Title

EFFECTS OF ENVIRONMENTAL ENRICHMENT AMD IMPOVERISHMENT ON RAT CEREBRAL CORTEX

Authors

Diamond, Marian C.
Rosenzweig, Mark R.
Bennett, Edward L.
et al.

Publication Date

1972-11-01

Published in the Journal
of Neurobiology, 3, No. 1,
pp. 47-64, 1972

LBL-1507

EFFECTS OF ENVIRONMENTAL ENRICHMENT AND IMPOVERISHMENT ON RAT CEREBRAL CORTEX

Marian C. Diamond, Mark R. Rosenzweig,
Edward L. Bennett, Bernice Lindner,
and Lennis Lyon

November 1972

Prepared for the U.S. Atomic Energy Commission
under Contract W-7405-ENG-48

LBL-1507

DISCLAIMER

JOURNAL OF NEUROBIOLOGY, VOL. 3, NO. 1, PP. 47–64

EFFECTS OF ENVIRONMENTAL ENRICHMENT AND IMPOVERISHMENT ON RAT CEREBRAL CORTEX

MARIAN C. DIAMOND,* MARK R. ROSENZWEIG,† EDWARD L. BENNETT,‡ BERNICE LINDNER,* AND LENNIS LYON*

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

SUMMARY

The first part of this paper deals with rat brain anatomy as affected by variations of starting age and of duration of exposure to enriched (complex) or impoverished (restricted) environmental conditions. Cerebral cortical depth measurements and weights of brain samples are compared. The second part of this report is concerned with an attempt to establish the extent to which the enriched condition or the improverished condition is responsible for the histological brain changes; this is done by comparison with brains from animals in the standard colony environment. The results indicate that both larger and more extensive cortical depth effects are found in rats exposed for 30 days rather than 80 days to their respective environments. The effects of enrichment are more prominent in the dorsal cortical segments, whereas the effects of impoverishment are more evident in the lateral cortical segments. More precise localization of cortical changes is obtained by measuring the depths at many segments than by taking the weights of relatively large blocks of tissue.

INTRODUCTION

We have previously reported significant differences in cortical depth between littermate rats raised in enriched (EC) or impoverished (IC) environments from 25 to 105 days of age (Diamond et al., 1964, 1966, and Diamond, 1967). The present paper expands and amplifies these results in three main ways: (1) Both starting age and duration of experimental periods are varied. (2) In an attempt to determine whether the experimental effects were caused by the enriched or the impoverished condition, a third condition is introduced intermediary between the other two conditions. (3) We have compared cortical depth measures with cortical weight values from enriched and impoverished animals.

* Department of Physiology–Anatomy.
† Department of Psychology.
‡ Laboratory of Chemical Biodynamics.

Part I. EC–IC Experiments Varying Duration and Age. These experiments were designed to provide precise localization of cerebral cortical depth differences on transverse brain sections from rats exposed to EC or IC for each of the three following periods: 25–55 days, 60–90 days and 105–185 days, and to compare the results with data previously obtained for the 25- to 105-day period (Diamond, 1966, 1967). One reason for this extended study was our finding that the cortical weight differences between EC and IC rats increase with 30 days of exposure to the respective environments and then diminish if the animals are retained in the conditions for a longer period, for example, 80 days (Bennett et al., 1970). We had also found that rats exposed to the two environments from 105 to 185 days of age developed differences in brain weights and brain chemistry, as much as did the 25–105 day animals (Rosenzweig et al., 1971). We then asked whether cortical depths would show similar effects.

METHODS

Littermate pairs of male rats of the S_1 strain from the University of California Psychology Department were used in these investigations. The detailed description of the EC and IC has been previously published (Bennett et al., 1964), as have the histological methods with data from the 25- to 105-day groups (Diamond, 1967). Only a brief description of the behavioral condition and histological methods will be presented here.

Behavioral conditions. From each littermate pair of rats, one animal entered EC and one IC. The animals in the EC lived together, 10–12 animals to a large cage (70 by 70 by 46 cm), and had access to a daily selection of toys, e.g., ladders, tunnels, swings, etc. (Rosenzweig and Bennett, 1969) and explored a Hebb-Williams maze for 30 min each day.

The IC animals lived one to a small cage (34 by 20 by 20 cm) with solid side walls so that animals could see or touch no other rat. The IC cages were placed in a separate, rather quiet room. Food and water were available to all rats *ad libitum*.

The environmental conditions were similar for all experiments. With the age and duration being the independent variables, 3 new groups of EC–IC experiments were designed for comparison with the previous results for the 25- to 105-day period (11–29 pairs, depending upon the sections taken for examination) (Diamond, 1967): I. 25–55 days of age (31 pairs of rats, 3 experiments). II. 60–90 days of age (50 pairs of rats, 4 experiments). III. 105–185 days of age (18 pairs of rats, 2 experiments). Animals in II and III lived 3 per colony cage from weaning at about 25 days until assignment to experimental conditions at either 60 or 105 days of age.

Histological procedures. At the time of autopsy all animals were given code numbers so their previous behavioral conditions were not known to the anatomists. The rats were anesthetized with ether, perfused with formol–saline, and the brains carefully removed from the skull. After being placed in the fixative for several days, the brains were prepared for either celloidin or frozen sections. We usually employ the celloidin technique in the event that more precise cytological measures, such as cell size or cell counts, are planned. Frozen sections are taken when only cortical depth measures are intended. Previous work has indicated that environmentally induced depth changes can be detected equally by either the frozen or celloidin methods (Diamond et al., 1966). Ten micra transverse sections were cut from frontal, somesthetic,

and occipital cortex using subcortical landmarks to insure uniform sampling. The subcortical landmarks were identical to those used in a previous publication (Diamond, 1967), but the distance between the sections has been more accurately placed on the figures in this paper, so different spacing is presented than on those figures previously published. Section 4 was not included in the present paper, but the numbering was kept the same as before in order to prevent confusion in locating sections. Each section was divided into segments B, C, D, and E as shown in Figures 1a–1b.

