Marian Cleeves Diamond

ANATOMICAL BRAIN CHANGES INDUCED BY ENVIRONMENT

Twenty-six years ago I began to study the structure and function of the brain, because I believed that the greatest plague inflicting mankind was initiated by the brain. Ecclesiastes tells us that vanity and vexation of spirit are the characteristics of man, whether he be rich or poor. And only a creature with a brain would invent and suffer from such cognitive ills. If the biological basis of behavior were found, perhaps man's existence could be a better lot. By understanding the structural and functional mechanisms of behavior, man might even solve his ancient emotional problems. Just how far have we come? Only after joining the team of Krech, Rosenzweig, and Bennett did I think I was taking steps in the right direction toward correlating learning behavior with brain anatomy. The purpose of this chapter is to present a collection of information which attempts to understand how the microscopic structure of the forebrain changes during the lifetime of one mammal, the rat, whether it be during normal development and aging or during life in artificially provided environments. Both external and internal environmental manipulations affect brain morphology. The experiments to be reported here involve primarily external factors which were manipulated by exposing animals to various degrees of environmental complexity. Since neurons are extraordinarily difficult to study *in vivo* without interfering with the subject, most information is accumulated from preserved material, and, for the same reason, neurons are studied from lower animals and not from human beings.

Marian Cleeves Diamond • Department of Physiology-Anatomy, University of California, Berkeley, California.

The Young, Adult, and Aging Forebrain

The normal development and aging pattern is presented first to serve as a standard for comparison with brain changes induced by environment. A literature search for information on quantitative changes in the anatomy of the forebrain during development and aging indicates a sparsity of complete data. In 1917, Sugita measured the thickness of the rat cortex during aging, but his samples were small for each age group, and the sections measured did not correspond to those commonly used today. A recent study in our laboratory (Diamond *et al.*, 1975*a*) showed the changes in depth during development and aging, using at least 15 rats per age group from 6 to 650 days, of not only the cortex but the hippocampus and diencephalon as well (Figure 1). (Figure 2 designates the positions of the areas measured.) Figure 1 shows that all parts of the cortex, anterior, posterior, medial, and lateral, increase in depth rapidly until 26 days of age, and then there is a 9% decrease in cortical depth between 26 and 650 days of age. A search for hemispheric dominance in the rat showed the right hemisphere to be thicker than the left in all age groups with but a few small reversals. The right medial (B) and the lateral (D) segments (Figure 2) are on the average between 2 and 3% greater than the comparable left hemisphere segments, whereas the dorsal (C) segment is between 5 and 7% greater in the right than in the left hemisphere. These quantitative hemispheric differences are apparent throughout the lifetime of the rat.

In contrast to the cortex, the hippocampal depth continues to increase until 650 days of age (Figure 1, middle section). The initial rise in depth from 6 to 10 days is very similar to the pattern of cortical growth, but instead of reaching a maximum at 26 days a slow 9% increase is seen by 650 days.

As in the cortex and the hippocampus, the greatest increases in diencephalon dimensions occur between 6 and 10 days of age, and then, like the hippocampus, the diencephalon continues to increase until 650 days. Apparently both the hippocampus and diencephalon are increasing in dimensions (by about 9–10%) with aging, while the cortex is decreasing by 9% or possibly stretching over the increasing subcortical mass (Figure 1).

In an attempt to understand the changes occurring in the constituents of the cortex with development and aging, different measurements have been taken on cell number and size. At birth, the rat has 94–97% of the adult number of neurons (Schade and Ford, 1965). In 1968, Brizzee and colleagues found an absence of any significant decrease in neuron density between 109–113 days and 763–972 days of age, but an increase in number of glial cells was noted. There was no difference in the distribution of neurons through cortical layers as a function of aging. The glial population was greater in the aged brain at all levels of the cortex except between the two

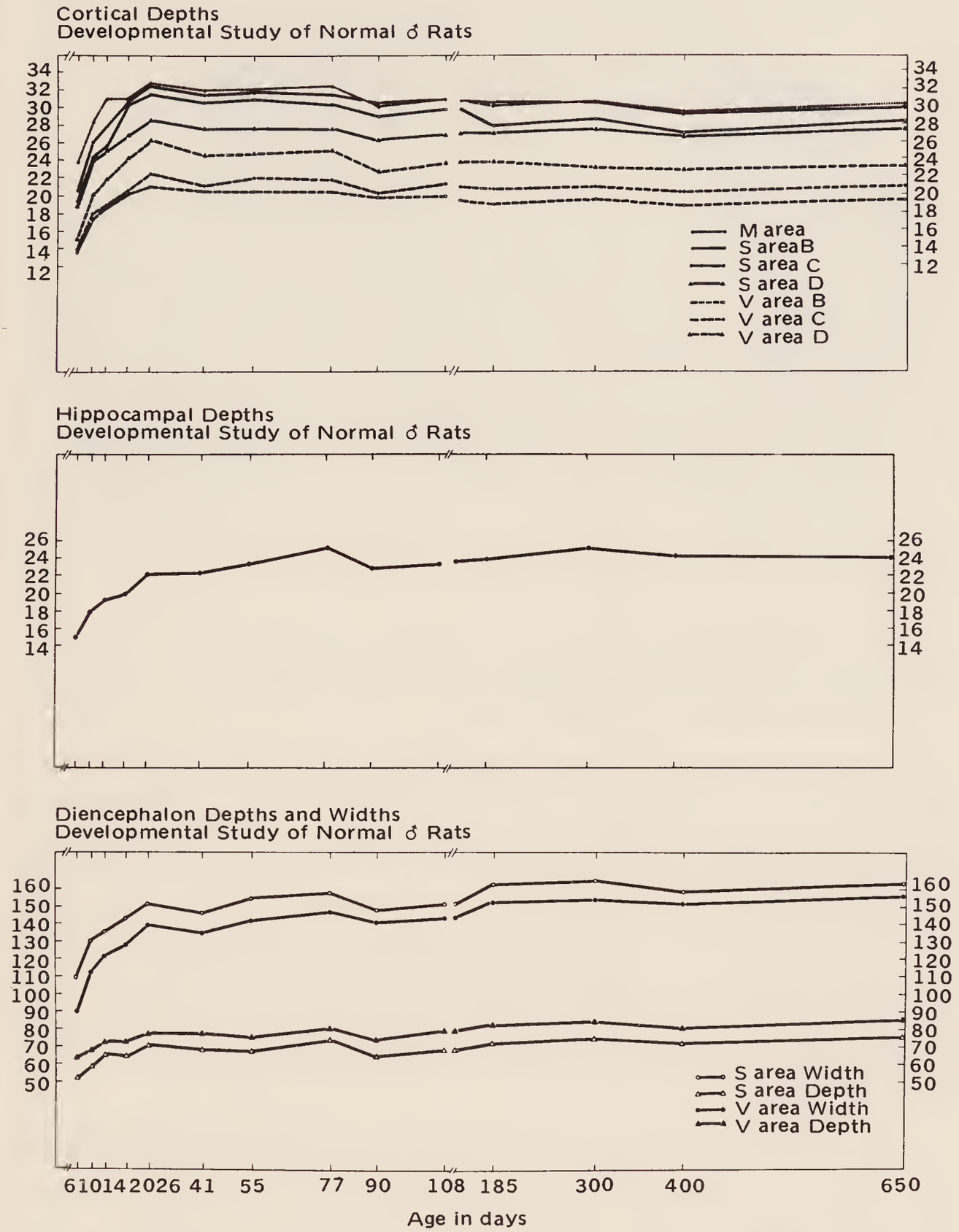

Figure 1. Developing and aging curves of the Long-Evans rat, cortical depth, hippocampal depth, and diencephalon width and depth from 6 to 650 days of age. The ordinate shows the measurements in millimeters at 22.5× magnification. The M area represents a tissue sample taken immediately before the anterior crossing of the corpus callosum. The S area is at the level of the crossing of the anterior commissure and the V at the level of the posterior commissure. The hippocampal depths were taken at the V level. From Diamond *et al.* (1975*a*).

