

out that the brain and cranial cavity might enlarge even after the sutures were completely closed. He believed that it was possible for the cranium to grow by the *internal absorption of bone and the laying down of bone on the external surface*. Intracranial capacity might, thus, be able to increase in enriched young adult animals, which exhibit greater thickness and weight in the cerebral cortex. However, since no intracranial differences were found between the two experimental conditions, it appeared that either the slight growth of the brain could be accommodated by a decrease in the volume of cerebrospinal fluid between the meninges surrounding the brain, or the internal ventricles decreased in size, or the cranial cavity did enlarge but our methods were not sensitive enough to detect it.

Though the internal cranial dimensions were not measurably changed by the environmental conditions, the effects on the external skull dimensions were quite marked. The isolated animals had the larger external skulls. Even in as short a period as 8 days, there were two differences which were statistically significant at the 0.05 level: the width of the upper jaw (bimaxillary width) and the width of the cheek bones (bizygomatic width). After 77 days in the environmental conditions, both the bimaxillary and bizygomatic widths were greater in the isolated than in the enriched by as much as 10% ($p < 0.001$). However, these external skull dimensions simply reflected changes in body weight; not anything occurring within the brain was causing these changes. This fact was confirmed by the high correlations between body weights and facial bone measurements after both 8 and 77 days in the experimental conditions.

In other 80-day experiments (on rats that had lived in the experimental conditons between the ages of 25 and 105 days), the body weights of the impoverished animals were always greater than those of the enriched. It was only when the duration of the experiments was shortened to 30 days that the body weight differences disappeared. In this present experiment dealing with skull measurements, the impoverished animals were significantly greater in both body weight and size of the facial bones. Yet their intracranial dimensions were similar to those of the enriched rats.

An explanation for this regional difference in skull development is offered by Weidenreich (102), who reported a negative correlation between size of brain case and size of face: the larger the former, the smaller the latter, and vice versa. Since the jaw was the essential constituent of the face, he reported, a larger face implied the development of a large and strong masticatory apparatus. It is very possible that the isolated rats

had little to do but chew on the cage as well as on their hard food pellets and, thus, developed a greater body weight with an accompanying larger jaw.

Not only were the facial bones of the isolated animals larger than those of the enriched rats, but the skulls of the isolated animals were heavier. The difference may have resulted from an overall increase in skeletal weight, which in turn may have accounted in part for the total body weight increase noted in the isolated animals. It is well known that restricted activity is a method used in agriculture to fatten animals, and obesity has been produced in laboratory animals by restriction methods (103). However, Donaldson (104) reported that isolated animals grow less well than those kept together. From our experiment, it is apparent that the isolated animal, in its smaller cage, becomes heavier in bone weight as well as in weight of soft tissues than the more active, enriched animal in the larger cage.

Our data indicate that the length of time the animals spend in their condition is important for the reliability of a study of body weight differences. Weight differences were considerably more marked in the initial experiment than in the replication. In the initial experiment the body weights of the two experimental groups diverged greatly after 8 days, whereas in the replication experiment the early separation did not take place. In an attempt to understand this apparent inconsistency between the data from the two experiments, body weights from two additional experiments were examined. In one, the body weight differences were obvious very early, and in the other, they were not. It appeared that no consistent large difference in weight existed in the early stages of the experiments, but that eventually each isolated group became significantly heavier than the corresponding enriched group.

Our skull measurements have indicated that the intracranial dimensions of enriched and impoverished young adult animals (25 to 102 days of age) are identical. When the animals are older (112 to 142 days), we have clear evidence from studying a baseline group that the cortex grows in response to an enriched environment. In our younger groups (e.g., those living in enriched or impoverished conditions between the ages of 25 and 105 days or 60 and 90 days), we do not have the baseline group for comparison. With the conditions we have studied at the present time, it can be said that though the intracranial cavity is stable in relation to environmental conditions, the cortex does have the capacity to increase in dimensions. Body weight is positively correlated with external skull dimensions but not with intracranial size.

5

ENRICHMENT AND IMPOVERTISHMENT OVER THE LIFESPAN

The Prenatal Period

Because the brain is constantly changing through development and aging, environmental stimuli must have different effects throughout a lifespan. Now we had the opportunity to examine these effects based on the encouraging results obtained from our initial experiments. These findings in 1964, showing brain alterations after enriching or impoverishing the environment postnatally from 25–105 days of age, set the stage for the others to follow.

The first experiments established that many measurements taken on the cortex, from cortical thickness to glial cell number, neuronal cell size, and blood vessel dimensions, could change with experience during this particular time period. With our initial results in hand, we began to examine such factors as age, duration of exposure to different environmental conditions, and sex to determine the potential extent of brain plasticity under a wide variety of conditions.

To start with environmental influences during prenatal development, while the nervous system is actively forming, seemed a reasonable beginning. The concept of "intrauterine education" can be traced back many centuries. It is found in Chinese literature of over 2000 years ago. Today in Japan, women still practice *Taikyo*, a type of intrauterine enrichment which is believed to have a beneficial effect on the postnatal life of the yet unborn child (1). Our initial studies from the prenatal period have proved that it is possible to influence the structure of the cortex with environmental enrichment at this very young age.

We designed an experiment in the early 1970s to study the effects of enriched environments on the intrauterine development of our rats (2). Long-Evans rats used in this study were weaned at 21 days of age and then grouped three of like sex to single standard colony cages. At 60 days of age the animals of each sex were separated into enriched and impoverished conditions. At 90 days of age, males and females of like environments were selected at random and placed together in small cages, one pair to a cage, for mating. (Vaginal smears were taken after a few days to determine whether pregnancy had occurred. No significant differences in the first appearance of sperm were noted between enriched and impoverished animals.) After 5 days the male-female pairs were separated, and all animals were returned to their original conditions, either enriched or impoverished, for most of the 21-day gestational period. But one day before they were to give birth, the females from the enriched condition were placed individually into the standard laboratory cages to allow privacy during parturition. The females already in the impoverished condition during pregnancy remained in their single cages while giving birth.

The parents lived in the experimental conditions between the ages of 60 and 116 days, a total of 56 days. Maze experience was still being used as part of the enriched environment when this experiment was performed. We found it necessary to stop this experience for both the males and females after 15 days of enrichment, because the pregnant females climbed over the maze barriers and squeezed between the tops of the barriers and the metal screen covering the whole maze. We were not certain how this activity would affect the fetal pups, and so we terminated the maze running.

Immediately after birth, the pups, as well as the parents, from all conditions were sacrificed, and the brains were prepared for histological study. At the same time, the adults' adrenals, thyroids, and testes were weighed; no significant differences in these organ weights were noted between the enriched and impoverished rats. There were no significant differences in the number of pups produced by enriched and impoverished parents or in the number of implantation or reabsorption sites in the uteri of the enriched and impoverished mothers. Such sites are one of the things we examine in studying rat reproduction: occasionally an imperfect embryo is formed and the developing pup is reabsorbed, leaving an identifiable site.

Though there was a trend for the cerebral cortices of the neonatal pups from the enriched mothers to be thicker than the cortices of those from the impoverished, there were no significant thickness differences between the two groups of pups. However, the body weight differences

between the pups from the enriched and impoverished parents led us to pursue this type of experiment further. The pups from the enriched parents weighed 6% more ($p < 0.001$) than those from the impoverished parents. This difference was identical in both the male and female neonate pups.

We initially speculated that we would find an accelerated somatic maturation to correlate with this greater body mass at birth in the offspring of the enriched parents. The advanced maturation, if it took place, might allow the offspring a greater opportunity to interact with the environment at an earlier age than was possible for the less mature offspring from the impoverished animals. If this too were true, we supposed we might be able to demonstrate an increase in cortical thickness in the pups from enriched parents resulting from early somatic maturation and, ultimately, from greater birth weight.