The positions of our sections can be related to Krieg's (1946) cytoarchitectonic designations of the rat cerebral cortex as follows: Sections 1–2 represent parts of Krieg's areas 8 and 10 from which motor responses can be elicited. Sections 3–7 encompass Krieg's area 4, part of the somatic motor cortex, and areas 3, 2, and 2a including the general somatosensory regions of the parietal cortex. The dorsal and dorsolateral portions (B and C) of sections 8–10 include areas 18, 17, and 18a; the lateral portions (D and E), 39 and 41. Area 17 is the primary visual receptive area and 18 and 18a are association visual areas. Area 41 is the auditory receptive area, and area 39 is an association cortical region. Thus, segments B and C in the occipital region are more closely associated with the visual system whereas the more lateral areas, D and E, are associated with auditory and association functional regions.

The brains to be prepared for frozen sections were removed from the fixative and placed in 30% sucrose for 48 hr prior to freezing with CO_2. Twenty micra frozen sections were cut from each brain and nine sections were taken at the identical landmarks used for celloidin sections.

The methods for taking samples for wet weight measures have been reported previously (Bennett et al., 1964). Histological sections 3–7 transverse the somesthetic cortex (S area) and sections 8–10 transverse the occipital cortex (O area), so that values from these sections can be combined for comparison with similar areas which have been taken for wet weight. That is, to obtain a single value for depth relatively comparable to the value for wet weight from a cortical area, cortical depths for sections 3, 5, 6, and 7 were averaged over segments B, C, and D for a somesthetic sample (see Figs. 1a–1b). Depths for sections 8, 9, and 10 were averaged over segments B, C, and D to represent an occipital cortical sample.

All tissues were stained with Windle's thionin stain (Windle, 1943). Microslide projected outlines were traced (22.5 X) and cortical depth readings were measured with a millimeter rule directly on the outlines; depth measures extended vertically from the corpus callosum to the dorsal surface of Layer II (Diamond et al., 1966). (Previously, in the 25- to 105-day period, Layer I was measured separately and did not show an EC–IC effect. Again, in the present experiments no differences were found in measuring Layer I, so all depth measures begin with Layer II.) The measurements began immediately lateral to the elevation of the corpus callosum (line 11, Fig. 1, Diamond et al., 1966) and were taken at each 6 mm interval for the celloidin sections. They were taken at 8–9 mm intervals for the frozen sections, which are larger because the tissue has not been affected by shrinkage. The measurements continued laterally to the widest dimension of the brain section, so that there were more readings in the posterior, wider sections. No measures were taken in the E segment of the occipital cortex in the 25- to 105-day group. The A segment, medial to the elevation of the corpus callosum, was not measured in the present studies, because there is more variability in the dimensions of this area than on the dorsal surface of the cortex. For each segment, depths from the right and left hemispheres were averaged.

The results were analyzed by a two-way analysis of variance testing the effects of environmental treatment and differences among litters. Detailed statistical comparisons between EC and IC are available upon request; these compare EC and IC differences for each segment from each section separately for each individual experiment from each age group.

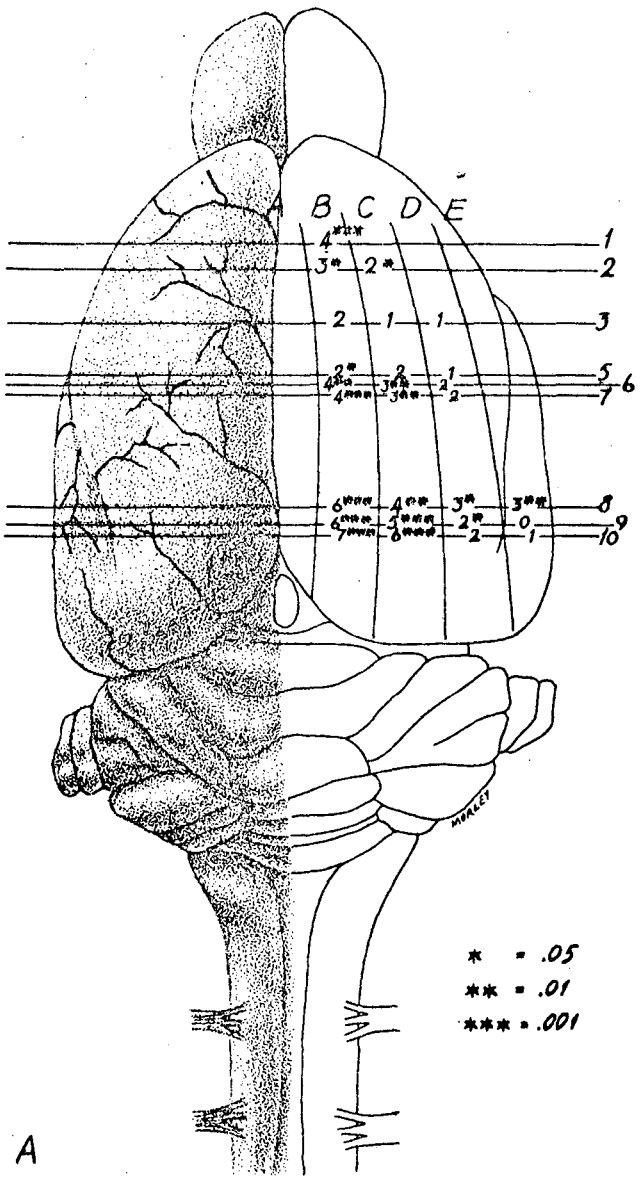

Fig. 1 (*continued*)