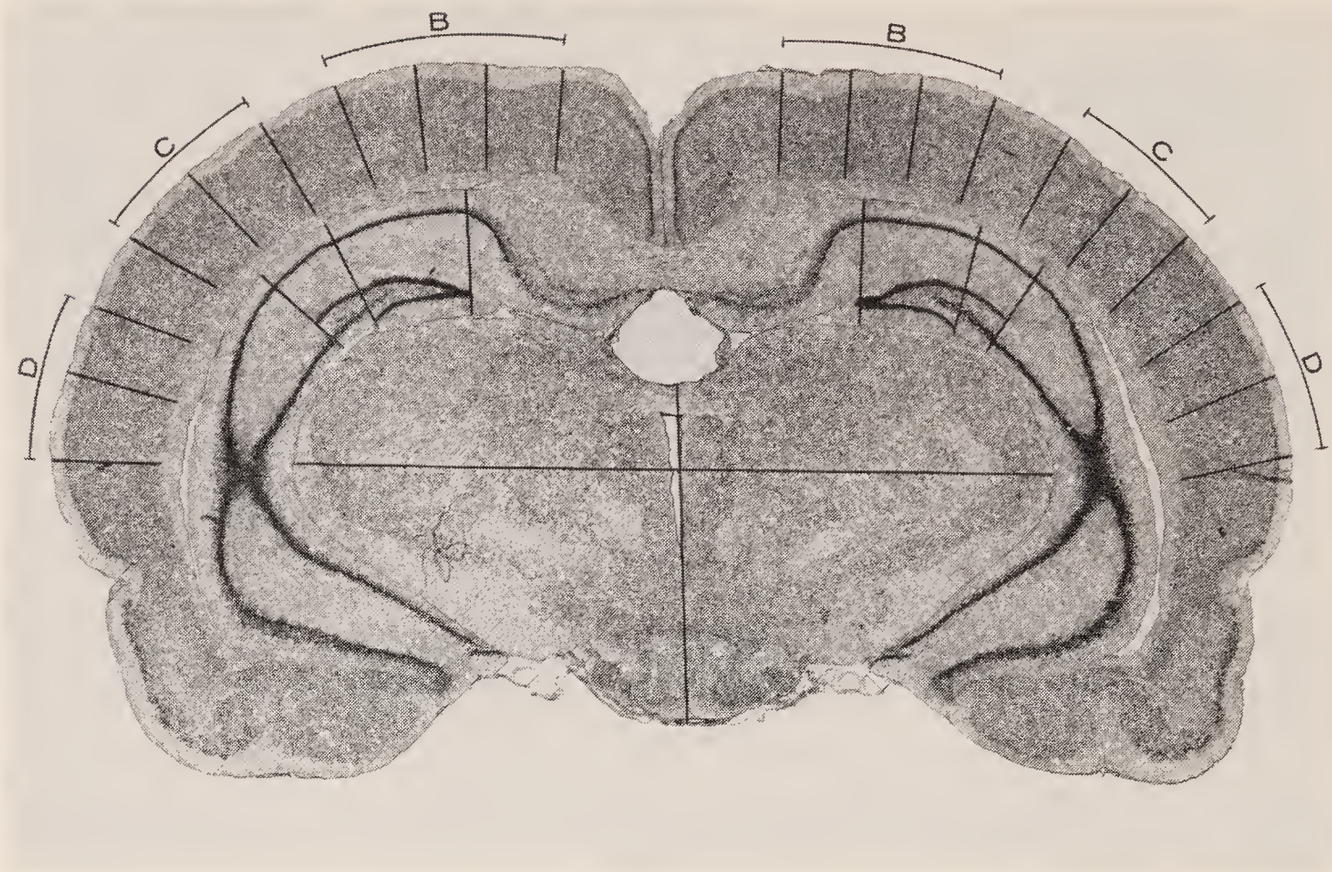

Figure 2. A transverse section of the rat brain at the level of the posterior commissure to indicate the position of the measurements for cortical depth, B, C, and D segments; hippocampal depth; and diencephalon width and depth. From Diamond *et al.* (1975*a*).

deepest levels measured. In a more recent study (Diamond *et al.*, 1976*a*), we counted neurons on photomicrographs of segment B of the occipital cortex (Figure 2), enlarging an area of cortex from about one millimeter square to one meter square. From these counts we found, as did Brizzee *et al.* (1968), no significant loss (4% N.S.) in cortical neuron number after 108 days of age, in the period from 108 to 650 days of age. However, between 41 and 108 days of age an 18% ($p < 0.001$) decrease in neuron number was counted. We hypothesize that the rat is endowed with many more neurons than needed, and that if not used early, the greatest neuron decrease will occur early in the rat's life.

In contrast to Brizzee *et al.*, we did find differences in rates of neuron loss in upper versus lower halves of the cortex. Between 41 and 108 days of age, a 9% loss in neurons occurred in the upper half; whereas, in the lower half, a 24% loss took place. Between 108 and 650 days of age, the upper layers lost 7%, and the lower layers, only 1%. Thus, the association layers apparently lose cells continuously with aging; whereas, cells in the projection layers are lost only in the early years.

At birth, the nuclei and perikarya are less than half the adult size (Schade and Baxter, 1960). By 6 days of age, the mean perikaryon volume has increased by 60% compared to that of the 1-day-old animal (Brizzee and

Jacobs, 1959). Measurements of neuronal nuclear volume in the visual cortex show a sharp decrease, about 30%, between 26 and 41 days of age, and thereafter a 13% decrease until 650 days (Diamond *et al.*, 1976*a*).

With regard to glial number and aging, Brizzee *et al.* (1968) found a moderate increase in packing density of the glial cells in the cerebral cortex throughout the life-span of the animal from 109–113 to 763–972 days. Glial counts were completed more recently on the photographs used for neuron determinations (Diamond *et al.*, 1976*a*). By counting about 450 glial cells/animal in 13 animals/age group, we found a decrease of 34% ($p < 0.001$) between 41 and 108 days of age, but only a 4% (N.S.) decrease between 108 and 650 days of age. The neuron and glial aging pattern was identical in this latter period. Using a DNA analysis, Enesco (1967) and Mandel and Bieth (1952) suggest the possibility that no change in total cell numbers occurs in the rat brain after the fourteenth postnatal day. These data show that the analyses of DNA content in whole brains are not really a very meaningful approach to following patterns of glial and neuron populations.

Brizzee *et al.* (1968) found the total rat brain weight and the weight of the major subdivisions greater in aged than in young adult rat brains. The ratio of forebrain weight to total brain weight increased significantly from young adult to old rats. Thus brain weight measures, including all parts of the forebrain, do not necessarily agree with measures on histological preparations (Diamond *et al.* 1975*a*). Loss of water during the histological preparations of the tissues may be one significant factor.

In summary, from examination of the data on the developing and aging forebrain it appears that the rat cerebral cortex increases in depth until 26 days of age and then slowly decreases until at least 650 days, whereas the hippocampus and diencephalon both continue to increase in dimensions until 650 days of age. The right cerebral hemisphere is thicker than the left in every cortical region measured between the ages of 6 and 650 days. The rat has about 95% of its neurons at birth and appears to lose or redistribute them most rapidly in the first 100 days. Both the volume of cortical neurons and the cortical depth show a considerable decrease during this time period. Between 100 and 650 days of age, there is very little difference in neuronal and glial number per unit area of rat cortex.

Effects of Environment on the Preweaned Rat Cortex

Having examined some data available on the growth and development of the postnatal forebrain of the rat, it is of interest to learn how some extrinsic factors from the environment influence these brain dimensions. During the

first 26 days of the rat's life, the cortex is growing most rapidly, and it was during this preweaning period that we (Malkasian and Diamond, 1971) placed rats in modified enriched and impoverished environments. Before the pups were 6 days of age, we found that the mothers disturbed the nest too violently to allow several mothers and their pups to live together in a single cage. So at 6 days of age three groups of animals were formed: (1) one mother with her three male pups remained in a standard colony cage (Unifamily Environment, UFE); (2) three mothers with three male pups each were placed together in a single large cage (Multifamily Environment, MFE); (3) three mothers with three male pups each were placed together in a single large cage with various metal objects (Enriched Condition–Multifamily Environment, EC-MFE). The UFE vs. EC-MFE cohort was divided into three age groups to be sacrificed at 14, 19, and 28 days of age. The results showed no cortical depth differences between 28-day-old UFE and MFE rats in either the somatosensory or occipital cortices, but the EC-MFE had significantly greater cortical depth than the UFE at all age groups (Figure 3 shows such changes in the occipital cortex). At 14 days of age, there were cortical depth differences as large as 16% in the EC-MFE animals compared to the UFE animals, and as large as 12% at 28 days. These findings suggest that some regions of a neonate cortex may be morphologically more responsive to the external environment than is the adult brain, which has been reported to demonstrate changes in the range of 5–10%. In addition, the neuronal nuclear and perikaryon areas in the somatosensory cortex were significantly greater in the EC-MFE animals than in those from the UFE in the 28 day old group.

Not only did the cortical differences between the UFE and the EC-MFE rats show marked differences at 14 days of age, the earliest period measured, but also, in the EC-MFE animals, the eyes opened 1 day earlier, giving an additional measure indicating that maturation of the nervous system was enhanced by the enriched environment. Increased neurogenesis may have resulted in increased rate of innervation of the orbital structures facilitating earlier eye opening (Eayrs, 1951).

Thus these results, when compared with the rapid growth curve of the cortex from the preweaned rat, indicate that maturation can be enhanced during the preweaning period.

Effects of Environment on Postweaned Male Rat Cortical Depth

In order to study the effects of environment on the rat brain, three types of environmental conditions are commonly used in our laboratory: Enriched (EC), Impoverished (IC), and Standard Colony (SC). EC animals were housed in a large cage (70 by 70 by 46 cm) and had access to stimulus objects (toys) which were changed daily. The EC animals were placed for 30 min in

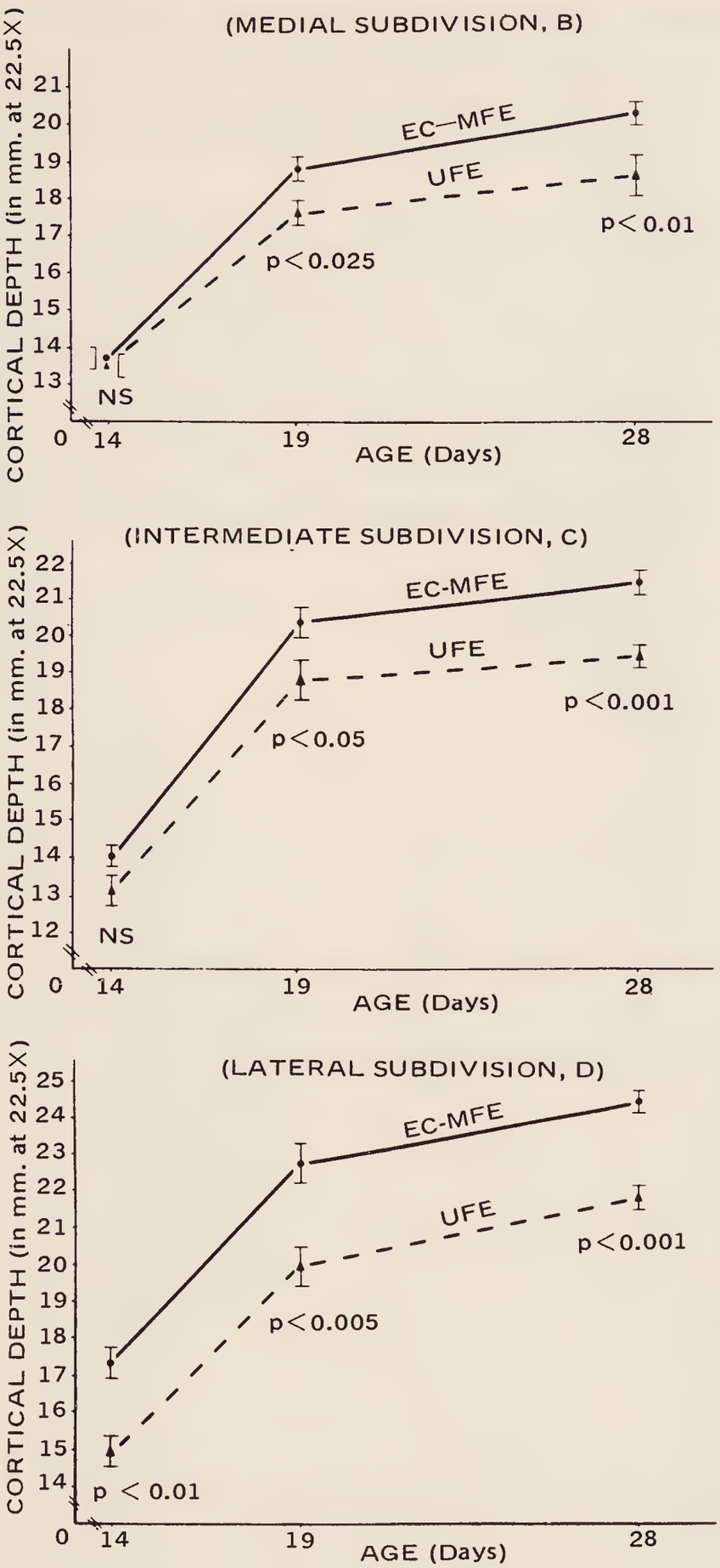

Figure 3. Depth of the occipital cortex in the preweaned Long-Evans rat as a function of age, comparing the Uni-family Environment with the Enriched Condition–Multi-family Environment rats. See Figure 2 for the approximate position of the B, C, and D segments in this experiment. From Malkasian and Diamond (1971).