With this hypothesis in mind, we designed a new set of experiments. In the new experiments, we studied the brains of the first generation of pups (which we labeled the F_1 generation) from enriched or impoverished parents, but those of pups from the next two generations (F_2 and F_3 generations) as well (see Figure 21). The design of this new experiment was similar to that of the previous experiment but with several major differences: (1) The parents were enriched in similar enrichment cages for similar periods of time, 30 days, but no maze experience was offered, and no impoverished animals were studied. (2) Instead of examining the F_1 pups' brains on the first postnatal day, the pups were nursed by their

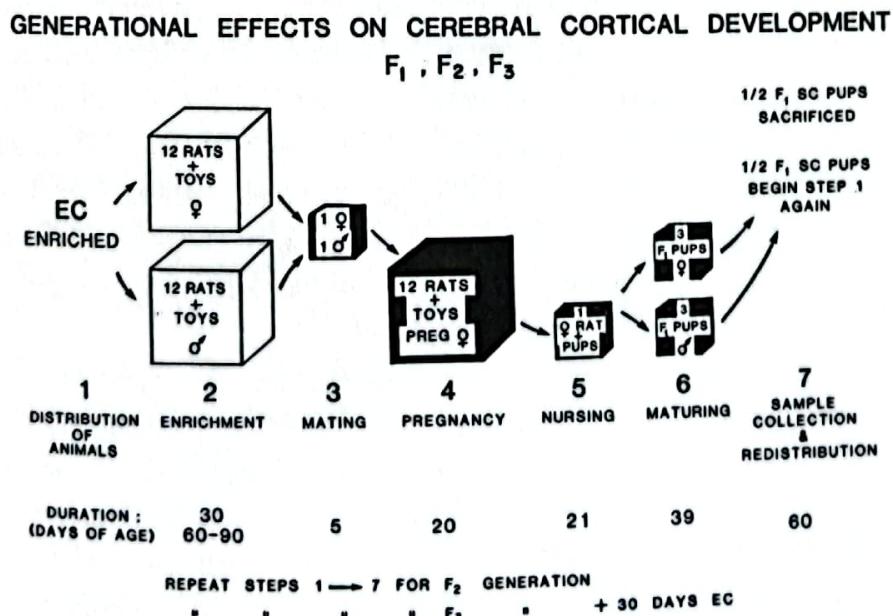


FIGURE 21. Experimental paradigm to show generational and enrichment effects on cerebral cortical development in F_1 , F_2 , and F_3 rats.

mothers now housed in individual cages until weaning at 21 days of age. The pups were then separated by sex and lived 3 to a standard cage until they were 60 days of age. (3) At the age of 60 days, half of the male and half of the female F₁ pups were placed into the enriched condition for a 30-day period to start the cycle for the F₂ generation. The other half of the F₁ animals were sacrificed in order to take the tissue samples needed for cortical thickness studies in their generation.

The cycle was repeated to obtain the F₂ generation and the F₃ as well. However, all of the animals of the F₃ generation were put into an enrichment cage for 30 days when the animals had reached 60 days of age. Since the F₃ animals were exposed postnatally to the enriched condition, they cannot be justly compared with the F₁ and F₂ animals that were not directly enriched. Nevertheless, the results from the F₃ animals are important in light of the results of our developmental studies showing that cortical dimensions normally decrease slightly between 60 and 90 days of age. Therefore, even though the F₃ animals were autopsied at an age 30 days older than the F₁ and F₂ rats, their results are included to demonstrate the cortical response in these slightly older rats after benefiting from their prenatal enrichment and from direct enrichment themselves.

The results from our continued breeding experiment before and during enrichment showed that between the F₁ and F₂ generations, it was the male pups that were affected the most. We found significantly greater thicknesses in the male F₂ cortex than in the F₁ in all three cortical sections sampled: frontal, somatosensory and occipital. Lateral area 10, in both right and left hemispheres, showed F₂ significantly greater than F₁ (by 5 and 6%, respectively); area 3, in both the right and left hemispheres, showed F₂ significantly greater than F₁ (by 4 and 5%, respectively); area 2, in both right and left hemispheres, showed F₂ was significantly greater than F₁ (by 7 and 13%, respectively). Areas 4 and 39 in the right hemisphere showed F₂ significantly greater than F₁ (5% and 8%, respectively). There was a nonsignificant trend showing F₂ greater than F₁ in all other male cortical areas sampled.

In comparing the male F₂ and F₃ groups, we observed two different patterns of response. The effects of aging were apparent in the frontal and somatosensory areas; that is, the cortical thickness decreased between the F₂ and F₃ animals. Specifically, the frontal cortex of the F₃ animals was significantly thinner than that of the F₂ animals (by 6 and 8%, respectively in the left and right hemispheres). The somatosensory cortex of the F₃ animals was thinner than F₂ (by 2 to 4%) in both right and

left hemispheres, with area 2, governing sensory mechanisms, showing a strongly significant difference ($p < 0.005$).

There were additional effects of the environmental enrichment in the occipital section, which was thicker in the F_3 animals than in the F_2 animals. For example, it was significantly greater in the F_3 generation in area 17, left hemisphere (6%) and in area 18, right hemisphere (4%). There was also a nonsignificant trend showing F_3 greater than F_2 in several other areas we examined in the occipital cortex.

The cortical differences between generations were not as marked in the females enriched in utero as in the males. In the somatosensory cortex, area 3 in the right hemisphere showed the only significant difference, a 6% greater thickness ($p < 0.01$) in the F_2 females than in the F_1 ; and in the posterior cortex, the only significant difference was in area 39 in both hemispheres, where the F_2 females also exhibited a 6% greater thickness ($p < 0.01$). Comparisons between F_2 and F_3 females revealed no significant differences, although some of the areas of the frontal and somatosensory cortex in the females did show positive effects of enrichment.

To summarize these results, the changes in the occipital cortical samples are particularly illuminating because of their consistency in the response to the experimental conditions. Figures 22 and 23 illustrate the F_1 , F_2 , F_3 enrichment incremental changes noted in the male and female Long-Evans rats for the right and left hemispheres. On the whole, it is clear that the male exhibited more extensive differences than did the female, both significant and nonsignificant.

A recent experiment by Kiyono et al. (3), has demonstrated that enriching the environment of the pregnant rat can enhance the maze-learning ability of the offspring. Their results suggest that the thicker cortex we found in successive generations in our experiments may contribute to better maze learning. It should be noted, however, that the parents in our experiment were exposed to 30 days of enrichment as adults as well as the enrichment the female subsequently received during pregnancy. Our females thus received twice the amount of enrichment that the mothers in the Kiyono experiment received. It is quite possible that our rats' maze-learning abilities would be even greater than those documented by Kiyono.

In order to assess the contribution of maternal care to the behavioral results they had observed, Kiyono et al. also cross-fostered the pups so that some were nursed by their enriched biological mothers and some by nonenriched foster mothers. The Kiyono group found that there was no

**Cortical Thickness Values
in F_1 , F_2 and F_3 Generations — Males**

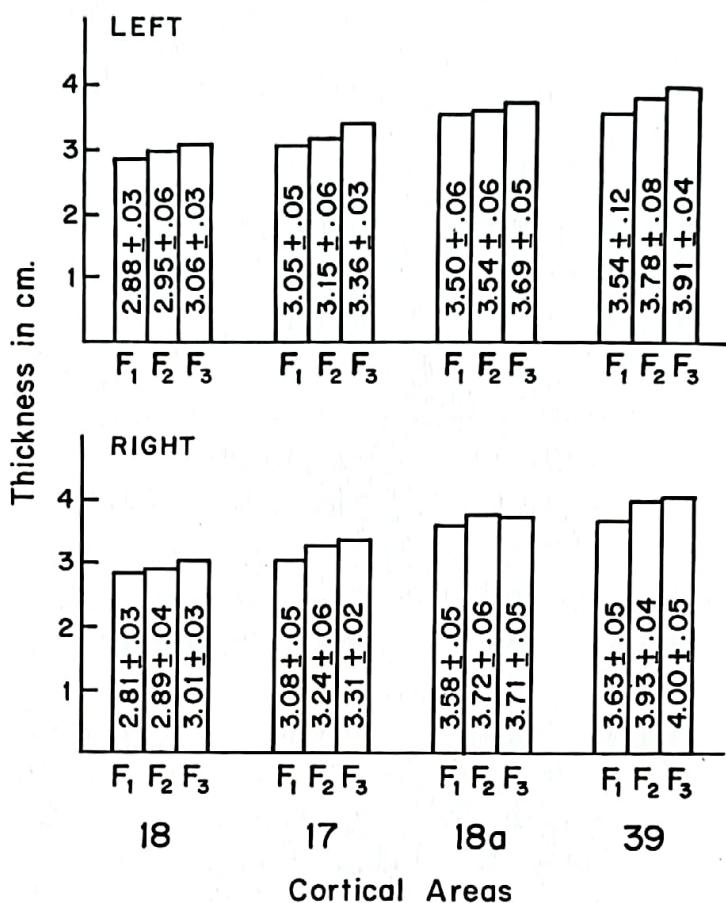


FIGURE 22. Posterior cerebral cortex (occipital cortex + area 39) in F_1 , F_2 , and F_3 generation males from enriched parents.

significant difference in the behavioral test results for the two groups of pups.

