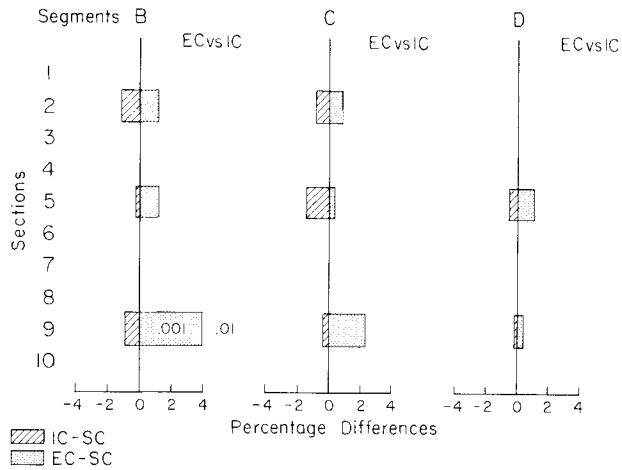

Fig. 5. The results are presented from 24 sets of adult rats in EC–SC–IC from 60 to 64 days of age to compare with the young, EC–SC–IC rats from 26 to 30 days of age. See Fig. 2 for details.

fect of impoverishment is manifest through the cortex, whereas, in the older rats, no impoverished effect is seen for this short duration.

Since four days are sufficient to bring about a structural change in the cortex, is it possible in still a shorter period, for example, in only one day, to show EC–SC–IC differences? The answer is no, at least in the age group studied. From the four day experiments, the effects of the EC are most marked in the older animals, therefore, older animals were chosen for the one day experiment, from 60 to 61. From 24 sets of EC–SC–IC animals no cortical depth differences were noted after one day.

Hippocampal depth

Table 2 summarizes the results on the measurements of the hippocampal depths from rats in EC and IC. No significant differences were recorded from the means from the combined data of initial and replication experiments. In one initial experiment from 25 to 105 days, a 7% ($p < .05$) difference was noted in five out of five pairs. However, upon replication for this age group, in three additional experiments, with 26 pairs of animals, the hippocampal depth differences were not significant.

DISCUSSION

These experiments have shown that the dimensions of the rat cerebral cortex can be measurably modified by the environment in as short a period as four days. Since cortical depth was the only measure used, it is not possible to say if changes occur at the ultrastructural level in less than four days. For the short-term experiments, when adult animals were studied, the effects of enrichment were more pronounced than those caused by impoverishment.

TABLE 2
Percent Differences in Hippocampal Depths between Environmentally Enriched and Impoverished Rats

Duration in days	Ages	% Diff.	#EC > IC (pairs)	*P*
1	60–61	1.1	13/24	NS
4	60–64	0.6	11.5/24	NS
4	26–30	0	12/20	NS
7	25–32	−1.2	10/22	NS
15	25–40	−0.5	9/23	NS
30	25–55	1.0	10/20	NS
30	60–90	2.0	11.5/20	NS
80	25–105[a]	1.9	17/31	NS
80	105–185	−1.3	10/18	NS
160	25–185	−0.6	11/22	NS
		TOTAL	115/224	

[a] Age group most comparable to Walsh et al. (1969).

In the immediately postweaned animals, the effects of impoverishment were most pronounced. In retrospect, it seems reasonable to assume that taking a rat pup away from the nest at weaning and placing it by itself is a traumatic behavioral experience. That the structure of the cerebral cortex should respond so readily to this trauma is somewhat surprising. Perhaps the experiments with the one day exposure to the environmental conditions might have shown depth differences if these experiments had been run from 25 to 26 days rather than from 60 to 61 days, since marked isolation effects developed in the young rats immediately after weaning. However, the younger rats were not used, since at the time, understanding effects of enrichment were of greater concern than understanding effects of impoverishment.

Undoubtedly, the preweaned rat would be a better choice for studying the shortest period necessary to bring about a morphological change due to enrichment, for the cortex is developing most rapidly during this period and also marked cortical depth differences have been reported from the preweaned rat after living 20 days in the enriched environment (Malkasian and Diamond, 1971).

Data from the 60 to 90 day group have indicated that both the enriched and the impoverished conditions affect brain structure (Diamond et al., 1972). In the present four day experiment from 60 to 64 days, only the enriched condition affected the brain. These results suggest the following: in older animals short-term exposure to the impoverished condition does not affect brain structure; yet during longer periods, impoverishment effects become evident. In young, postweaned animals, the impoverished condition is most effective early and the benefits of enrichment develop as the animal remains for a longer period in the enriched condition, for example, 25–55 days.

Flechsig (1901) stated an important principle for the brain in man and Bailey and von Bonin (1951) found the same to be true for subhuman primates, namely, that there are no significant direct interconnections between the limbic regions, and the motor, auditory, somesthetic, and visual cortexes. In an

excellent review of the structure and proposed functions of the hippocampus, Altman et al. (1973) report that the hippocampus is not directly linked either to primary sensory or primary motor components, indicating that no recent evidence has contradicted the statement of Flechsig (1901). Even though the primary cortical areas and the hippocampus are not directly connected, interest in the interplay between the two areas in learning and memory still exists. Most studies agree that lesions in the hippocampus of the rat, cat, and monkey do not affect learning a simple 2-choice simultaneous discrimination task, whereas, deficits in learning are seen using the Y-maze with spatial discriminations. Hippocampal damage has been reported to retard performance on complex mazes, discrimination reversal, and alternation, as shown, for example, by Adey (1967), Thomas, Hostetler, and Barker (1968), Kimble (1968), and Isaacson and Kimble (1972).