the field of a Hebb-Williams apparatus (75 by 75 cm) where the pattern of barriers was changed daily. The IC animals were housed individually in small cages (32 by 20 by 20 cm) with no stimulus objects or maze experience. The SC animals were housed three to a small cage also with no stimulus objects or maze experience.

In the original experiments dealing with anatomical measures and enriched and impoverished environments, 80 days was considered as a reasonable time to bring about changes in brain structure. Since those first experiments, the duration has been decreased gradually from 80 days to 30 to 15 to 7 to 4 days and to 1 day, even though no changes were found during the 1-day experiments (Diamond *et al.*, 1976*b*).

Figures 4 and 5 show the dorsal view of the rat brain, with the lines 1 through 10 representing the positions of transverse histological sections

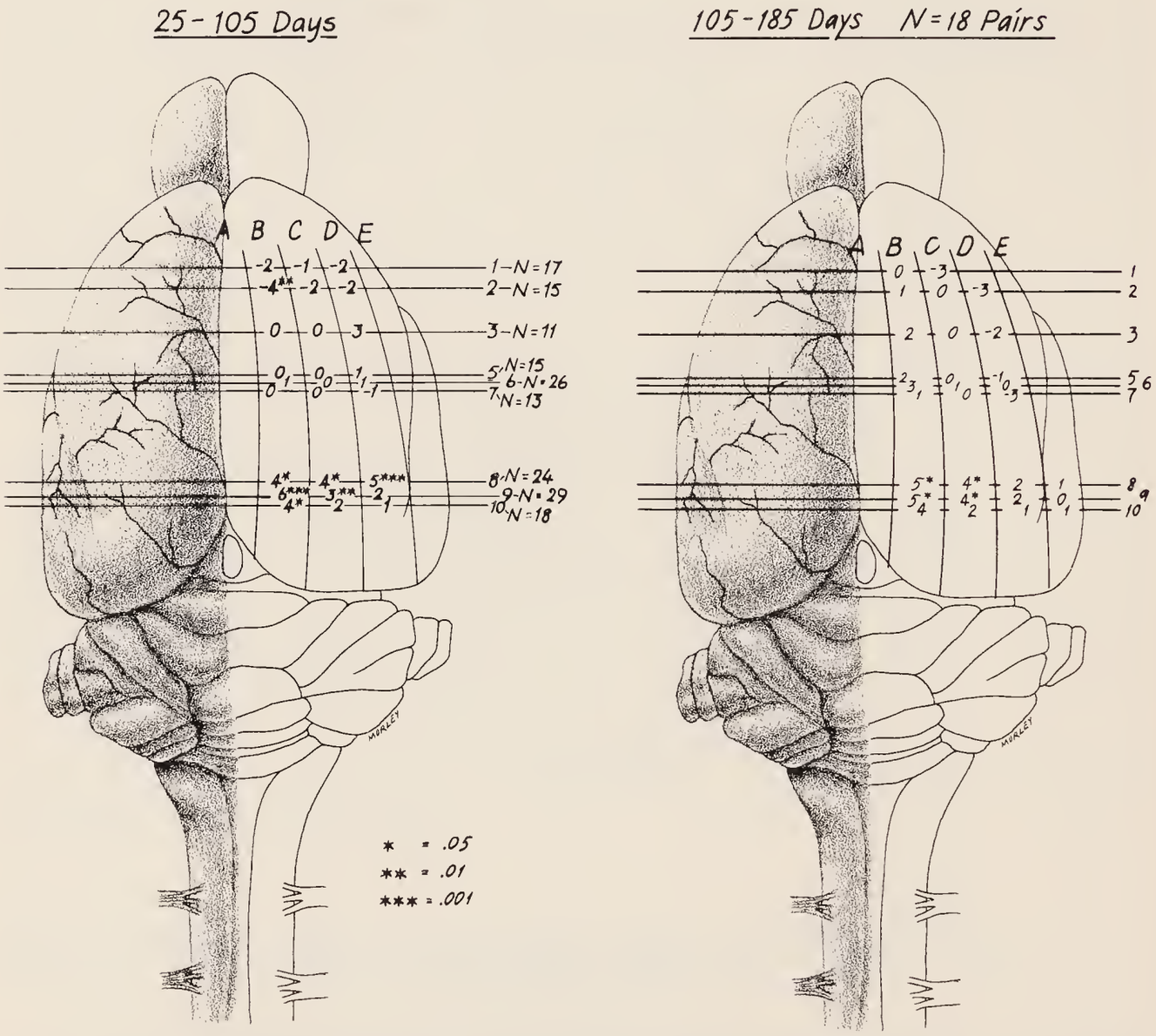

Figure 4. Left: Percent differences in cortical depth measures between rats in enriched or impoverished environment from 25 to 105 days of age. Right: Percent differences for rats in the same conditions also for 80 days, but from 105 to 185 days of age. From Diamond *et al.* (1972).

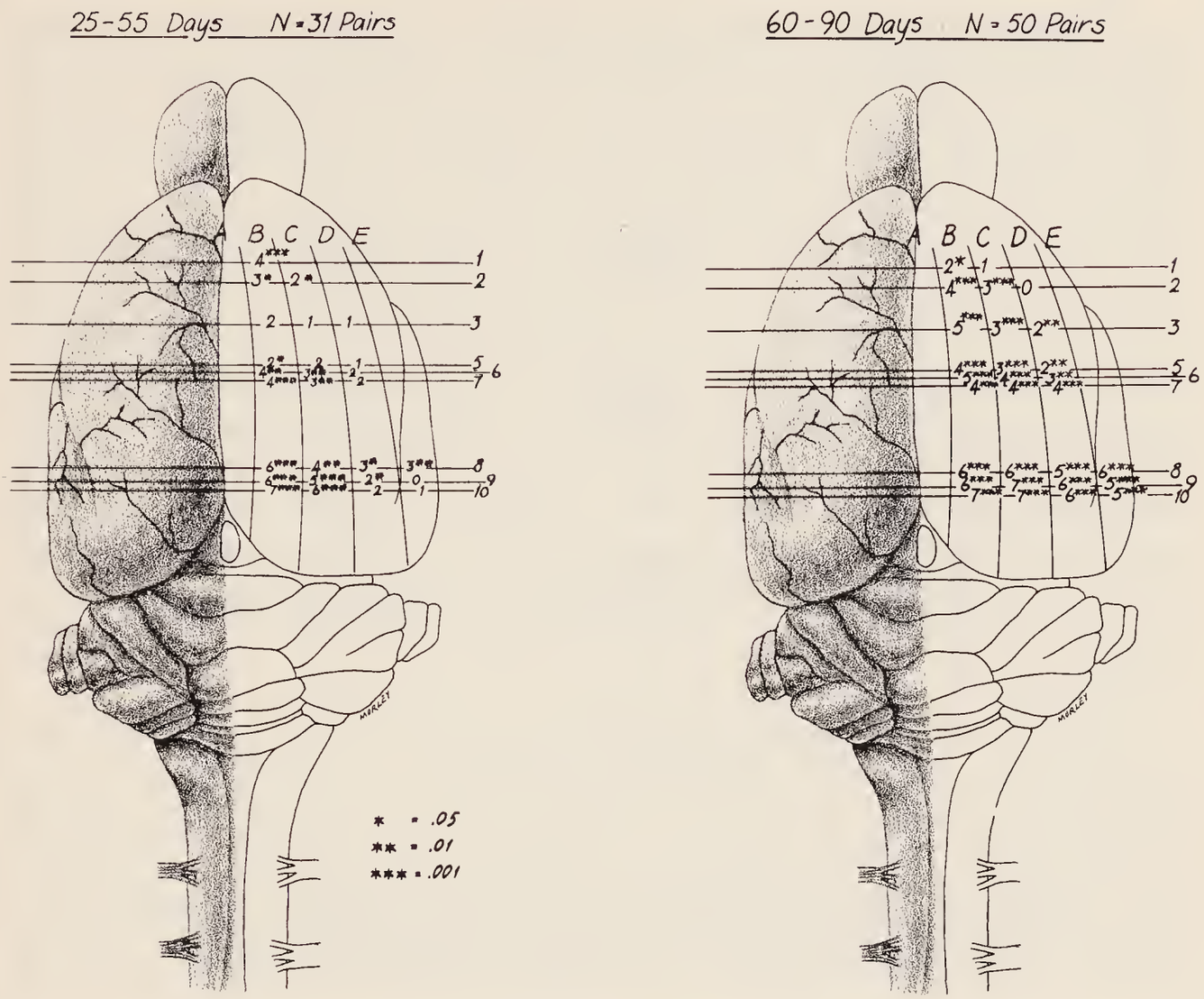

Figure 5. Left: Percent differences in cortical depth measures between rats in enriched or impoverished environment from 25 to 55 days of age. Right: Percent differences for rats in the same conditions also for 30 days, but from 60 to 90 days of age. From Diamond *et al.* (1972).

measured for cortical depth changes. The numbers on the brain represent the percent differences in cortical depth changes between enriched and impoverished environments. Figure 4 represents the cortical depth changes after two different 80-day periods, from 25 to 105 days and from 105 to 185 days. In both of these periods, the greatest cortical depth differences are seen in the occipital cortex, but in none of the sections compared was the cortical depth significantly different between the two 80-day groups. Figure 5 shows the depth changes for two different 30-day periods, from 25 to 55 days and from 60 to 90 days. Even though it appears that the cortical depth changes are greater in the 60–90 day group, the results of the two 30-day groups do not differ significantly from one another except in the lateral occipital cortex (lines 8, 9, and 10, segments D and E). The young 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 most of the segments compared.