Other studies, however, suggest that there are lasting effects of both maternal care and enrichment in utero. Ivanksy and Homewood (4) put mothers and their pups into an enriched environment at different times between pregnancy and the time of weaning. When tested at 64 days of age, those pups exposed soon after birth to an enriched condition had significantly better scores on behavioral tests than controls.

To our knowledge, our experiments provide the first evidence that the dimensions of the cerebral cortex can be altered without directly enriching the offspring, i.e., by enriching the parents before pregnancy and the female during pregnancy. With the experimental design we used we still do not know whether the enrichment before pregnancy had any effect. The experiment should be repeated by separating the two variables, either enriching the parents or enriching the pregnant female.

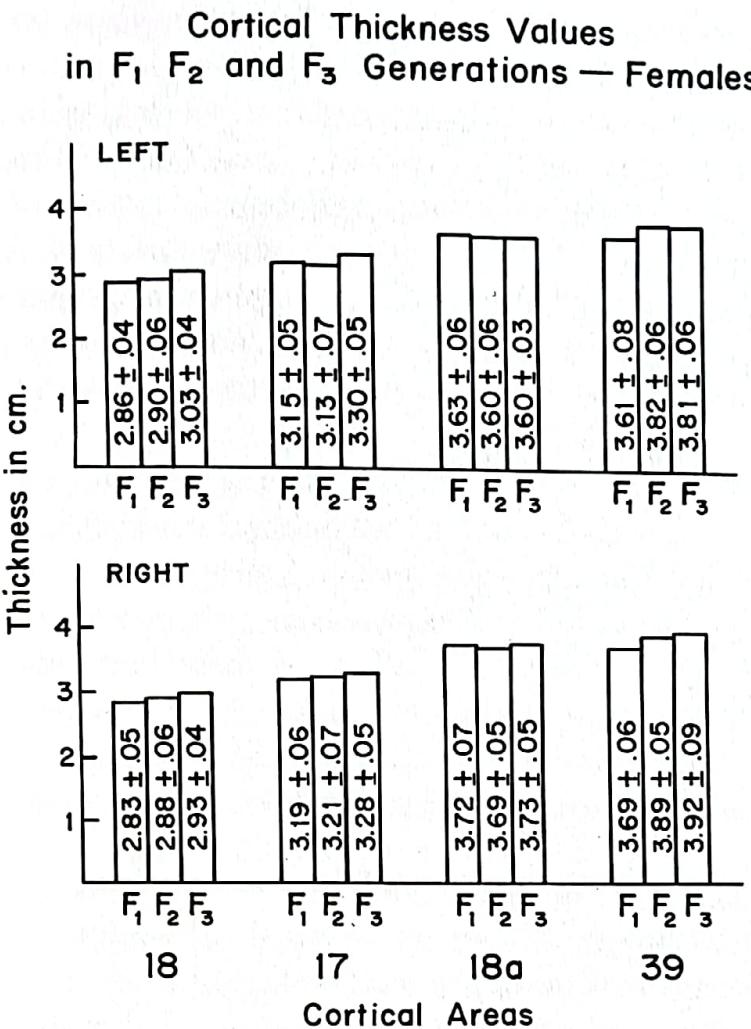


FIGURE 23. Posterior cerebral cortex (occipital cortex + area 39) in F_1 , F_2 , and F_3 generation females from enriched parents.

For these first experiments we were anxious to learn whether we could change the cortical thickness dimensions in the offspring of enriched parents and thus, gave enrichment in several stages, before pregnancy and during pregnancy. The results indicate that parental enrichment can produce positive results in some regions of the cortex.

For now, the hypothesis we offer to explain these effects is that the sex steroid hormones that are present at high levels in the mother during pregnancy might more readily cross the placental barrier if the mother is a more physically active, enriched female, thereby "priming" the cortex of the developing fetus. The enriched F_2 animals would then have an advantage over the enriched F_1 animals, having been subjected to higher than usual levels of sex steroid hormones during two pregnancies.

An explanation for the changes in the female fetal cortex is suggested by the results of Pappas et al. using young adult females. Pappas et al. injected exogenous progesterone intraperitoneally (2 mg/kg body

weight) into neonatally ovariectomized rats from 45 to 90 days of age. They found increases in cortical thickness throughout the cortex, with significant differences in areas 10, 4, 3, 2, and 18. It is of interest to note that in our experiment, four of these same areas (area 18 being the exception) responded to the generational enrichment conditions in the male rat, whereas in the female area 3 was the only one to show significant differences. The results of Pappas et al. suggest that progesterone has an increased metabolic effect on cortical tissue and thus increases its dimensions. It is possible that such an effect occurred in the rats enriched in utero.

Other experimental results show that progesterone increases protein uptake in intact female rats. This interaction could help influence the increase in cortical thickness seen in our experiments.

The influence of either progesterone or estrogen on the developing fetus is obviously complex. The effect of these hormones on cortical development may depend on the age and sex of the animal being exposed, the duration of exposure, and the amount of maternal circulating hormone as well as existing ratios of progesterone and estrogen in the animal's system.

Factors other than steroids circulating in the blood play a role in altering the structure and chemistry of the developing brain. For example, direct maternal control of the fetal biological clock in utero has been measured in terms of the rhythm of glucose metabolism in a part of the brain called the suprachiasmatic nucleus (5). This nucleus is found in the hypothalamic region of the forebrain. Investigators have demonstrated that the mother coordinates the phase of the fetal biological clock to her own circadian rhythm, which in turn is determined by the ambient lighting conditions. If the mother can transmit something that indicates ambient lighting conditions to the fetus, it is possible that she also transmits something about the effects of other environmental stimuli she receives, for example, increased tactile stimulation. Our findings, which show significant generational increases in somatosensory areas 2 and 3 for the males and in area 3 for the females, suggest that extra tactile stimulation either in utero or during lactation can affect cortical development of the young rats.

We have no rational explanation for the responsivity of area 39 in both sexes to the generational effects, yet the differences seem too consistent to be accidental. It is just this area which responds most to enrichment in the youngest unweaned animals' brains that we have measured (after 8 days of enrichment between the ages of 6 and 14 days). This area, a lateral posterior part of the brain, continues to be responsive to

the enriched environment until the animal is 105 days of age. But in the older animals, 185 and 904 days of age, no significant changes are noted as a consequence of living in the enriched condition. It appears that the young animal's responsiveness in area 39 is lost with age.

Although the F₁ and F₂ comparisons are the most valid to make because the experimental conditions were similar, the measurements made in the occipital region of the F₃ group were consistent with the pattern of incremental increases in cortical thickness observed in the first two generations. As mentioned earlier, the F₃ group could not be directly compared with the other two groups because the F₃s were exposed to an enriched condition between 60 and 90 days of age and thus the F₃ animals were older at autopsy. The impact of this exposure was evident in the fact that, despite their being older than the other two groups at autopsy, the F₃ rats' cortices were thicker in the occipital cortical sections.