105-185 Days N=18 Pairs

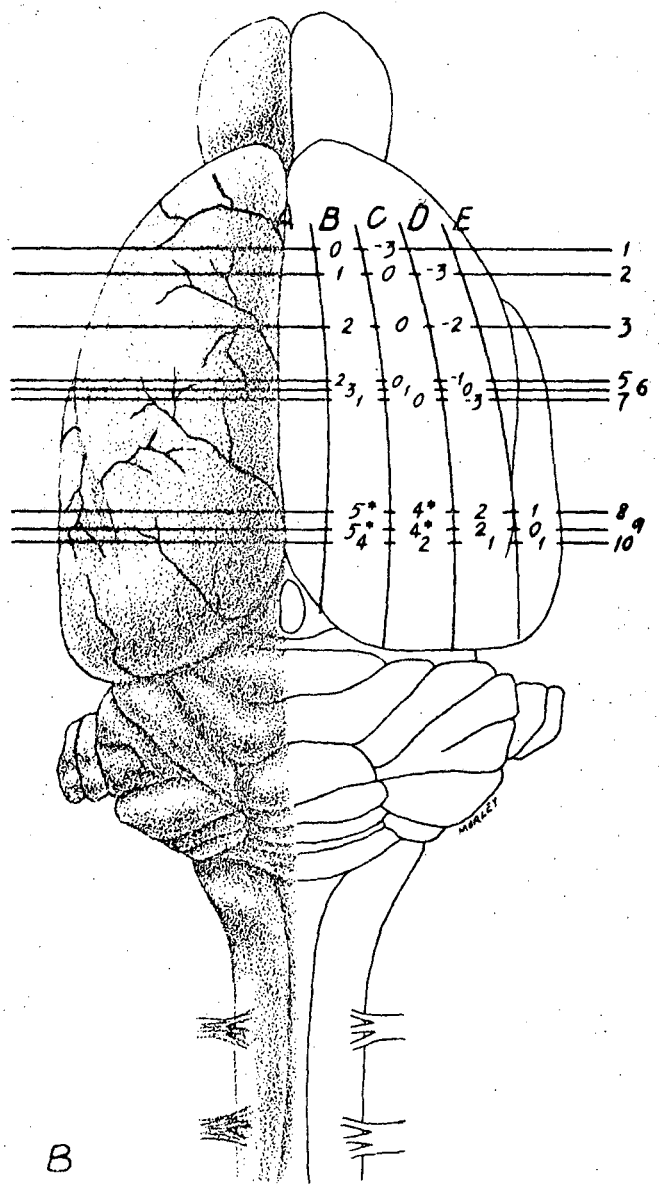

Fig. 1. Dorsal views of rat brain showing significant differences between EC and IC in depth of cortex; the differences are presented as percentages: EC minus IC/IC × 100. The percent value for each segment was obtained by averaging depths from the right and left hemispheres. The numbers 1–10 indicate the positions of the sections taken, and A, B, C, D, and E represent standard arbitrary segment divisions of each section. Figure 1A represents results from animals in the EC–IC conditions from 25 to 55 days of age and 1B from 60 to 90 days of age.

RESULTS

Figures 1 and 2 include the mean percent depth differences between EC and IC groups for each segment, B, C, D, and E from the combined experiments for each age period. Differences significant at beyond the

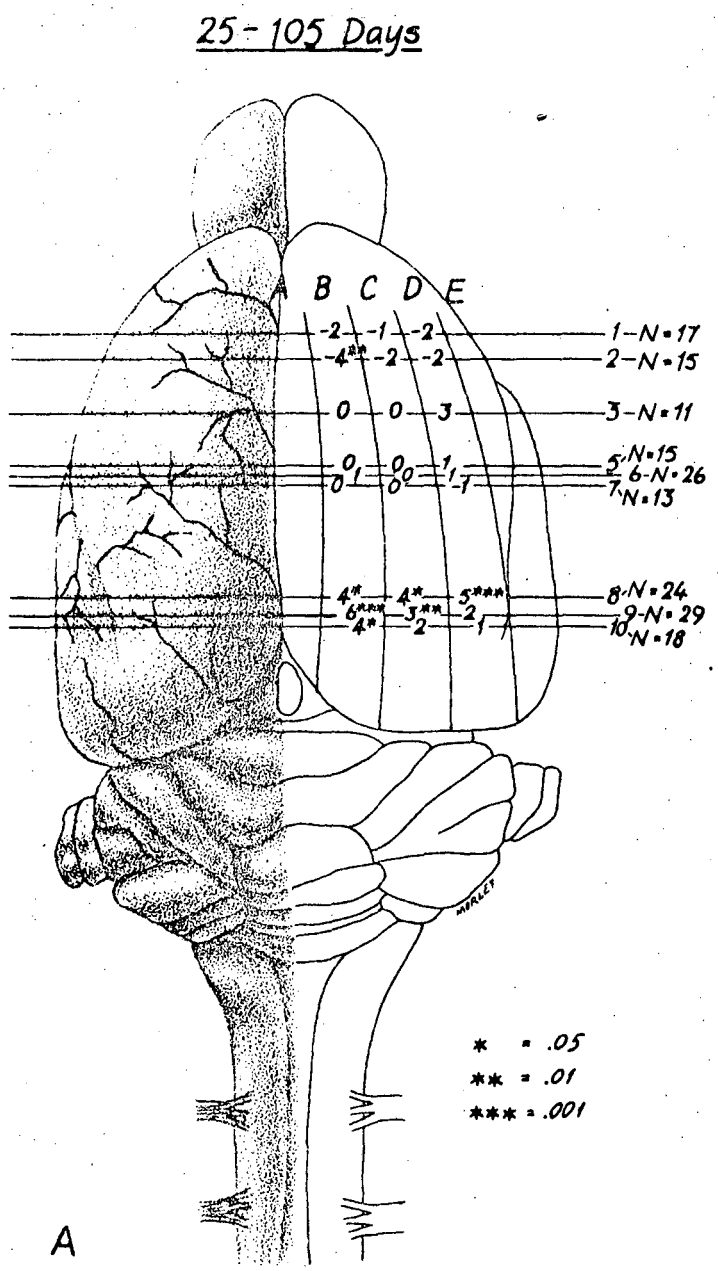

Fig. 2 (*continued*)

0.05 level are starred. Figure 2a, a modification of a figure on 25- to 105-day animals published previously, Diamond, 1967, is presented for comparison with the more recent data. Figure 2a differs from the published