Figures 6, 7, and 8 compare the enriched and impoverished conditions with a third condition, the standard colony. In Figure 6, the two 30-day groups are presented, showing that impoverishment is manifest by a thinner cortex than that of the standard colony animal in most sections measured, whereas the effects of enrichment are most pronounced in the occipital cortex, sections 8, 9, and 10. The effects of enrichment appear to be greater, but are not significantly so, in the 60–90 day group than in the 25–55 day group. Figure 7 shows that, with 15 days' duration, effects of both enriched and impoverished environments are seen, but primarily effects due to impoverishment. With 4 days of experience, environmental effects are evident but for different reasons dependent on the age of the group. For example, the top part of Figure 8 shows that if animals are placed in their respective conditions at 26 days of age, primarily the effect of impoverishment is manifest; however, if adult animals enter their conditions at 60 days, the results, as seen in Figure 8 (bottom), indicate primarily an effect due to the enriched condition.

These data indicate the importance of several factors to be taken into consideration in correlating anatomy and behavior. The effects of age and duration of exposure to various environments have been known previously to bring about different patterns of behavior, but that brain structure is altered in different areas depending on age and duration is something new. Animals isolated immediately at weaning demonstrate abnormal behavior

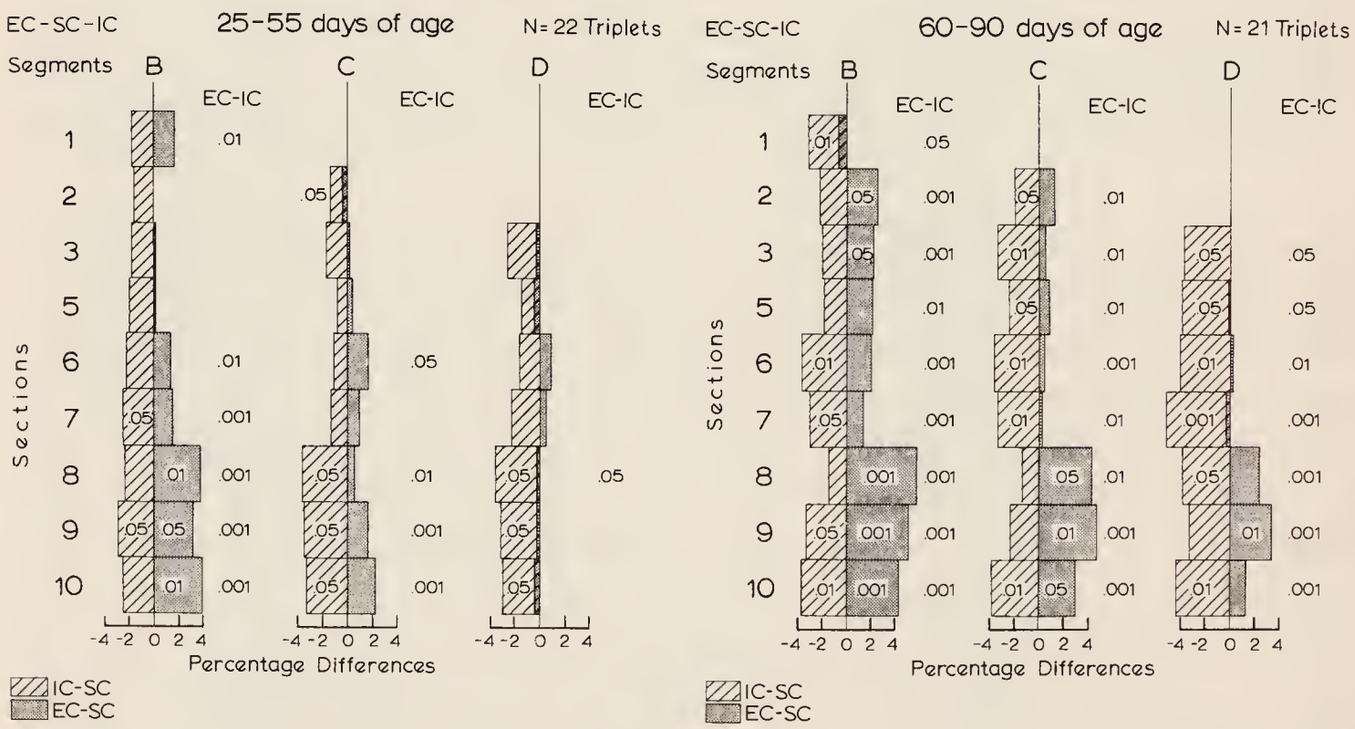

Figure 6. Left: Comparisons are made between the percent cortical depth differences from enriched, standard colony, and impoverished rats in their conditions from 25 to 55 days of age. The sections 1–10 are shown in Figures 4 and 5. The diagonally striped bars represent the effects of impoverishment compared to the standard colony rat and the stippled areas the effects of enrichment. Right: Percent cortical depth differences for another age group, from 60 to 90 days of age. From Diamond *et al.* (1972).

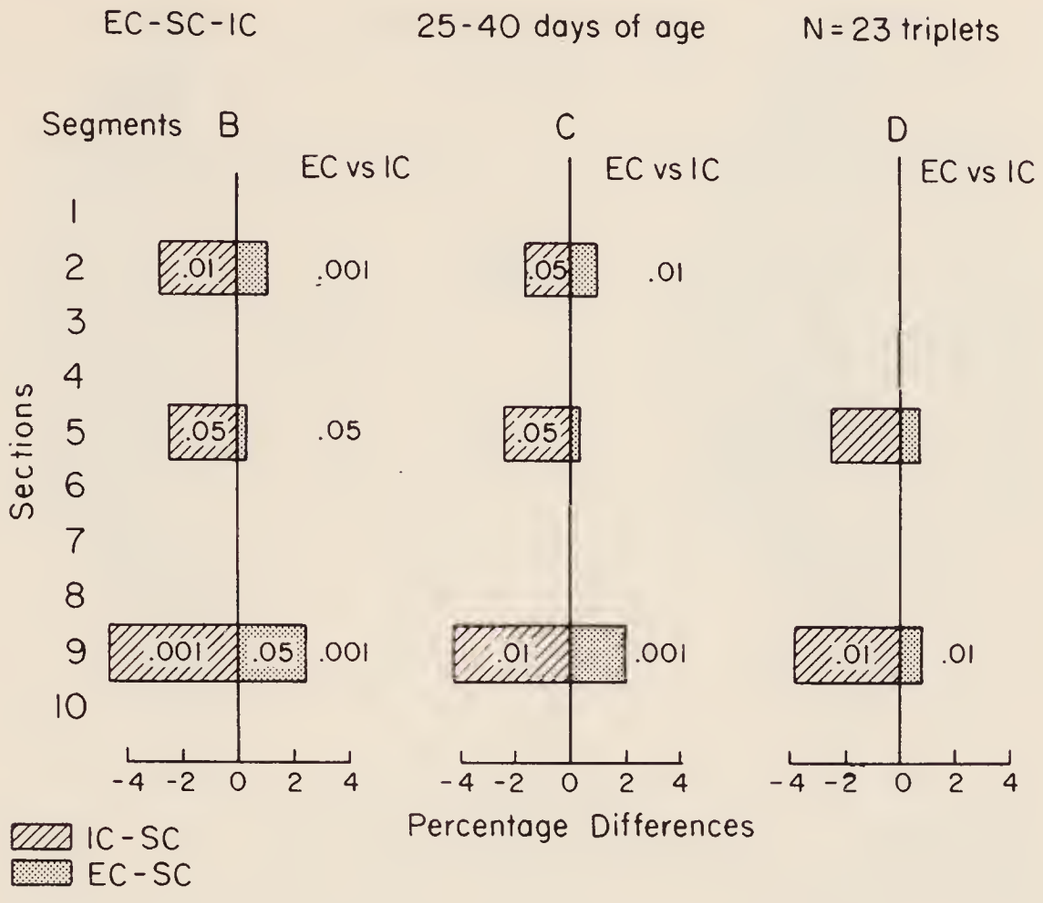

Figure 7. Percent cortical depth differences for three sections, 2, 5, and 9, from enriched, standard colony, and improverished rats in their environments from 25 to 40 days of age. From Diamond *et al.* (1975*b*).

during adulthood. The present results with rats have shown that isolation for 4 days after weaning reduces cerebral cortical structure, but if the rats are continued in the enriched or the impoverished environment for 30 days the effects of isolation remain and the effect of enrichment eventually develops. Similarly, if rats enter the enriched or the impoverished condition at 60 days of age, only the effects of enrichment are measurable after 4 days, but after 30 days effects of isolation also appear.