Since the thickness of the occipital cortex normally decreases in animals living in the standard laboratory conditions between 55 and 90 days of age, the larger size of the F₃ cortex compared with that of the F₁ and F₂ was particularly noteworthy. The areas of the F₃ occipital cortex were apparently benefiting from the intrauterine effects or direct enrichment or both. It should be noted that it is the occipital cortex that most frequently increases in thickness during postnatal enrichment.

In conclusion, our experiments have demonstrated incremental increases in cortical thickness over two successive generations of rats experiencing parental enrichment and enrichment in utero. The third generation displayed some increases as well, albeit with additional exposure to the enriched conditions. Undoubtedly, many factors could be behind these findings. We have suggested the possible role of progesterone. The high concentration of nerve growth factor in the placenta might conceivably be another consideration in stimulating cortical dimensions in the fetuses of the enriched mothers with a possible increased placenta circulation. The role of nerve growth factor in cortical development is only recently becoming established. We are anxious to continue with these types of experiments to gain a better understanding of the mechanisms involved.

From Birth to Weaning (1-28 days)

Having found that it was possible to influence the prenatal cortex, we now wished to learn whether the early postnatal cortex (that of rats before the age of weaning) could also be affected by enriched or impover-

ished environmental conditions. (Weaning for these experiments was at 28 days rather than at 25 as for the others.) We have noted that the plot of normal cortical development has essentially two definite slopes. The first slope is a steep increase immediately after birth until the animal is 26 to 41 days of age; it reaches a peak and is followed by the second, a slow decline throughout the rest of the lifetime of the animal. We were now interested in determining whether we can change the cortex of rats during the normal increasing slope of the curve between birth and the age of 26 to 41 days. Usually, rats are weaned between 21 and 25 days of age.

In order to learn about the effects of a stimulating environment on the rapidly growing phase of the cortex, we conducted an experiment that required a new living arrangement: young animals had to be housed with mothers (6). Long-Evans male pups had an advantage at the beginning of these experiments compared to pups in the experiments after weaning because the litter size was reduced to three pups per mother at birth instead of the usual eight. When the pups were 6 days of age, three environmental conditions were formed: (1) One mother with her three pups remained in the standard colony cage; this condition was referred to as the unifamily environment. (2) Three mothers with three pups each were placed together in a single large cage, 46 × 71 × 69 cm (the multifamily environment). (3) Three mothers with three pups each were placed together in a single large cage with "toys" (the multifamily enriched condition). The toys were many different kinds of objects, such as ladders, small mazes, wheels, and swings (see Figure 24). Unifamily and multifamily enriched groups were sacrificed at 14, 19, and 28 days of age. The unifamily and multifamily but not enriched cohort was sacrificed at 28 days of age.

By the time one succeeds with an experiment it appears straightforward, but we arrived at the final experimental design just described only after several unsuccessful attempts. For example, in our first multifamily enriched environments, the three mothers with their three pups each were put into the enriched cage immediately after the birth of the pups. By the second day, the mothers had eaten the babies, possibly because of parental anxiety due to this unfamiliar grouped environment. Step by step we learned that not until the pups reached 6 days of age would the mothers tolerate the group enriched living condition.

Some behavioral observations of these groups were noted during the course of the experiment. The multifamily animals were more docile than those raised in the unifamily environment. Often isolated animals are quite disturbed when handled. During the night (the 12-hour dark pe-

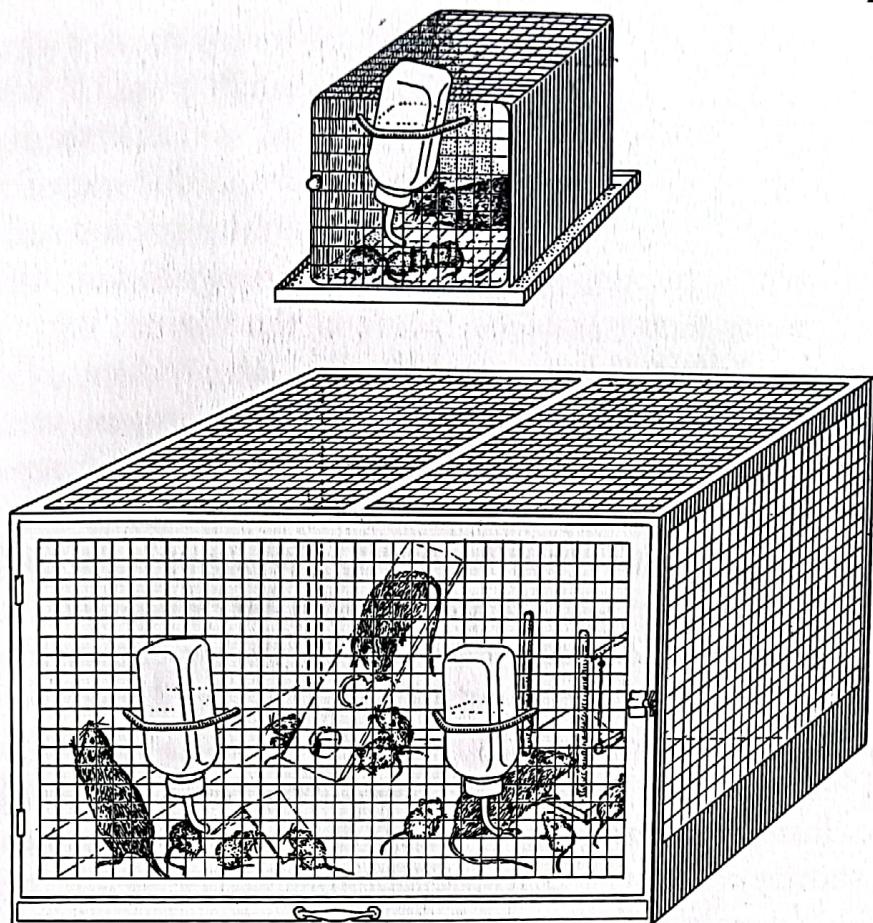


FIGURE 24. Living conditions for the unweaned rats: (below) multifamily enriched condition; (above) unifamily condition.

riod we subjected the rats to), the enriched multifamily animals explored their cages more than the unifamily animals; and like other multifamily animals, the enriched multifamily rats were more docile than the unifamily at autopsy. The greater interaction that the enriched mothers and pups had with other rats may have prepared them for more docile responses to the experimenters.

When we compared the rats housed in the unifamily condition with the multifamily rats, the former were significantly heavier (6%, $p < 0.05$) in body weight than those living with several families. This is consistent with other findings that the body weight of small litters (5 rats) is greater than that of larger litters (8 rats) during the weaning period (7, 8). But there were no significant differences in adrenal weights and in the time of eye opening between the unifamily and multifamily pups.

At 14 days of age, the unifamily and enriched multifamily rats differed significantly in body weight, head-to-tail length, and testicular weight. The differences were 10% ($p < 0.05$), 15% ($p < 0.001$), and 7% ($p < 0.05$), respectively, the values for the unifamily rats always

being greater. Rats become sexually mature by 60 days of age. Because of the increased testicular weight in the unifamily pups, it would be of interest to follow some of these little rats until sexual maturity to learn which group, the enriched or nonenriched, reached this stage first. When enriched and impoverished males were mated with enriched and impoverished female animals respectively, there were no significant differences in the time of the first appearance of sperm in the vagina, suggesting that exposure to the different environments did not produce differences in sexual maturation. In examining other endocrine organs, we found no differences in adrenal weights between the experimental groups, signifying that stress was not a major factor in these experiments.

The time of eye opening was observed carefully in order to determine the rate of maturation of the nervous system. Normally, rats' eyes open on day 14. However, the eyes opened on day 13 for the enriched multifamily rats and on day 14 for the unifamily animals. This one-day difference was statistically significant ($p < 0.05$). At least two factors can be considered here: (1) that the enriched condition was accelerating maturation, a conclusion supported by cortical thickness measurements (to be described shortly); and (2) that the possible increased androgen levels produced by the enlarged testes delayed the perforation of the palpebral fissure (between the eyelids), preventing early eye opening. Though either of these suggestions is feasible, the former is more strongly supported by our other experiments.