It is not clear why Walsh et al. (1969) were able to show hippocampal differences with eight pairs of animals in enriched and impoverished environments, whereas we did not find significant hippocampal differences with 31 pairs in the same age group. If, in the present experiment, the results had been reported from the one initial experiment with five out of five cases demonstrating an effect of environment on hippocampal depth, the results would have been misleading. Upon further examination of a total of 224 pairs of brains, no significant changes in hippocampal depth were seen. However, this finding does not mean that cellular changes did not take place. We did not look for cellular changes in our laboratory, for such measures have been made only if depth readings indicate an experimental response. The fact that Walsh et al. (1969) found changes in both hippocampal depth and cellular sizes remains a puzzle yet to be solved.

Altman, Brunner, and Boyer (1973) discuss the possibility of a two stage developmental sequence in the postnatal hippocampus, an immature stage and the adult stage. In the former, the hippocampus is immature because in altricial species the bulk of the granule cells in the dentate gyrus of the hippocampus are acquired during infancy. Perhaps, hippocampal depth differences will be manifest if the animals are placed in their respective environments during the preweaned period rather than during the postweaned or adult period. This hypothesis is presently being tested.

CONCLUSIONS

Cortical depth differences were found between male rats exposed to enriched and impoverished environmental conditions for successively shorter times for 15 days (from 25 to 40 days of age), for seven days (25 to 32 days of age), and for four days (26 to 30 and 60 to 64 days of age).

In the experiments begun at weaning (at 25 days of age), the cortical depth changes were caused primarily by impoverishment. If the animals were young adults, 60 days of age upon entering their respective conditions, the cortical changes were induced by enrichment.

No cortical depth differences were found between enriched and impoverished rats after 1 day in the experimental environments from 60 to 61 days.

In every age group and for every duration, except for the one day group, the dorsal–medial segment of the occipital cortex responded to the environmental conditions.

No significant hippocampal depth differences were noted between enriched and impoverished animals.

The authors thank the National Science Foundation for supporting this research, Grant No. GB-30368XB. They are also grateful to the many students who helped with the morphological measures: Richard Gilliam, Bill House, Vicki Johnson, Laurie Kneith, and Leslie Kruth.

REFERENCES

ADEY, W. R. (1967). Hippocampal states and functional relations with corticosubcortical systems in attention and learning. *Prog. Brain Res.* **27:** 228–245.

ALTMAN, J., BRUNNER, R. L. and BOYER, S. A. (1973). The hippocampus and behavorial maturation. *Behav. Biol.* **8:** 557–596.

BAILEY, P. and VON BONIN, G. (1951). *The Isocortex of Man.* University of Illinois Press, Urbana, Illinois.

BENNETT, E. L., DIAMOND, M. C., KRECH, D. and ROSENZWEIG, M. R. (1964). Chemical and anatomical plasticity of brain. *Science* **146:** 610–619.

DIAMOND, M. C., LAW, F., RHODES, H., LINDNER, B., ROSENZWEIG, M. R., KRECH, D. and BENNETT, E. L. (1966). Increases in cortical depth and glia numbers in rats subjected to enriched environment. *J. Comp. Neurol.* **128:** 117–125.

DIAMOND, M. C. with technical assistance by LINDNER, B. and RAYMOND, A. (1967). Extensive cortical depth measurements and neuron size increases in the cortex of environmentally enriched rats. *J. Comp. Neurol.* **131:** 364–375.

DIAMOND, M. C., ROSENZWEIG, M. R., BENNETT, E. L., LINDNER, B. and LYON, L. (1972). Effects of environmental enrichment and impoverishment on rat cerebral cortex. *J. Neurobiol.* **3:** 47–64.

FLECHSIG, P. (1901). Developmental (myelogenetic) localization of the cerebral cortex in the human subject. *Lancet* **II:** 1027.

GLASSMAN, E. and WILSON, J. E. (1972). Brain function and RNA. In: *Macromolecules and Behavior,* 2nd ed. John Gaito, Ed., Appleton-Century-Crofts, New York, pp. 39–59.

ISAACSON, R. L. and KIMBLE, D. P. (1972). Lesions of the limbic system: their effects upon hypotheses and frustration. *Behav. Biol.* **7:** 767–793.

KIMBLE, D. P. (1968). Hippocampus and internal inhibition. *Psychol. Bull.* **70:** 285–295.

MALKASIAN, D. R. and DIAMOND, M. C. (1971). The effects of environmental manipulation on the morphology of the neonate rat brain. *Int. J. Neurosci.* **2:** 161–170.

ROSENZWEIG, M. R., BENNETT, E. L. and DIAMOND, M. C. (1971). Chemical and anatomical plasticity of brain: Replications and extensions. In: *Macromolecules and Behavior,* 2nd ed. J. Gaito, Ed., Appleton-Century-Crofts, New York.

THOMAS, G. J., HOSTETLER, G. and BARKER, D. J. (1968). Behavioral functions of the limbic system. In: *Progress in Physiological Psychology,* vol. 2. E. Stellar and J. M. Sprague, Eds., Academic Press, New York.

WALSH, R. N., BUDTZ-OLSEN, O. E., PENNY, J. E. and CUMMINS, R. A. (1969). The effects of environmental complexity on the histology of the rat hippocampus. *J. Comp. Neurol.* **137:** 361–366.

WINDLE, W., RHINES, R. and RANKIN, J. (1943). A Nissl method using buffered solutions of thionin. *Stain Technol.* **18:** 77–86.

Accepted for publication June 12, 1975