Effects of Environment on the Cortical Depth of Castrated Male Rats

Since it had been shown in the Long-Evans strain of rats that the occipital cortical depth differences induced by enriched or impoverished environment in the male rat are greater than those in the female rat (Diamond *et al.*, 1971), we (McDonald and Diamond, unpublished data) were interested in learning if hormones secreted by the testis were responsible for this cortical depth difference. Two groups of animals were studied, those castrated at birth before the brain was essentially male, and those castrated at 30 days of age. Both groups were placed in an enriched or impoverished environment

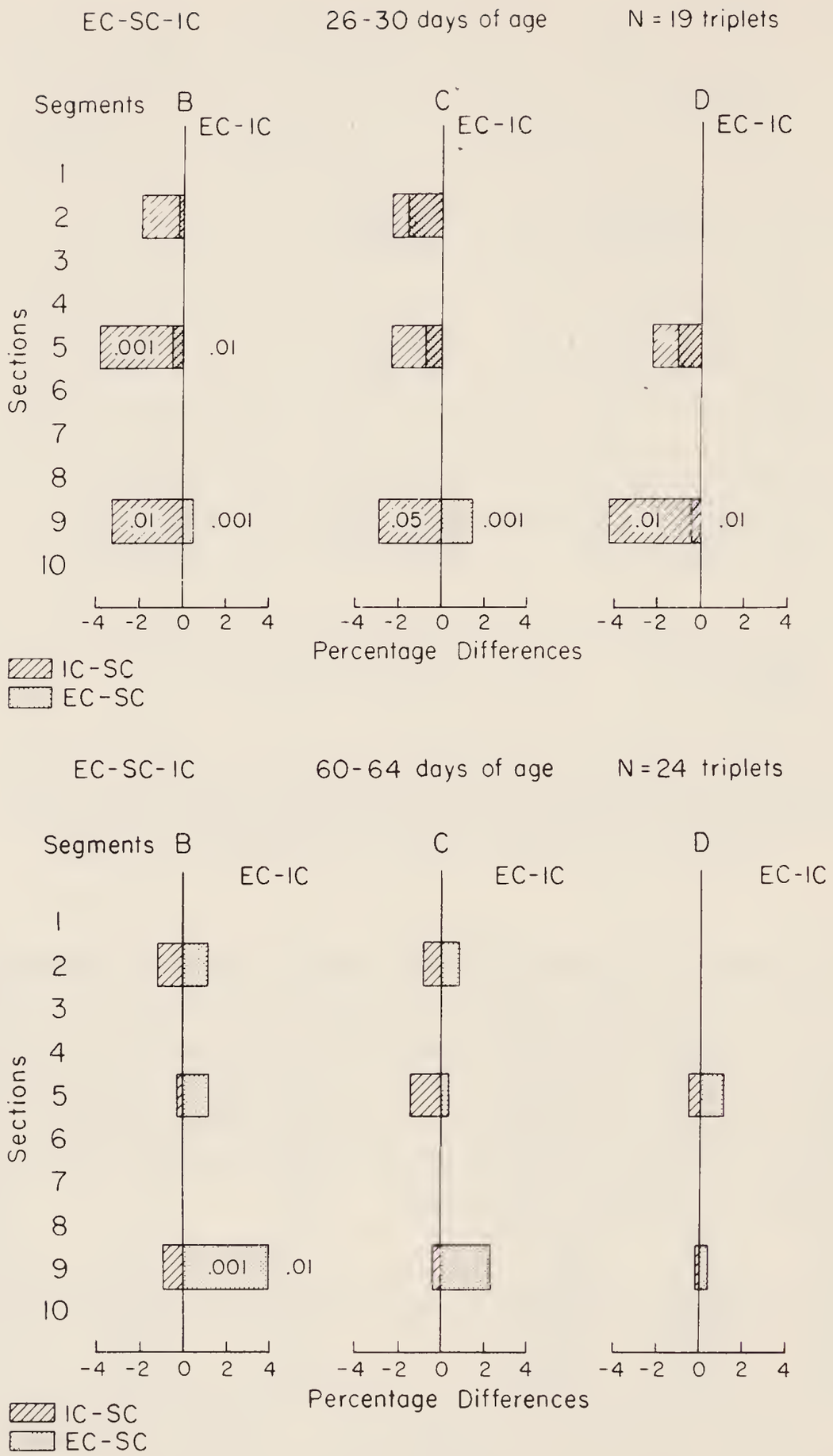

Figure 8. Top: Percent cortical depth differences for three sections, 2, 5, 9, from enriched, standard colony, and impoverished rats in their environments from 26 to 30 days of age. Bottom: Same comparisons made for animals in their environments also for 4 days, but from 60 to 64 days of age. From Diamond *et al.* (1975*b*).

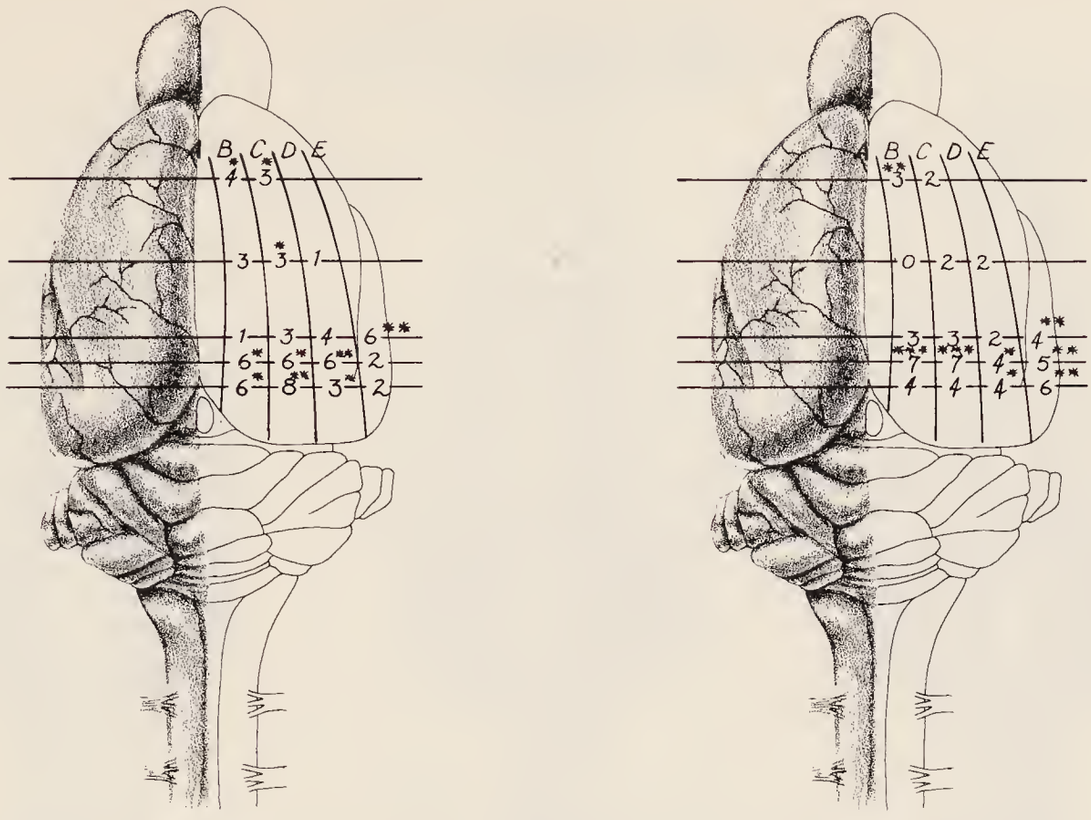

Figure 9. Left: Percent cortical depth differences between enriched and impoverished animals castrated at birth (N = 11 pairs). Right: Percent cortical depth differences between enriched and impoverished animals castrated at 30 days of age (N = 12 pairs). From McDonald and Diamond (1974).

from 60 to 90 days of age, and both groups clearly displayed the environmental effects seen with the intact male animal (Figure 9), indicating that something other than the male hormones was responsible for the male and female cortical depth differences. Experiments are now in progress to determine the brain changes in ovariectomized enriched and impoverished rats.

Effects of Environment on the Cortical Depth of Female Rats

That sex hormones may change the brain is not a new notion. Some 90 years ago, Gowers (1885) suggested that sex hormones affect brain function, when he noted that epileptic seizures varied with the phase of the menstrual cycle. In our research dealing with environment and brain changes, we did not anticipate sex hormones to play an important part, for in 1971 Rosenzweig, Bennett, and Diamond showed that enriched and impoverished conditions can cause brain changes in the absence of the pituitary gland in male rats when the hormones stimulating the gonads are reduced. That sex hormones may play a role in our environmental changes was discovered quite by accident. The experiment shedding light on this subject derived from a study designed to examine the brains of offspring from parents experiencing varied environmental conditions. Before examining the brains of the off-