Body measurements were taken on the 14, 19, and 28 day groups of rats. Only the 14 day group showed somatic differences between the multifamily and the unifamily impoverished animals. In experiments with rats at 19 and 28 days of age, the somatic differences noted at 14 days were no longer exhibited. In these experiments, however, once again the eyes of the enriched multifamily rats opened earlier than did those of the unifamily animals, this time by 2 days ($p < 0.01$).

Cortical thickness measurements were taken on all of the pups in the various conditions. No significant differences were noted between the unifamily and the multifamily animals at 28 days of age. In other words, the animals (3 mothers plus 9 pups) living together in the large cage without toys did not develop cortical thickness changes which differed from the animals living in the unifamily condition (1 mother plus 3 pups). The importance of the presence of stimulus objects or toys with which the animal can interact will be borne out in the next comparisons.

In as short a period as 8 days (the animals were in the experimental conditions from 6 to 14 days), the somatosensory cortex of the multifamily enriched animals developed strikingly significant differences from that of

the unifamily rats (Figure 25). The differences in thickness ranged from 7% ($p < 0.025$) to 11% ($p < 0.001$), with the enriched animals having the thicker cortices. The importance of the presence of toys in influencing cortical structure is clearly confirmed in this experiment. Interestingly, the visual cortical regions did not show significant differences at this early stage, when the rats were 14 days old. Evidently, since the eyes had not opened until 13 days of age in the enriched and 14 days in the unifamily group, not enough visual input had occurred to induce cortical changes between the two groups. But in the most lateral segment of the sample of the occipital cortex, area 39, a multisensory integrative area, by 14 days of age a thickness difference of 16% ($p < 0.01$) had developed between the multifamily enriched and the unifamily group. In these young rats receiving a good deal of input, clearly evident in the changes found in the somatosensory area, the active recruitment of information from many sensory regions apparently created the changes seen in area 39. This 16% difference in cortical thickness is the largest we have encountered in rats either before or after weaning.

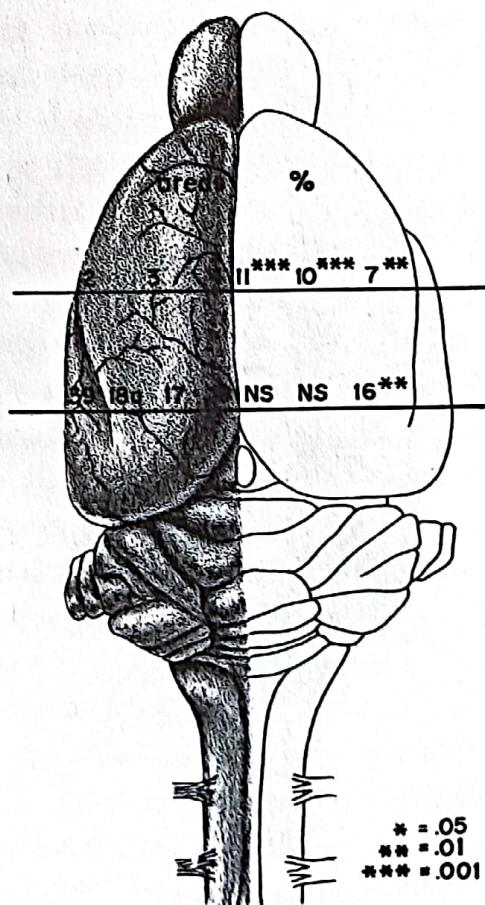


FIGURE 25. Percentage cortical thickness differences between multifamily enriched and unifamily rats living in their respective conditions from 6 days to 14 days of age.

By the age of 19 days, the enriched multifamily rats had significantly thicker somatosensory and occipital cortices than the unifamily animals (Figure 26). One area, area 2, did not show a significant change at this age. This area often does not follow patterns that other cortical regions show in response to development or environment.

In the animals autopsied at 28 days of age, all subdivisions of the somatosensory and occipital cortices showed significant differences in thickness between the enriched multifamily and the unifamily animals. At 28 days, the occipital cortex appeared to have shown more response than it had during either of the shorter periods. In fact, the differences ranged from 9% ($p < 0.05$) to 12% ($p < 0.001$), the largest changes seen throughout the occipital cortex with any age group, whether before or after weaning. The 16% change reported at 14 days was in one region only while other, adjacent regions were nonsignificant; in the 28-day-old group, the differences represent significant changes in three adjacent regions.

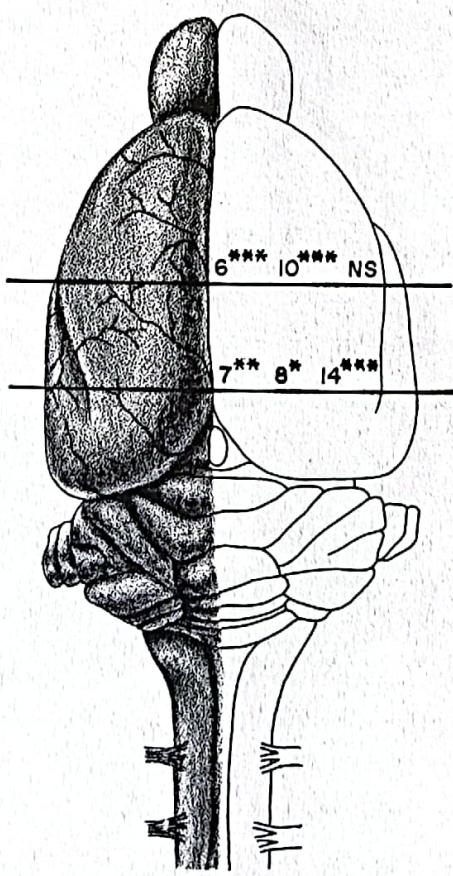


FIGURE 26. Percentage cortical thickness differences between multifamily enriched and unifamily rats living in their respective conditions from 6 to 19 days of age.

The cortical changes in these not-yet-weaned male rats indicate that it is possible to accelerate measurable morphological cortical maturation in very young animals. In many segments of the cortex, we achieved cortical thickness differences greater than those we have seen in animals past the age of weaning. The results also suggest to us that the somatosensory cortex is more plastic before the eyes open, but once visual input begins, it is the visual cortex that shows greater changes.

From Weaning to Adulthood (25-185 days)

In studying the impact of the environment on the cortex of rats during the period from weaning to adulthood, we wondered whether the age of onset of the experimental conditions was an important consideration. We made our first comparisons between animals living in their environments from 25 to 105 and from 105 to 185 days of age (9). In none of the cortical sections we compared was the change in thickness significantly different between the two 80-day groups. As we found when we established normal cortical thickness developing and aging curves, the cortex is decreasing during both of these age periods. Although one comes in the early phase of descent and the other a little later, the effects of different environmental conditions are similar in the two age periods in counteracting the decrease. Whether we started enrichment at 25 days or 105 days, the greatest changes occurred in the occipital cortex, with the magnitude of the change similar for the two groups, between 2% and 5 to 6% (see Figure 27). These results show that an 80-day exposure to enriched or impoverished conditions is as effective whether it starts during the early part of the decreasing slope of the cortical curve or 100 days later.