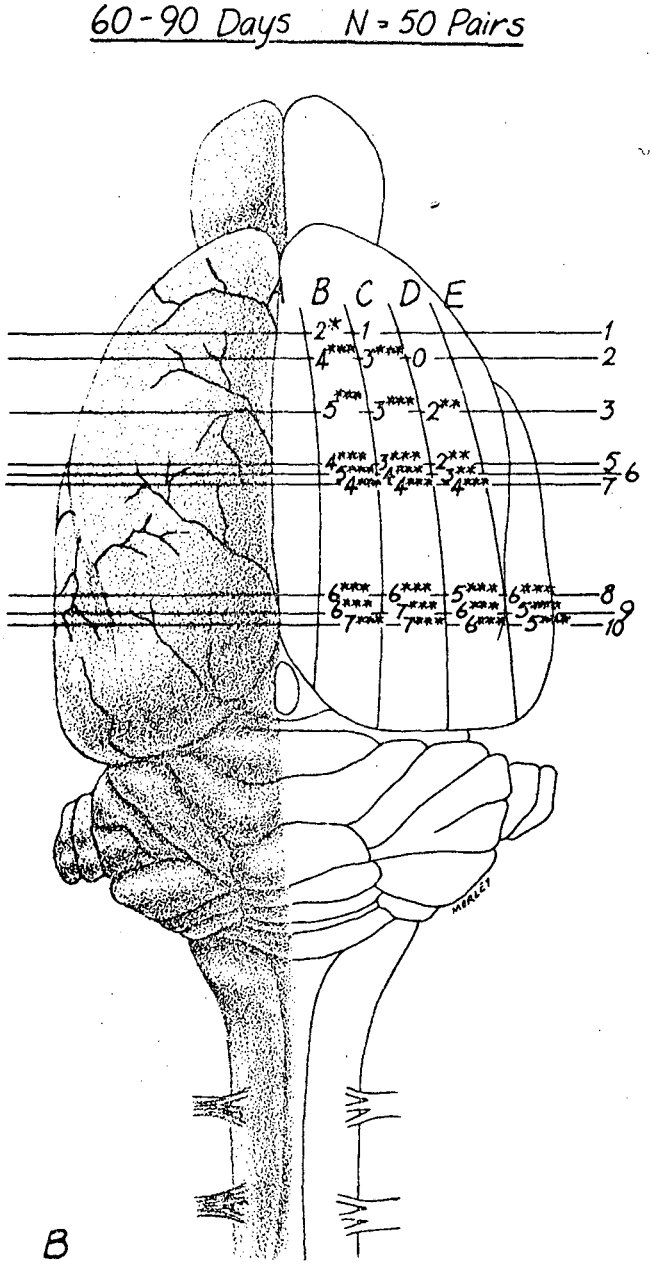

Fig. 2. Dorsal views of rat brain showing 80-day EC–IC effects as percentage differences. Symbols and abbreviations are as in Figure 1. Note in Figure 2 that some of the differences are negative, i.e., the IC is greater than the EC.

figure in that the non-significant values as well as the significant ones are included.

A. 25- to 55-day EC–IC. Figure 1a shows that the greatest depth differences occur in the occipital cortex (sections 8–10). The EC–IC differences are more pronounced in the B and C segments than in the more lateral D and E segments in the occipital cortex. In the somesthetic cortex a similar pattern is shown with the EC–IC effects being greater in the B and C segments than in the D segment.

The EC–IC effects in each of the 9 sections from the present 25- to 55-day period (Fig. 1a) were compared with each of the 9 sections from the previously reported 25- to 105-day period shown in Fig. 2a. Although the 25- to 55-day treatments showed larger effects, in 23 out of 24 segments compared, in only 4 out of the 24 comparisons were the differences significant.

B. 60- to 90-day EC–IC. The 60- to 90-day period yielded the most positive results in depth measurements encountered throughout the set of experiments, as shown in Fig. 1b. Every segment of occipital cortex gave differences significant at beyond the 0.001 level for the four experiments combined. Figure 1b also showed larger EC–IC differences than have been previously noted outside the occipital region. The 25- to 55-day treatment (Fig. 1a) had given evidence that the somesthetic cortex was responding to a 30 day experimental condition, but there is no question that the 60–90 day treatment had a marked EC–IC effect in the somesthetic cortex.

A statistical comparison of cortical depth effects was made between section 5 and section 9 on four 60–90 day experiments in order to test whether the EC–IC effect was greater in a sample from the occipital cortex (section 9) than in a sample from the somesthetic cortex (section 5). The effect in each segment in the occipital cortex was significantly greater than in the somesthetic cortex (Rosenzweig et al., 1969).

We compared the EC–IC differences from the 60- to 90-day period with both the 25- to 105-day and the 25- to 55-day period. The effects for the 60- to 90-day period were significantly larger than those for the 25- to 105-day period in 23 out of 26 segments. The differences between 60–90 and 25–55 did not reach statistical significance except in two cases, although in 22 out of 27 segments compared, the 60- to 90-day period showed greater EC–IC effects than did the 25- to 55-day period.

C. 105–185 day EC–IC. In the 80 day experiments with the older animals, 105–185 days (Fig. 2b), the cortical depth changes were not as marked as in the two 30-day experiments. The B and C segments of sections 8 and 9 from the older animals were the only areas with significant differences, 4–5%, and these were significant only between the <0.05 and 0.01 levels in contrast to the strongly significant <0.001 differences in the 25–55 and 60- to 90-day periods. In part, the lower level of significance of 105- to 185-day results is due to the smaller number of cases

tested at this age range. The EC–IC results were not significantly different in any of the sections compared between the two 80-day groups.

DISCUSSION

We have now mapped enriched vs. impoverished cortical depth effects during 4 conditions: two 80-day groups, 25–105 and 105–185 days; two 30-day groups, 25–55 and 60–90 days. In making comparisons among these groups, there are several questions to be asked.

A. Are the EC–IC effects similar for the two 80-day groups where the ages at onset differ? We can answer a definite Yes, for in none of the sections compared was the cortical depth significantly different between the two 80-day groups.

B. Are the EC–IC effects similar for the two 30-day groups when their ages at onset differ? In comparing the 25- to 55-day experiments with the 60- to 90-day experiments, the lateral occipital cortex (segments D and E) was the only area showing significant differences, with the 60- to 90-day group having higher values than the 25- to 55-day group. With this one exception, the results of the two 30-day periods do not differ.

C. Do the EC–IC cortical depth effects found from 80 days of treatment differ significantly from those found from 30 days? The younger 30-day group (25–55) does not differ markedly from the two 80-day groups. However, the older 30-day group (60–90) is significantly greater than both the 80-day groups in several of the comparisons made.