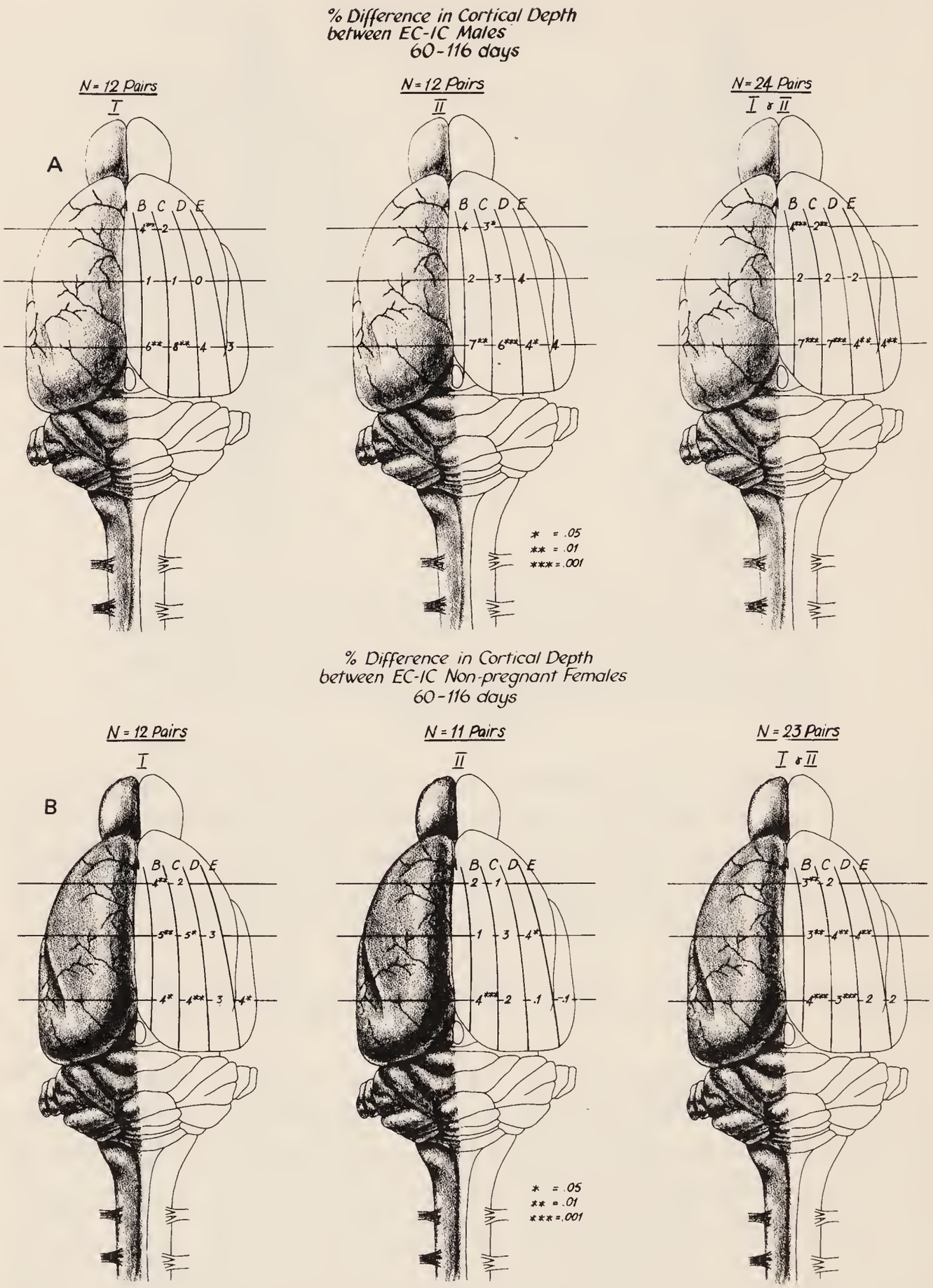

Figure 10. This figure is divided into three sections, A, B, and C. A: Percent cortical depth differences between enriched and impoverished Long-Evans male rats in their conditions from 60 to 116 days of age. I represents results from the initial experiment, II results from the replication experiment, and III the combination of results from I and II. B: Percent cortical depth differences for nonpregnant Long-Evans female rats. C: Percent cortical depth differences for the postpartum females. From Diamond *et al.* (1971).

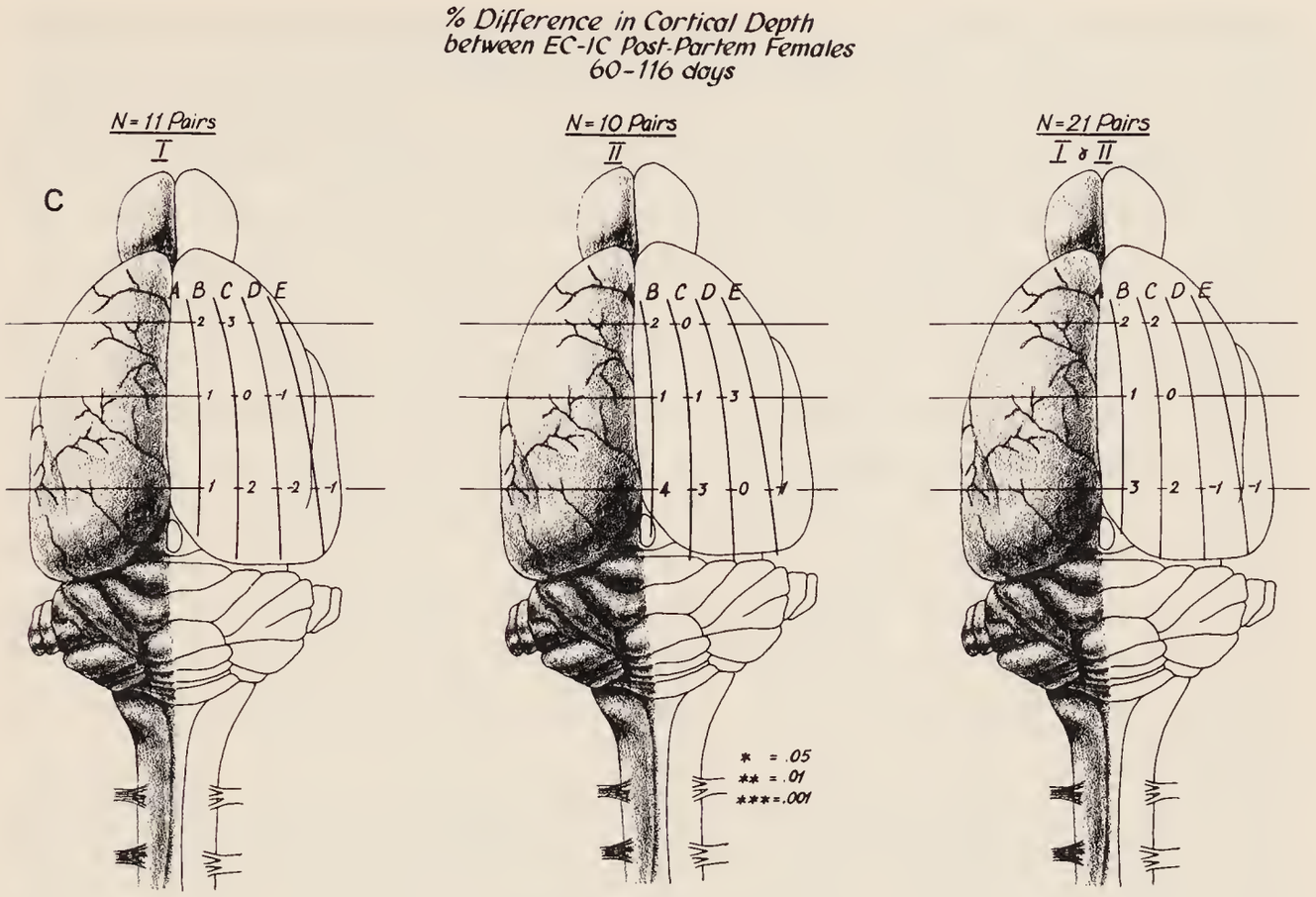

Figure 10 (continued).

spring, it was necessary to confirm morphological changes in the brains of both sets of parents. The changes in the male brains were confirmed, but an unexpected result concerning the postpartum female brain altered the direction of the experimental approach. A lack of experimental difference between the enriched and impoverished postpartum females was observed (Diamond *et al.*, 1971) (Figure 10). In the nonpregnant female, significant depth differences were found in the medial area of the frontal, somesthetic, and occipital cortex. The differences found in the occipital cortex in the nonpregnant female were significantly smaller than those found in the Long-Evans male of the same age. However, in the nonpregnant female significant differences were seen in the somesthetic area that were not present in the male of the Long-Evans strain (Figure 10). Why the male occipital cortex responds more than does the female occipital cortex while the female somesthetic cortex responds more than the male somesthetic cortex is not clear. Of course, these results offer opportunity for speculation about differences between male and female brains, with one being more responsive to visual sensations and the other to peripheral sensations, but at present no valid scientific measures support these speculations.

To return to the lack of cortical depth differences between enriched and impoverished postpartum rats, more information is now available than at the time of the original publication in 1971. In an attempt to understand the reasons for the changes in the impoverished pregnant rat's brain, we (Hoover

and Diamond, 1976) have just completed a preliminary study measuring the effects of norethynodrel, a synthetic progesterone-like compound, on Na, water, Cl, and K content in the occipital cortex of enriched and impoverished rats. Half of the animals in each environment were given norethynodrel, and the other half served as controls. Norethynodrel administration altered the response of the cortex to environmental enrichment. Norethynodrel-treated animals in the enriched environment had a thinner cortex and less Na in the visual cortex than did the EC controls. No differences in cortical water and electrolytes were seen between control and norethynodrel-treated animals in the impoverished environment. These data suggest that norethynodrel altered the ability of the cortex to respond to the enriched environment but had no effect on the cortex of the impoverished animals. Obviously, more work needs to be done here to determine the meaning of these important implications. In the meantime, all of these data have indicated that different external environments influence hormonal action on brain structure and chemistry.

From the original report on effects of pregnancy on rats in different environments, it was evident that the brain from the impoverished pregnant rats had reached the depth of not only the enriched female cortex but also the impoverished male cortex. That the impoverished pregnant female cortex reached the depth of the male cortex but did not surpass it causes one to question whether there is a "ceiling effect" in cortical depth. At no time have the environmental conditions stimulated the cortical depth of the postweaned rat to go beyond 10%. As mentioned previously, a 16% difference in cortical depth was encountered between the enriched and the impoverished preweaned rat, at the time when normal maturation was apparently being accelerated. In the future, postweaned rats could possibly be exposed to more rigorous environmental conditions to determine if larger depth differences can be induced. (I have always said that one of my dreams was to cause the rat cortex to form its first convolution!)

Effects of Environment on the Hippocampus of Male Rats

The hippocampus is known to be associated with learning and memory. For example, hippocampal damage has been found to retard performance on complex mazes, discrimination reversal, and alternation (Thomas *et al.*, 1968; Kimble, 1968; Isaacson and Kimble, 1972). Thus it appeared a natural area of the brain to examine in relation to enriched and impoverished environments. Walsh *et al.* (1969) found a 6% ($p < 0.05$) difference in hippocampal depth between eight pairs of enriched and impoverished rats. More recently, we have completed an extensive study with 224 pairs of postweaned rats

comparing the hippocampal depth between enriched and impoverished animals for many age groups and varied durations. At no time were significant changes in hippocampal depth encountered (Diamond *et al.*, 1975*b*) (Table 1). It is not clear why Walsh *et al.* (1969) were able to show hippocampal differences; whereas, no significant differences were found in our laboratory. Perhaps the entorhinal cortex and not the hippocampus should be measured in enriched and impoverished animals, for McLardy (1970) reported that only when the entorhinal cortex was removed was a deficit seen in recent memory; no deficit occurred with bilateral removal of the hippocampus or fascia dentata. Recent evidence does show the entorhinal cortex responds to an enriched environment (Diamond *et al.*, 1976*b*).