We also compared two other periods of onset, one at 25 days (25 to 55 day group) and the other at 60 days (60 to 90 day group). But in the experiments based on these starting ages, the animals were in their conditions for shorter periods of time, for 30 days instead of 80. The thickness of the cortex did not differ significantly between the groups with these two starting ages, with but one exception, the lateral occipital cortex (see Figure 28). Figures 28 and 29 include 10 sections through the cortex instead of the 3 shown in Figure 1. Even with a shorter duration (30 days), and with different starting ages (either 25 or 60 days), the enriched and impoverished changes were, in general, not significantly different. That is, the effects of the enriched or impoverished conditions

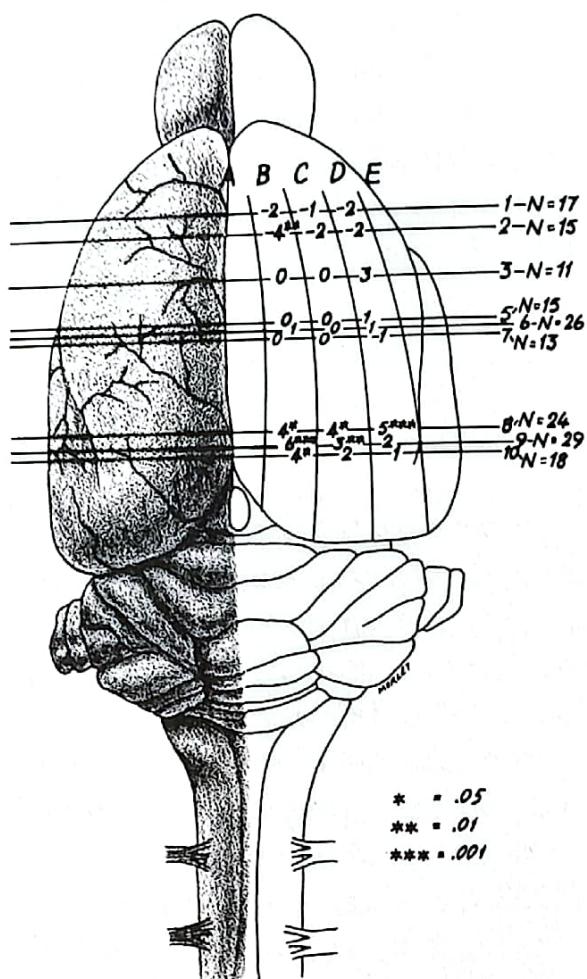
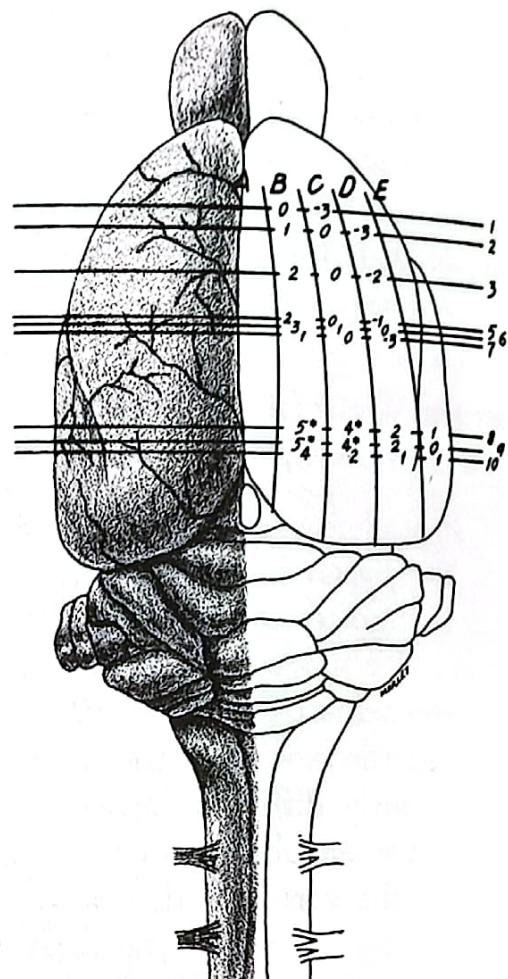
25 - 105 Days105 - 185 Days N=18 Pairs

FIGURE 27. Cortical thickness percentage differences between enriched and impoverished rats living in their respective environments for 80 days, either 25 to 105 days or 105 to 185 days.

were not significantly different (with one exception) between the 25 to 55 day group and the 60 to 90 day group.

Figure 29 shows the effects of enrichment and impoverishment compared with standard colony conditions. For the younger 30-day group, subjected to the conditions between the ages of 25 and 55 days, the differences are primarily due to the impoverished condition. Slight enrichment effects are seen in the occipital cortex, sections 8, 9, and 10. It is apparently detrimental to isolate the young pup immediately after weaning at 25 days of age; at the same age, the effect of environmental enrichment is not very strong. But by waiting until the animals are 60 days old to place them in their respective environments for a 30-day period, we get a different cortical response. The effects of enrichment become stronger, especially in the frontal and occipital cortex, as do the effects of impoverishment. A reduction in stimuli to the cortex appar-

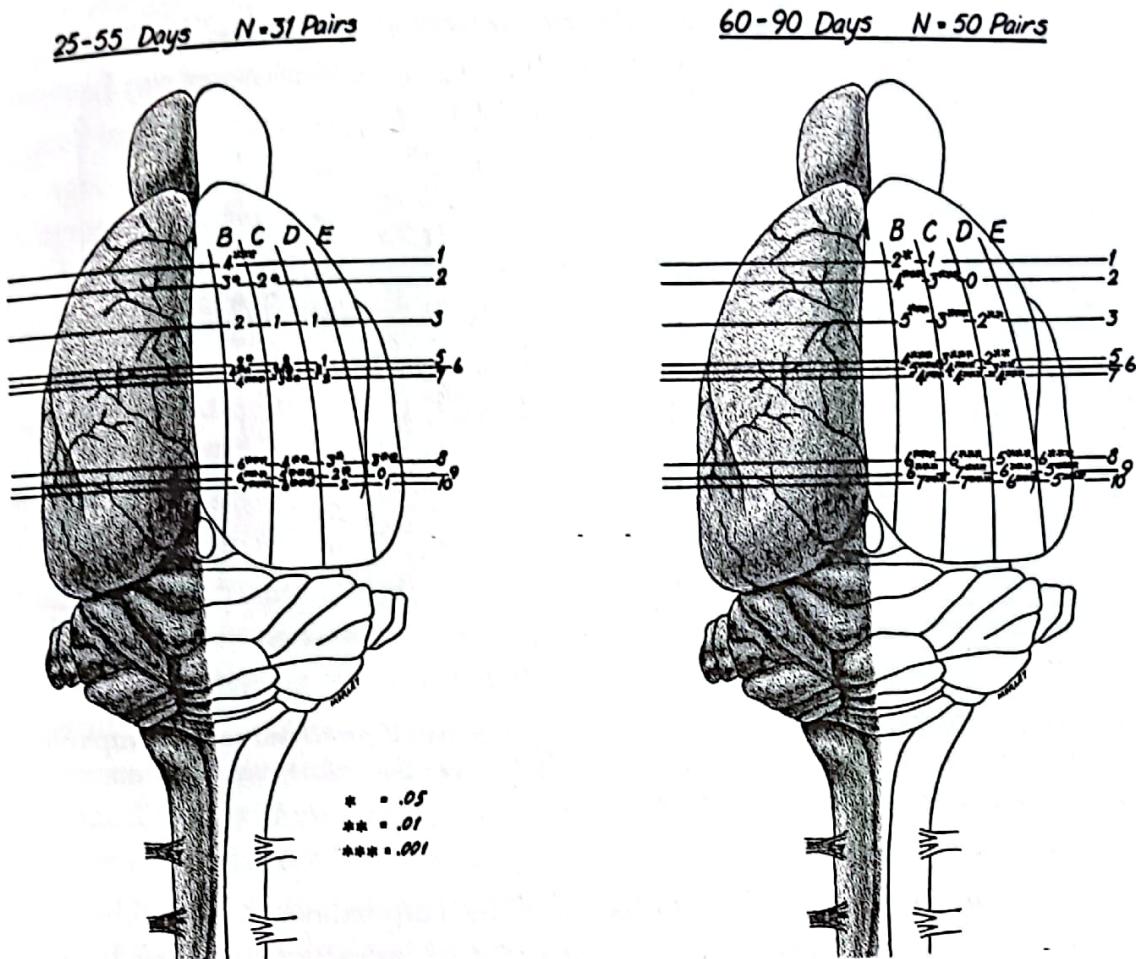


FIGURE 28. Cortical thickness percentage differences between enriched and impoverished rats living in their respective environments for 30 days, either 25 to 55 days or 60 to 90 days.

ently creates more deleterious effects than increased stimuli enhance cortical structure. In other words, the effects of impoverishment are stronger than the effects of enrichment in creating cortical structural changes in both the 25 to 55- and 60 to 90-day groups.