The region of the cortex which shows enriched vs. impoverished differences in the greatest number of comparisons is the caudal region of the cerebral cortex including the occipital cortex, a fact which has been reported previously (Rosenzweig et al., 1969). In the present study, we have supported our earlier data and in addition have shown that the frontal and somesthetic regions demonstrate morphological changes when the duration of the environmental treatment is for 30 days instead of 80 days. The cortical depth measures indicate that some areas such as the motor and somesthetic cortex respond to the environmental conditions for only a certain period, and then these brain differences diminish as the enriched vs. impoverished treatments are prolonged.

Figures 3 and 4 present wet weight data collected over a 3-yr period to study the variability of the EC–IC effect during several seasons in a single year and over several years. These figures summarize data extending from 1966 to 1969 from 13 experiments for the 25- to 55-day period and from 8 experiments for the 60- to 90-day period. Although the EC–IC differences are small in some cases, they have been found in all experiments. There are no apparent trends with regard to seasonal effects or in comparing recent values with those from the earlier experiments. The extensive data on weight differences can be used to find the sampling distribution of EC–IC effects.

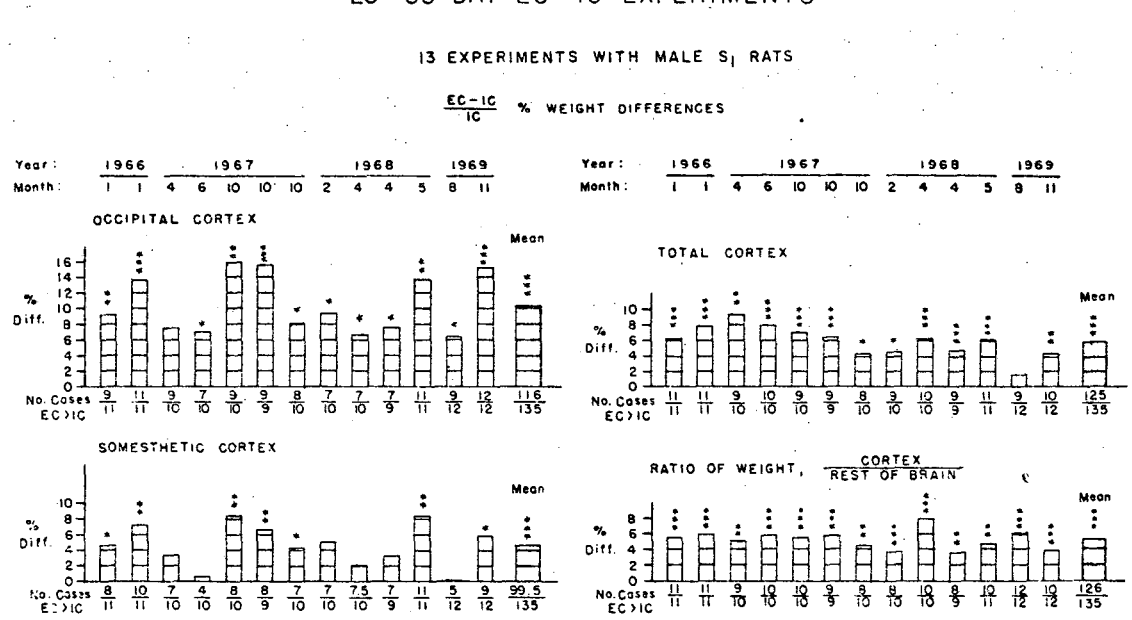

Fig. 3. The percentage differences in cortical weight from S_1 animals in EC–IC from 25 to 55 days of age. The data were collected over a 3-yr period for individual experiments, but this compilation of all the data has not been published previously The month and year of sacrifice are shown.

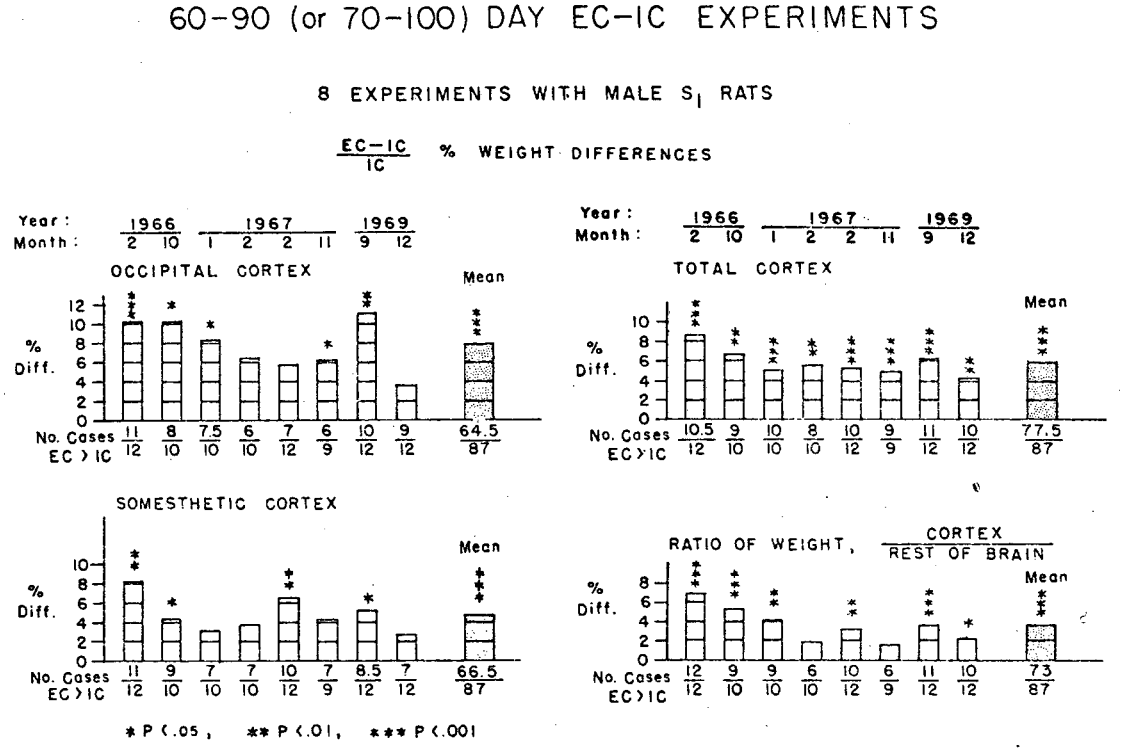

Fig. 4. The percentage differences in cortical weight from S_1 animals in EC–IC from 60 to 90 days of age.