Effects of Environment on Cortical Cell Number

The changes occurring in the cerebral cortical depth measurements as a consequence of environment having been observed, a logical next step is examination of the constituents within the cortex which could be responsible for such depth changes. Determining the number of cells in an area of the cortex is one consideration. We hope that mechanized techniques will soon be used accurately to differentiate cell types in the brain. At the present, nothing is more effective than the human eye to detect the subtle differences in size and density of the cell bodies in the cerebral cortex. The classification of all the glia in between the morphologically clearly distinguished glia creates a major problem. The differentiation between endothelial cells and certain glia

Table 1. Percent Differences in Hippocampal Depth between Environmentally Enriched and Impoverished Rats

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

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

is another hindrance to precise, automated brain cell counts. In the meantime, three methods of cell counts have been employed on brains from animals in their environments from 25 to 105 days of age: (1) The number of neurons and glia were counted in each microscopic field, proceeding from the pia surface to the corpus callosum (Diamond *et al.*, 1964). (2) An autoradiographic examination was made of the rate of glia multiplication due to an enriched environment (Altman and Das, 1964). (3) Overlapping photomicrographs were combined into one composite picture of an area of the occipital cortex 1.00 by 0.75 mm. Differential cell counts of neurons and glia were made from each animal (Diamond *et al.*, 1966). Although neurons were counted in both the microscopic and the composite picture methods, no significant differences in the number of neurons were detected between the two experimental groups of animals. By the initial method of counting cells in each microscopic field, a higher glia/neuron ratio in the enriched brain was seen, for the decrease in glia per microscopic field was significantly less than the decrease in neurons. By the autoradiographic technique, the investigators reported an increase in labeled cells in the cortical radiation and in the corpus callosum of the enriched animals but did not attempt to differentiate types of cells. By the examination of the cells in the enlarged, composite picture, an absolute increase in glial cells in the enriched brain was found, compared to the impoverished brain, giving a higher glia/neuron ratio of 16% ($p < 0.02$). Essentially all three methods have led to the same conclusion that there is a difference in glia count between the brains of enriched and impoverished animals. It is yet to be determined whether enrichment or impoverishment is responsible for this difference by comparing the counts with those from a standard colony littermate. For the 25–105 day age group, the differences in depth measures are due more to the enriched condition than to the impoverished condition, so one might predict the glia differences also will follow this direction. The difference in glia counts may be attributed to any one of a number of functions mentioned previously (Diamond *et al.*, 1966): to supply additional neuronal nutritional needs, to support newly formed fibers, to regulate ionic movement in the brain, to give additional specificity to synaptic membranes, to respond to axoplasmic movement, to regulate vascular flow, or to encode experience.

Effects of Environment on Neuronal Nuclear and Perikarya Areas

In one initial experiment (Diamond *et al.*, 1966), we expressed concern over the lack of increase in perikarya area in the enriched rats, for we had hypothesized that the cortical depth increases might be due in part to dendritic branching, which presumably would demand greater perikarya dimen-

sions. But from camera lucida drawings we could find no significant differences between the enriched and impoverished groups. However, with the projection of the cells using a microfilm reader, with the larger image to measure, significant differences in both perikarya area and in the nuclei of the perikarya were found, 17% ($p < 0.001$) and 20% ($p < 0.01$), respectively (Diamond, 1967). These large cellular changes were found in brains from animals in enriched and impoverished conditions from 25 to 105 days of age. In 1971, Malkasian and Diamond reported perikarya and nuclear changes of 16% and 19%, respectively, in the somatosensory cortex of preweaned rats living in enriched and impoverished environments from 6 to 26 days of age. In more recent experiments (Diamond *et al.*, 1975*b*), perikarya and nuclear dimensions have been measured in rats living in the environmental conditions for 30 days, from 25 to 55 and from 60 to 90 days of age, and in an older 80-day group, from 105 to 185 days of age. However, in none of these groups were significant differences between enriched and impoverished animals found in the nuclear area. Only nuclear area was measured because of the high positive correlation found between it and perikarya area in the 25–105 day group. In only two durations measured, from 25 to 105 and from 60 to 90 days, do the perikarya and nuclei show changes in response to external environment; in the former age group both nuclei and perikarya show changes, and in the latter only the perikarya.

Effects of Environment on Dendritic Branching

In 1966, Holloway investigated whether the dendritic branches of stellate neurons in the occipital cortex were responding to environmental manipulation from 25 to 105 days of age. He found that they were. In a later experiment in small, medium, and large pyramidal neurons and in stellate neurons, enriched rats consistently had more higher-order dendritic branches than their impoverished littermates (Greenough and Volkmar, 1973). The standard colony rats were closer to the impoverished animals in higher-order branching. In the pyramidal neurons, the branching differences occurred primarily in the basal dendrites. No consistent differences in the length of branches were found by either Holloway or Greenough and Volkmar. In examining areas other than the occipital cortex, Greenough *et al.* (1973) could not detect any dendritic differences in the frontolateral cortex, but in the temporal cortex smaller but reliable differences were seen in layer 5 pyramidal cell basal dendritic branching. We are presently collaborating with Smit and Uylings, Brain Research Institute, the Netherlands, to determine if dendritic branching occurs in older rats as a consequence of the enriched and impoverished environments (from 112 to 142 days of age). In this experiment, the enriched

rats have been exposed to the seminatural enriched condition at the University of California Field Station for Research in Animal Behavior rather than the usual laboratory enrichment cages, in an attempt to produce significantly greater anatomical changes than those formed in the laboratory. The seminatural condition consists of outdoor enclosures having a dirt-covered concrete base 30 by 30 ft and a screen over the top. Significant increases in dendritic branching have been found in older enriched animals (Uylings *et al.*, 1976).

Effects of Environment on Dendritic Spines and Synapses

Speculations have been made for a long time pointing to the synapse as a primary site concerned with memory mechanisms. Many of the synapses in the cerebral cortex are found on dendritic spines. In an attempt to understand the role of the dendritic spine in the brain's response to enriched and impoverished environments, Globus *et al* (1973) counted spines on basal, apical, and oblique dendrites from the occipital cortical pyramidal neurons. Only the basal dendrites from the enriched animals had a greater number of spines when compared with basal dendrites from impoverished animals, implying an increased number of intracortical connections. The logical next step was to measure the environmentally induced morphological change at the region of functional interaction between two nerve fibers, namely at the synapse. The first experiments of Mollgaard *et al.* (1971) examined synapses at the electron microscopic level and provided evidence that the structure of the synapse can be altered by environment. The length of the postsynaptic thickening in asymmetrical synapses in layer III of the occipital cortex was shown to be considerably longer in the enriched animal than in the impoverished. Not only were the synapses longer but also they were fewer per unit area in the enriched animals compared to the impoverished animals exposed to their environments from 25 to 55 days. These results, showing the plasticity of the synapse, were encouraging, but they did not indicate clearly whether the enriched or the impoverished condition was responsible for the synaptic changes, so it was necessary to repeat the experiment comparing both conditions with a third, the standard colony condition.

Recent (Diamond *et al.*, 1975*b*, 1976*b*) initial and replication experiments studied synapses in enriched, impoverished, and standard colony animals from 25 to 55 days of age. Although the large differences shown by Mollgaard and colleagues were not repeated, an 8% ($p < 0.01$) difference in postsynaptic thickening length in layer IV between 675 synapses from enriched rats and 680 synapses from impoverished rats was found. This synaptic difference was due primarily to the impoverished condition, as shown by comparison with standard colony littermates (Table 2). A smaller sample from layer III

Table 2. Percent Difference in Postsynaptic Length for EC-SC-IC Using All Animals Studied

	Number of animals	Number of synapses	Length of synapse (mm)	Condition compared	Percent difference	*P*
Expt. I						
EC	7	306	11.4	EC-SC	0.1	N.S.
SC	7	306	11.3	SC-IC	6.7	<0.10
IC	8	359	10.6	EC-IC	6.8	<0.05
Expt. II						
EC	10	369	12.2	EC-SC	1.5	N.S.
SC	9	312	12.0	SC-IC	5.4	N.S.
IC	8	321	11.4	EC-IC	6.9	<0.10
Expts. I + II						
EC	17	675	11.8	EC-SC	1.1	N.S.
SC	16	618	11.7	SC-IC	6.3	<0.05
IC	16	680	11.0	EC-IC	7.5	<0.01

showed changes of the same order of magnitude as those in layer IV. The number of synapses per unit area of neuropil was 15% ($p < 0.01$) more in the impoverished than in the enriched animals, and this difference was primarily due to impoverishment. Thus, environmental experience does significantly affect synaptic length and number. That synapses can be modified by environment has also been supported by West and Greenough (1972), who measured not only the postsynaptic thickening length but also the bouton diameter and the length of the region of tight aposition of the pre- and postsynaptic processes in type 1 synapses in layers I, II, and VI of the occipital cortex. In these studies, the only measure showing significant differences was the length of the postsynaptic thickening, with the enriched rats having the greater length by 14%.

The results of Mollgaard *et al.* (1971), West and Greenough (1972), and Diamond *et al.* (1975*b*) all indicate that synapses respond to environmental changes by either increasing or decreasing their dimensions. In 1971, Soleto and Palay also concluded that synaptic rearrangements are continuously taking place in the brain even under so-called normal conditions. Raisman (1969) demonstrated that the central nervous system is capable of synaptic rearrangements after a destructive lesion has been placed in the brain. Along similar lines, Lynch *et al.* (1973) have provided data indicating different forms of postlesion growth in the hippocampus of the rat. All of these combined sources support the idea that the synapse is constantly subject to change in response to the external environment.

Duration of Anatomical Effects

By examining one region of the cortex, the medial occipital area, from animals exposed to the different environments at different ages and for varying periods of time, the periods of maximum response to the environments become evident (see Table 3). Animals in the environments for 4 and 7 days develop 3–4% enriched vs. impoverished differences, but by 15 days a 7% difference is seen which is as large a difference as that found with the longer durations, 30 or 80 days. However, if animals are kept in their conditions for 160 days, differences greater than 7% do not develop, but actually are less, becoming more similar to the values seen with the short durations, 4–7 days. From these data, it is apparent that the occipital cortical depth difference between enriched and impoverished rats becomes diminished after 80 days.

If one examines the data from the somesthetic cortex in contrast to the occipital cortex, for the various age groups and for the different durations, only two groups, from 25 to 55 days and from 60 to 90 days, show significant depth differences between enriched and impoverished environments. No distinct increase in cortical depth is seen in the somesthetic area as the duration of the experiment increases, for at 4 days a 3% significant difference is seen and at 30 days a 3–4% significant difference is present, with no further increase for a longer duration. Evidently, one cannot speak of general overall cortical changes due to environmental stimulation for different periods, for some cortical regions indicate greater differences with longer durations, and some do not.