We have found that both 30-day and 80-day periods of enrichment or impoverishment alter the cortex, but is one duration as effective as the other? The younger 30-day group (25 to 55 days of age) did not differ markedly from the two 80-day groups (25 to 105 and 105 to 185 days of age). However, the older 30-day group (60 to 90 days of age) gave significantly greater measurements than both of the 80-day groups in several of the areas compared. From these results, we can conclude that the animals living for 30 days in their respective environments as young adults developed the most significant cortical changes. During the 80-day period, the rats may have become "bored" with the selection from a common pool of toys, and the cortex decreased in its dimensions

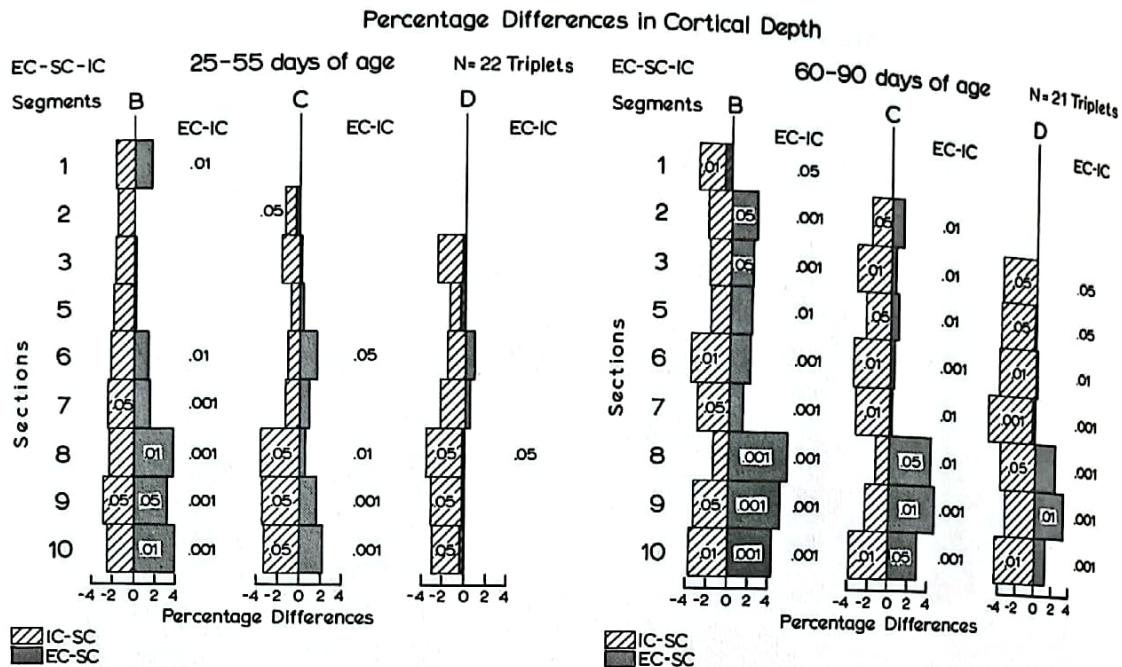


FIGURE 29. Effects of enriched and impoverished environments compared with a standard, 25 to 55 days or 60 to 90 days. Segments and sections are as illustrated in Figures 27 and 28.

as a result of a lack of stimulation. The importance of keeping new variety of inputs to the cortex to maintain its increased thickness became evident from these experiments.

In both 30-day groups (25 to 55 days and 60 to 90 days of age), the enrichment effect was mainly in the occipital cortex, including the primary visual cortex. However, some scientists do not believe that vision plays an important role in the effects of enrichment and impoverishment. Studies by Krech et al. (10) and Rosenzweig et al. (11) showed that cortical weight changes occurred even if the enriched and impoverished experiments were run in the dark or with blinded animals. Yet, in our study (6), when we examined the lateral geniculate nucleus, a thalamic way station in the visual pathway, we found the proportion of neuronal cells per unit area to be 20% less in the enriched unweaned rats than in the nonenriched. These results suggested that the lateral geniculate nucleus from the enriched animals possessed more fiber connections or neuropil separating the nerve cells from each other than that of the nonenriched animals. Our results indicate that the visual system is involved in the cortical thickness changes.

In addition, other results, from our studies on rats before the age of weaning, have shown that vision may play a significant role in altering the enriched occipital cortex. It will be recalled that if the animals were placed in the enriched or nonenriched conditions between the ages of 6

and 14 days, before the eyes opened, the primary visual cortex did not respond to the environmental conditions. If, however, the animals were placed in their respective conditions for the period between 6 and 19 days of age, which includes at least 5 days with the eyes open, significant differences between the enriched and nonenriched rats were found in the thickness of the visual cortex. Thus, two of our experiments offer evidence that vision plays a considerable role in promoting the cortical changes.

Because so many brain regions send fibers to the occipital cortex, at the present time it is difficult to attribute to any one functional unit the full responsibility for the cortical changes in response to the environment. Fibers other than visual fibers associated with the occipital cortex include those from the basal ganglia (12), hypothalamus (13), hippocampus (14), and nonspecific thalamic fibers (15, 16). From these various sources of input it is not possible to determine which specific pathways are being stimulated to increase the dimensions of the occipital cortex.

For all of these studies on rats between weaning and adulthood, the animals lived in their respective conditions for either 30 or 80 days. We wondered if we could shorten these periods and still find measurable cortical changes. In the next series (9), the rats were exposed to their environmental conditions for successively shorter times, i.e., for 15 days (from 25 to 40 days of age), for 7 days (25 to 32 days of age), for 4 days (26 to 30 or 60 to 64 days of age), and for 1 day (60 to 61 or 80 to 81 days of age).

No significant cortical thickness differences were noted between the enriched and impoverished animals that had lived only 1 day in their respective environments, whether from 60 to 61 or from 80 to 81 days of age. However, we learned that after 4 days of exposure to the different living conditions, the cortical thickness differences were highly significant in area 18, the medial occipital area, by 3 to 4% ($p < 0.001$). As mentioned previously in this chapter, it is this visual association area of the cortex which consistently responds to the enriched condition, for reasons that are not yet fully understood.

In the 4-day groups, the other areas of the occipital cortex also showed differences related to environmental conditions; these were most significant in the medial areas and progressively less significant in areas farther laterally. The experiments on animals in the 26- to 30-day age group caused cortical changes due primarily to impoverishment, whereas those on the 60- to 64-day age group created differences which were mostly due to enrichment. As mentioned earlier in this chapter, taking the pups away from the parent early in life after weaning appeared to be

detrimental to cortical development. In the older animals, short-term exposure to the impoverished conditions did not negatively affect the brain structure, yet with longer-term exposure to impoverishment, detrimental effects became evident even in the older animals.

If the occipital cortex could change after 4 days in enriched or impoverished conditions, was the pattern of change any different after 7 or 15 days? Yes, after 7 days, the frontal, somatosensory, and occipital areas all showed statistically significant differences ranging from 2% ($p < 0.05$) to 5% ($p < 0.001$). Area 18, the medial occipital cortex, showed a 4% change after 7 days of enrichment, as it did after 4 days of enrichment. After 15 days of enrichment, all regions of the cortex that were measured again showed statistically significant changes. After 15 days, area 18 (the medial occipital cortex) reached a larger difference, a 7% difference ($p < 0.001$), in contrast to the 4% seen during the shorter time periods. The animals living in their environments for 30 to 80 days also differed by 7 to 8% in area 18, if they entered their environments before 100 days of age. With our environmental conditions, cortical area 18 appears to be most susceptible to change and reaches its greatest thickness after two weeks of enrichment. The effects of the 30- and 80-day periods on the cortical thickness were no greater than those of the 15-day period.

Once we learned that young animals living in their environments for 30 days (from 60 to 90 days of age) experienced thickness changes throughout the cortex, we then wondered if it were possible to change the structure of the cortex in adult animals during a 30-day period. We carried out a study in collaboration with Uylings et al. at the Brain Research Institute in Amsterdam (17). The design was as follows: At the age of 112 days (considered young adulthood) 12 sets of triplets were divided accordingly: 12 animals were sacrificed to serve as a baseline for cortical thickness at this age, 12 were placed in the enriched condition, and 12 were separated 3 per cage in the standard colony condition. After 30 days, the cortical thickness was measured on the enriched and standard colony rats.