Table 1 compares EC–IC percentage differences in depth and weight values for two different age periods, 25–55 and 60–90 days. For the 60- to 90-day period the depth and weight values both show highly significant EC–IC differences, and the two measures do not differ statistically. For the 25- to 55-day period the EC–IC differences are twice as great for the occipital as for the somesthetic cortex on both measures but the magnitude of the weight difference is more than two times as great as the depth differences. The differences between the weight and depth measures are significant beyond the 0.001 level for the occipital cortex and beyond the 0.05 level for the somesthetic cortex.

Table 1
EC vs. IC % Differences Comparing Cortical Depths with Wet Weight Values

	30-Day experimental conditions							
	25–55 Days				60–90 Days			
	Depth		Weight		Depth		Weight	
	% diff.[a]	EC > IC[b]	% diff.	EC > IC	% diff.	EC > IC	% diff.	EC > IC
Somesthetic	2.2[c]	20/31	4.7[d]	99.5/135	3.6[d]	38/50	4.9[d]	66.5/87
Occipital	4.6[d]	23/30	10.4[d]	116/135	6.2[d]	45/50	7.8[d]	64.5/87

[a] % difference = EC minus IC/IC × 100
[b] number of cases EC > IC
[c] $P < 0.01$
[d] $P < 0.001$

After having presented data on cortical depth and cortical weight, one can now ask whether anything further is learned by carrying out the more tedious histological methods instead of simply weighing samples of cortical tissue. One obvious advantage in taking the histological measures is that we gain more detailed knowledge of the spatial distribution of the EC–IC effects in the cortex. The EC–IC differences are more pronounced in the dorsal and dorsolateral aspects of the sections than more laterally, with the exception of the 60- to 90-day period where the EC–IC differences appear more uniform (Fig. 1a–1b).

Part II. EC–SC–IC Experiments Varying Age of Onset

METHODS

The standard colony (SC) group was included in two of the 25- to 55-day experiments (22 sets of triplets) and in two 60- to 90-day experiments (21 sets of triplets). Here full EC–SC–IC results will be presented for these 4 experiments. Littermate triplets were separated into the EC, IC, or SC. In the SC, 3 rats were housed in a single cage comparable in size to the IC cage (34 by 20 by 20 cm), but the sides were wire bars instead of being solid metal as in the IC cages.

RESULTS

A comparison of relative effects of environmental enrichment (EC vs. SC), shown by the dotted area, and of impoverishment (IC vs. SC), striped bars, is given in Figures 5 and 6. The results for the 25- to 55-day period will be presented first in Figure 5.

For the dorsal B segment, both EC and IC contribute about equally to the cortical differences, i.e., they are equally far removed from the SC. In the dorsolateral C segment, the EC–IC cortical depth differences are brought about more clearly by the IC condition, with the SC–IC differences reaching a statistically significant level primarily in the occipital cortex. In the lateral D segment, only the IC gives significant results. The EC effect fades out as one progresses laterally.

In the 60- to 90-day group (Fig. 6) the EC appears to have a stronger influence in the occipital region than in the more anterior regions. The EC–IC differences appear throughout the cortex and are due to both EC

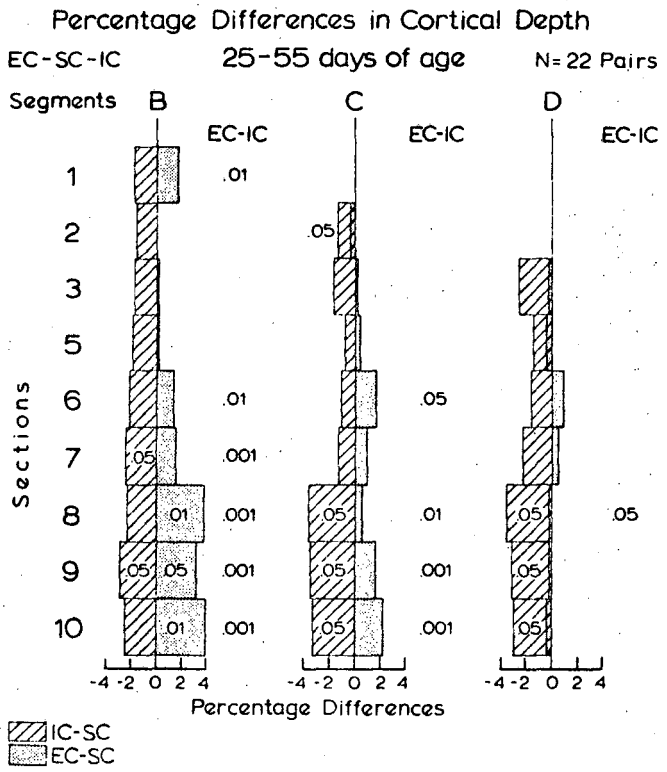

Fig. 5. This figure shows cortical depth differences between the enriched condition (EC) and the standard colony (SC), and between the impoverished condition (IC) and the SC from 25–55 days. (Fig. 1a compared only EC–IC from 25–55 days.) The histological sections are numbered on the left of each segment, extending from the anterior section 1, to the posterior section 10, as shown in Figs. 1 and 2. The *p* values for the EC–IC % differences are to the right of the segments, B, C, and D.