Table 3. Percent Differences in Medial Occipital Cortical Depths between Environmentally Enriched and Impoverished Rats

Duration in days	Ages	Percent differences	Number of EC>IC (pairs)	*P*
1	60–61	0.7	10/24	N.S.
4	60–64	2.9	17/24	0.01
4	26–30	3.7	15/20	0.001
7	25–32	3.8	19.5/22	0.001
15	25–40	7.1	22/23	0.001
30	25–55	6.6	25/30	0.001
30	60–90	6.7	45.5/50	0.001
80	25–105	7.9	22/22	0.001
80	105–185	5.0	13/18	0.05
160	25–185	3.0	16/22	0.05
		Total	205/255	
	Total minus the 1-day group		195/231	

Summary

What do all of these anatomical changes mean with regard to brain function? Knowledge of an increase or a decrease in the size of a structure is not necessarily of great value unless a functional correlation can be made. However, size changes by themselves within specific areas of the brain are useful as regional indicators of continued functional interaction. With the somesthetic cortex responding differently to an enriched environment than the occipital cortex, and the medial area differently from the lateral, there is little wonder that it is difficult to design a behavioral test to determine the functional significance of the effect of a multiple environment. In attempts to correlate behavioral performance with increased cortical structure, the most frequently cited finding indicates that the enriched rat makes fewer errors in less time in a reversal discrimination test than does the impoverished rat (Krech *et al.*, 1962). However, LeBoeuf and Peeke (1969) and Peeke *et al.* (1971) found no effect on Lashley III maze learning between animals from enriched and impoverished environments.

In a recent attempt to refine the experimental approach and to correlate anatomical changes with learning, rats were given a series of maze patterns in increasing complexity, and the cortical depth of a trained rat was compared with that of an untrained but maze-familiar littermate. Preliminary results indicate cortical depth differences between trained and untrained littermates (Diamond, Malkasian, and Johnson, 1974, unpublished). In one sense, the training in this experiment could be referred to as learning, and yet, on the other hand, it could be called enrichment of environment. How does one really establish an endpoint which is clearly defined as learning? With all of the effort that has gone into brain research correlated with learning, few sharply defined end points are available. It is, perhaps, at this time that one turns to simple animals, with a less complicated appearing system of nerve cells, but even here, with fewer cells, the limited number of cells must tackle some tasks carried on by many cells in the more complex animals. So which is actually the simpler system? At present, no one approach with invertebrates or vertebrates will clarify "how the brain learns." Investigators will continue to add peripheral and central knowledge to the pile and eventually the pieces will fit together, possibly to show a very simple mechanism.

In conclusion, the work concerned with enriched and impoverished environments and brain structure has shown that the brain grows with use (see Table 4). Since the brain consists partly of nerve cells, studies on the neurons have demonstrated that every part of the cell measured to date, from the nucleus to the synapse, can increase or decrease depending on whether the environment is stimulating or not. We have learned that certain parts of the cortex respond by changing size when the duration of exposure to

Table 4. Summary of Anatomical Changes Measured in the Medial Occipital Cortex

Measurement and condition	*N* (pairs)	Experimental duration in days	Percent difference	*P*
I. Cortical depth differences between enriched and impoverished rats				
A. Preweaned Long-Evans males	7	6–14	2	N.S.
	7	6–19	7	0.01
	19	6–26	9	0.01
B. Postweaned S_1 males	24	60–61	0.7	N.S.
	20	26–30	4	0.001
	24	60–64	3	0.01
	22	25–32	4	0.001
	23	25–40	7	0.001
	30	25–55	7	0.001
	50	60–90	7	0.001
	22	25–105	8	0.001
	18	105–185	5	0.05
	22	25–185	3	0.05
C. Castrated Long-Evans males, neonates	11	60–90	6	0.05
D. Castrated Long-Evans males, at 30 days of age	12	60–90	7	0.001
E. Nonpregnant Long-Evans females	23	60–116	4	0.001
F. Postpartum Long-Evans females	21	60–116	3	N.S.
G. Newborns from Long-Evans enriched and impoverished parents	30	95–116	0	N.S.
II. Cortical depth differences between enriched and standard colony postweaned S_1 males	20	26–30	0.5	N.S.
	24	60–64	4	0.001
	23	25–40	3	0.05
	22	25–55	3	0.05
	21	60–90	5	0.001
III. Cortical depth differences between standard colony and impoverished S_1 males	20	26–30	3	0.01
	24	60–64	1	N.S.
	23	25–40	5	0.001
	22	25–55	2	0.05
	21	60–90	4	0.05
IV. Difference in glial number between enriched and impoverished S_1 males	17	25–105	14	0.01
V. Difference in neuronal area between enriched and impoverished S_1 males	12	60–90	4	0.05
	20	25–105	12	0.001

Table 4. (Continued)

Measurement and condition	*N* (pairs)	Experimental duration in days	Percent difference	*P*
VI. Difference in neuronal nuclear area between enriched and impoverished S_1 males	20	25–55	2	N.S.
	19	60–90	2	N.S.
	20	25–105	12	0.001
	18	105–185	−1	N.S.
VII. Difference in postsynaptic length between				
Enriched and standard colony S_1 males	13	25–55	1	N.S.
Standard colony and impoverished S_1 males	13	25–55	6	0.05
Enriched and impoverished S_1 males	13	25–55	8	0.01

the environmental condition is long, and others when the duration is short. Essentially, the brain increases in size with exercise as does skeletal muscle, but size alone does not tell how the muscle functions. The mechanism by which actin and myosin interact explains how the muscle contracts. Is there any reason to believe that a nerve cell which has increased in size with use is a center for memory storage? Perhaps the increase in size in the nerve cell means that all existing components of the cell have increased in activity to form new connections which are possibly the site of memory. By constant use of a newly formed or enlarged, existing synapse, memory is retained, and with disuse it is lost. The memory mechanism cannot be as simple as this, for on this basis how does one explain the retrieval of long-term memory, memory not used for decades? Not only by the presence or absence of a synapse, for the synapse structure can be modified within days. Perhaps a synapse does not completely disappear with disuse. Is there a minimum size a synapse can reach and still serve as a memory site? An investigation on the persistence of synaptic changes might prove useful in helping to understand further the structural changes related to memory functions. We have only just begun, with the real questions and answers still ahead.

References

Altman, J., and Das, G. D. 1964. Autoradiographic examination of the effects of enriched environment on the rate of glial multiplication in the adult rat brain. *Nature (London)*, *204*, 1161–1163.

Brizzee, K. R., and Jacobs, L. A. 1959. Early postnatal changes in neuron packing density and volumetric relationships in the cerebral cortex of the white rat. *Growth*, *23*, 337–347.

Brizzee, K. R., Sherwood, N., and Timiras, P. 1968. A comparison of cell populations at various depth levels in cerebral cortex of young adult and aged Long-Evans rat. *Journal of Gerontology*, *23(3)*, 289–297.

Diamond, M. C. 1967. Extensive cortical depth measurements and neuronal size increases in the cortex of environmentally enriched rats. *Journal of Comparative Neurology*, *131*, 357–364.

Diamond, M. C., Krech, D., and Rosenzweig, M. R. 1964. The effects of an enriched environment on the histology of the rat cerebral cortex. *Journal of Comparative Neurology*, *123*, 111–119.

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 number in rats subjected to enriched environment. *Journal of Comparative Neurology*, *128*, 117–125.

Diamond, M. C., Johnson, R. E., and Ingham, C. A. 1971. Brain plasticity induced by environment and pregnancy. *International Journal of Neuroscience*, *2*, 171–178.

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. *Journal of Neurobiology*, *3(1)*, 47–64.

Diamond, M. C., Johnson, R. E., and Ingham, C. A. 1975*a*. Morphological changes in the young, adult and aging rat cerebral cortex, hippocampus, and diencephalon. *Behavioral Biology*, *14*, 163–174.

Diamond, M. C., Lindner, B., Johnson, R. E., Bennett, E. L., and Rosenzweig, M. R. 1975*b*. Differences in occipital cortical synapses from environmentally enriched, impoverished and standard colony rats. *Journal of Neuroscience Research*, *1(2)*, 109–119.

Diamond, M. C., Johnson, R., Ingham, C., Rosenzweig, M., and Bennett, E. L. 1975*c*. Effects of differential experience on neuronal nuclear and perikarya dimensions in the rat cerebral cortex. *Behavioral Biology*, *15*, 107–111.

Diamond, M. C., Johnson, R. E., and Gold, M. 1976*a*. Differential cell counts in young adult and aging rat cerebral cortex. Unpublished.

Diamond, M. C., Ingham, C. A., Johnson, R. E., Bennett, E. L., and Rosenzweig, M. R. 1976*b*. Effects of environment on morphology of rat cerebral cortex and hippocampus. *Journal of Neurobiology*, *7(1)*, 75–86.

Eayrs, J. T. 1951. The factors governing the opening of the eyes in the albino rat. *Journal of Anatomy*, *85*, 330–337.

Enesco, H. 1967. A cytophotometric analysis of DNA content of rat nuclei in aging. *Journal of Gerontology*, *22*, 445–448.

Globus, A., Rosenzweig, M. R., Bennett, E. L., and Diamond, M. C. 1973. Effects of differential experience on dendritic spine counts in rat cerebral cortex. *Journal of Comparative and Physiological Psychology*, *82*, 175–181.

Gowers, W. R. 1885. *Epilepsy and Other Chronic Convulsive Diseases*. William Wood & Co., New York.

Greenough, W. T., and Volkmar, F. R. 1973. Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Experimental Neurology*, *40*, 491–504.

Greenough, W. T., Volkmar, F. R., and Juraska, J. M. 1973. Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of the rat. *Experimental Neurology*, *41*, 371–378.

Holloway, R. L., Jr. 1966. Dendritic branching: Some preliminary results of training and complexity in rat visual cortex. *Brain Research*, *2*, 393–396.

Hoover, D., and Diamond, M. C. 1976. The effects of norethynodrel administration on the