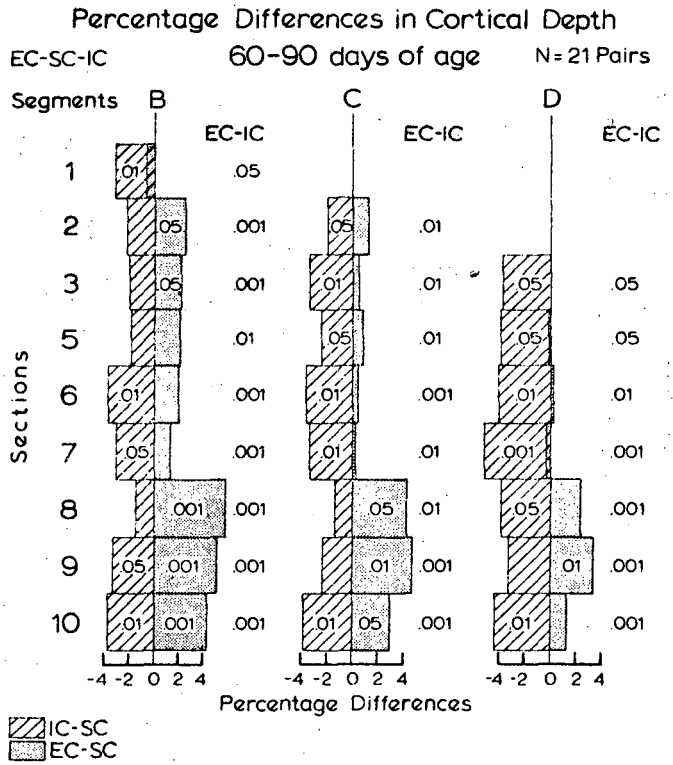

Fig. 6. This figure shows cortical depth differences between the enriched condition (EC) and the standard colony (SC), and between the impoverished condition (IC) and the SC from 60–90 days. The histological sections are numbered on the left of each segment, extending from the anterior section 1, to the posterior section 10, as shown in Figures 1 and 2. The p values for the EC–IC % differences are to the right of the segments B, C, and D.

and IC. In the dorsolateral C segment, the occipital area is more affected by the EC than by the IC. However, the more anterior somesthetic and frontal regions appear to be more affected by the IC. Once again, as in the B segment, the EC–IC differences are statistically significant throughout the cortex in the C segment for the 60- to 90-day period, but the differences are not as large in the B segment. As with the 25–55 day period, the IC effect in the lateral D segment is more dominant than the EC effect. In fact, there is only one section which shows a positive EC effect (Fig. 6, Section 9).

DISCUSSION

These data show different EC–SC–IC effects depending upon the age of the animals and the segments of cortex studied. The only uniform effect is found in the D segment where IC is predominantly responsible for the cortical depth difference in both 30-day groups.

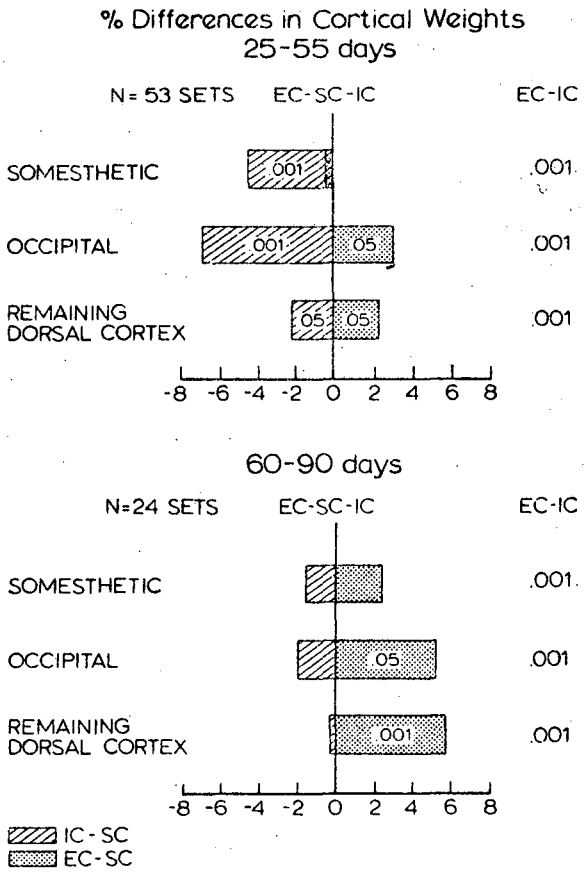

Fig. 7. EC vs. SC and IC vs. SC % differences in cortical weights for 25- to 55-day experiments (upper half) and 60- to 90-day experiments (lower half).

The lateral segments, predominantly affected by isolation, include the auditory cortex. Rats are very sound-oriented creatures. Raising rats in a quiet room may have caused the auditory area to receive less stimuli and, thus, the decrease in cortical depth in the posterior lateral cortex. The precise environmental factors which are responsible for these reported areal cortical changes are yet to be discovered.

We have previously reported EC–IC differences in differential glial counts and in neuron and neuron-nuclear size (Diamond et al., 1966; Diamond, 1967). Since all of our detailed histological measures were taken on 25- to 105-day experiments, we still cannot say if the impoverishment or the enriched conditions for this age group were predominantly responsible for the EC–IC cell differences. The detailed studies in the 25- to 105-day period were undertaken on the B segment on Section 9 in the occipital cortex, an area for which the 60–90-day experiments show predominantly an enrichment effect and for which the 25- to 55-day experiments show both EC and IC effects. Experiments are in progress to

study the EC–SC–IC differences on the 25–105 day period. Similarly, we have recently found EC–IC synaptic differences in a study utilizing the electron microscope (Møllgaard et al., 1971). It will be necessary to replicate these electron microscopic experiments with an SC group.

It is now important to compare the depth effects (Figs. 5 and 6) with weight effects (Fig. 7). For the 25- to 55-day periods the overall EC–IC differences for both weight and depth are due either to impoverishment or to both impoverishment and enrichment, but not due primarily to enrichment alone (Figs. 5 and 7). For the 60- to 90-day period, in the occipital cortex, the enrichment effect was greater than the impoverishment effect for both depth and weight. In the somesthetic section, weight and depth differences do not agree. The depth shows an isolation effect and the weight shows no difference. The new 25- to 105-day EC–SC–IC experiments will also be studied in this regard.

Once again the value of measuring cortical depth in addition to measuring wet weight is borne out by greater spatial resolution in the depth measurements. Take for example the 60- to 90-day period (Fig. 6) where the medial segment of the occipital cortex shows primarily an EC effect, whereas the lateral segment is influenced principally by the impoverished condition. The whole block of tissue taken for wet weight cannot be used to distinguish these areal differences.

That vision is not playing a primary role in the EC–IC effect has been supported by previous work showing that cortical weight effect occurs even when enriched and impoverished experiments are run in the dark or with blinded rats (Krech et al., 1963; Rosenzweig et al., 1969). It has been reported that fibers other than visual fibers are associated with the occipital cortex. These include connections with the basal ganglia (Lehmann and Koukkou, 1964), hypothalamus (Vanegas et al., 1969–1970), hippocampus (MacLean and Creswell, 1969) and non-specific thalamic fibers (Akimoto and Creutzfeldt, 1958; and Skrebitsky, 1969).

As part of our overall project, we have endeavored to determine what aspects of the experimental environments are responsible for the changes in cortical depth and in other brain measures. While this problem is not yet completely solved, we have demonstrated some factors to be of importance and others not to be required to produce the changes. What seems to be necessary is active exploration on the part of the EC animal with varied stimulus objects, the period of exposure being at least about an hour per day over a few weeks. Although the largest changes occur in the occipital region, we have found that visual experience is not required, since rats develop clear EC–IC differences even if they are kept in the dark or are blinded before the start of the experiment (Rosenzweig et al., 1969). Although animals are usually kept in a group in EC, social influences are not required, since rats exposed singly to EC develop the brain effects, especially if they are run in the dark period of the daily cycle or are primed by small doses of an excitant drug. Control experiments have shown that the increased locomotor activity and the handling that

characterizes the EC situation are also not required to induce the cerebral effects, nor is stress a factor (Rosenzweig, Bennett & Diamond, in press).

CONCLUSIONS

1. The somesthetic cortex shows a significant enriched (EC) vs. impoverished (IC) cortical depth difference for the 30-day experiments but not for the 80-day experiments. The occipital cortex shows the EC–IC effect for both durations.
2. The EC–IC effects on cortical depth for the 25- to 105- and 105- to 185-day experiments do not differ significantly from each other. Thus, age of onset is not a critical factor.
3. In most parts of the cortex the EC–IC effects for 25–55 days do not differ from those for 60–90 days.
4. The separate effects of enrichment and impoverishment were measured by comparison with standard colony (SC) littermates. The EC–SC effects are more pronounced in the dorsal cortical segment, whereas the IC–SC effects are more evident in the dorsolateral and lateral segments.
5. The EC–IC cortical weight differences are found in each of 13 25- to 55-day experiments and in each of eight 60- to 90-day experiments over a 3-yr period.
6. For the 60- to 90-day period the cortical depth and weight values show highly significant differences and the two values do not differ statistically from each other.
7. For the 25- to 55-day period the differences between weight and depth measures are significantly different from each other.
8. More precise localization of cortical changes is obtained by measuring the depths at many segments than by taking the weights of relatively large blocks of tissue.

REFERENCES

AKIMOTO, H. and CREUTZFELDT, O. (1958). Reaktionen von Neuronen des optischen Cortex nach elektrischer Reizung unspezifischer Thalamuskerne. *Arch. Psychiat. Nervenkr.* **196:** 494–519.

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

BENNETT, E. L., ROSENZWEIG, M. R., and DIAMOND, M. C. (1970). Time courses of effects of differential experience on brain measures and behavior of rats. In E. L. Byrne (Ed.), Molecular approaches to learning and memory. New York, Academic Press, pp. 55–89.

DIAMOND, M. C., KRECH, D. and ROSENZWEIG, M. R. (1964). The effects of an enriched environment on the histology of the rat cerebral cortex. *J. Comp. Neurol.* **123:** 111–120.

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. (1967). Extensive cortical depth measurements and neuron size increases in the cortex of environmentally enriched rats. *J. Comp. Neurol.* **131:** 357–364.

KRECH, D., ROSENZWEIG, M. R. and BENNETT, E. L. (1963). Effects of complex environment and blindness on rat brain. *Arch. Neur.* **8:** 403–412.

KRIEG, W. (1946). Connections of the cerebral cortex. *J. Comp. Neurol.* **84:** 277–323.

LEHMANN, D. and KOUKKOU, M. (1964). Neuronale Effekte der Caudatumreizung im visuellen Cortex. *Pflügers Arch. ges Physiol.* **280:** 297–315.

MACLEAN, P. D. and CRESWELL, G. (1969). Anatomical connections of visual system with limbic cortex of monkey. *J. Comp. Neurol.* **138:** 265–278.

MØLLGAARD, K., DIAMOND, M. C., BENNETT, E. L., ROSENZWEIG, M. R. and LINDNER, B. (1971). Quantitative synaptic changes with differential experience in rat brain. *Int. J. Neuroscience* **2:** 113–128.

ROSENZWEIG, M. R., BENNETT, E. L., and DIAMOND, M. C. (1967). Transitory components of cerebral changes induced by experience. *Proc. 75th Annual Convention Amer. Psychol. Assoc.*, 105–106.

ROSENZWEIG, M. R. (1968). Effects of experience on brain chemistry and brain anatomy. *Atti Acad. Naz. Lincei* (Italy), **109:** 43–63.

ROSENZWEIG, M. R., BENNETT, E. L., DIAMOND, M. C., WU, S.-Y., SLAGLE, R. W., and SAFFRAN, E. (1969). Influences of environmental complexity and visual stimulation on development of occipital cortex in rat. *Brain Research* **14:** 427–445.

ROSENZWEIG, M. R. and BENNETT, E. L. (1969). Effects of differential environments on brain weights and enzyme activities in gerbils, rats and mice. *Develop. Psychobiology* **2:** 87–95.

ROSENZWEIG, M. R., BENNETT, E. L. and DIAMOND, M. C. (1971). Chemical and anatomical plasticity of brain: replications and extensions, 1970. In Gaito, J. (Ed.), *Macromolecules and behavior*, 2nd ed. New York, Appleton-Century-Crofts. In press.

SKREBITSKY, V. G. (1969). Nonspecific influences on neuronal firing in the central visual pathway. *Exp. Brain Res.*, **9:** 269–283.

VANEGAS, H., FOOTE, W. E. and FLYNN, J. P. (1969–70). Hypothalamic influences upon activity of units of visual cortex. *Yale J. Biol. Med.* 191–201.

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

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720