This was an important experiment for at least two reasons. First, it did prove that the adult cortex could change positively in response to an enriched condition in a 30-day period. The cortical thickness of the adult enriched rat was thicker than that of the rat living in the standard conditions at 142 days of age. Second, and perhaps even more important, it showed that enrichment was actually increasing the dimensions of the cortex and not only inhibiting the normal decrease in cortical thickness. The enriched rat's cortex at 142 days of age was thicker than the baseline

rat's cortex measured at 112 days of age. This experiment, with its baseline control, thus offers sound evidence for actual growth due to enrichment in the adult animal.

Middle Age and Old Age (400-904 days)

We were now interested in learning whether the brain in middle and old age could show changes similar to those seen in the younger brain. Several studies were made over the years from the late 1970s to 1985, including those of older animals at 444 days, two groups at 630 days and 904 days of age. (As we mentioned earlier, the Long-Evans rat has been known to live for as long as 904 days in our laboratory.) For the 444-day-old animals and the second group of 630-day-old animals which consisted of only the middle-aged animals in the enrichment cage, the results were similar to those in the younger adult animals (112 to 142 days of age); i.e., the thickness of the occipital cortex was greater in the enriched than in the nonenriched rats (18). Even the 630-day-old animals, which were over halfway through their lifetime, could develop cortical changes in response to the enriched living conditions.

The first 630-day-old group of animals need to be described in greater detail, for their experimental design was somewhat different from other enriched animals. It was our first aging experiment. For this group, only seven animals lived to be 600 days of age. Thus, it was necessary to redesign our experimental conditions to suit so few animals. We decided for the first time to mix age groups, to bring the number of animals in the enriched condition up to 12 by using four 600-day-old animals and eight 60-day-old animals. We put the remaining three 600-day-old animals in the standard colony condition. This way, we had the usual number of 12 rats in the enriched condition—four middle-aged and eight young--and we had three middle-aged rats in the standard colony condition. We had several cages of 60-day-old rats in the standard colony to compare with their littermates in the enriched condition. All rats lived in their respective conditions for 30 days, from 60 to 90 days of age for the young, and from 600 to 630 days of age for the middle-aged.

When the cortical thickness measurements were made at the end of the 30-day period, each of the four 630-day-old enriched rats' cortices was thicker than those of the nonenriched. Since there were so few animals, the differences were not significant, but this experiment demonstrated that the cortices of rats two-thirds of the way through a lifetime could still grow in response to a stimulating environment.

Though the middle-aged (630-day-old) rats showed a positive response to the stimulating environments, the younger rats living with the older ones differed in their response to enriched conditions from young rats living in enriched cages without old rats. The young enriched rats—the 60- to 90-day group—living with the older enriched ones showed no significant differences in cortical thickness compared with the young nonenriched rats. In other studies, large differences had developed when young rats lived only with their age-mates from 60 to 90 days of age. Apparently, it was the old rats that dominated the mixed environment. They were the ones that lined up in the front of the cage to see what new toys would be introduced for the day. While the old ones awaited their toys, the young ones slept in the back of the cage. It appeared as if a dominant hierarchy had been established whereby the old animals were interacting with the toys and preventing the young ones from doing so.

We later learned that rats could live to the age of 800 days under normal conditions in some German laboratories. Our 630-day-old rats were not old by comparison. We needed to change our laboratory rat-raising procedure in an attempt to get our rats to live beyond 630 days of age. For the next study, my long-time assistant, Ruth Johnson, and I decided to take care of the rats ourselves instead of leaving their upkeep to others (19). As we changed the toys and cleaned the cages twice a week, we provided a little more attention to the animals than was normally given. We held them up against our laboratory coats for a short period. Perhaps as a result of this extra attention, the rats lived up to the age of 766 days in their standard colony conditions. Until this age (over two-thirds of their lifetime) they had lived in the colony condition, three rats in each small cage, before some of them entered a new, stimulating environment.

Specifically, the rats were taken from their standard colony conditions at 766 days of age and separated into either the enriched or the standard colony conditions, with new living partners. After 138 days in their respective environments, when the rats reached the very old age of 904 days, the experiment was terminated. Upon examining the cortical thickness, once again we found that the enriched animals had a thicker cortex than the nonenriched, especially in area 18, where the difference reached 10% ($p < 0.05$) (see Table 7 and Figure 30). In fact, the differences in cortical thickness were as great as those seen in the young rats. But it is essential to point out that for these old animals, the experimental conditions lasted for 138 days rather than for the 30-day periods we used with the young rats. How much of a change would have occurred in the old

TABLE 7
Percentage Cortical Thickness Differences between 904-Day-Old Male Long-Evans Rats Exposed to Enriched and Nonenriched Environments

AREA		ENRICHED*	NONENRICHED	%	<i>p</i>
		(N = 5)	(N = 8)		
Frontal cortex	10M	2.69 ± 0.187 ^b	2.53 ± 0.114	6	0.03
	10L	2.97 ± 0.210	2.75 ± 0.136	8	NS
Parietal cortex	4	2.68 ± 0.076	2.55 ± 0.152	5	NS
	3	2.92 ± 0.236	2.76 ± 0.184	6	NS
	2	2.68 ± 0.306	2.49 ± 0.107	8	NS
Occipital cortex	18	1.61 ± 0.115	1.46 ± 0.117	10	0.05
	17	1.84 ± 0.077	1.73 ± 0.076	6	0.01
	18a	1.93 ± 0.053	1.86 ± 0.082	4	0.05
	39	2.09 ± 0.116	2.08 ± 0.147	0.4	NS

*Exposure to the enriched conditions was from 766 days of age.

^bValues are $\bar{X} \pm SD$. Micrometers can be obtained by multiplying by 444.

rats after only the 30-day time period remains to be seen. Most important, however, the cortex of the very old animals did respond positively to the enriched condition.

We stopped the experiment when the rats reached 904 days of age because by this time we had lost two animals, both of them from the enriched condition. We were concerned that all of the enriched animals might die before we could measure their brains. But it was puzzling to find animals dying in the enriched and not in the nonenriched condition.

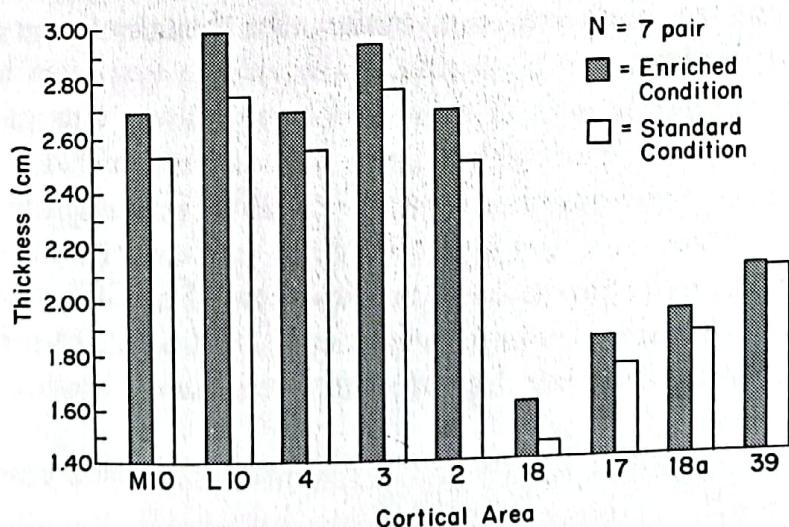


FIGURE 30. Environmental influences on the thickness of the cerebral cortex in rats exposed to standard or enriched conditions between 766 and 904 days of age.

We hypothesized that, perhaps, the stimulation of living in the larger group with the toys was not beneficial to such old animals.

It is possible that the old rats would survive better if they had access to more solitary living, free from interaction with other rats. Their food and toys could be in a large central enrichment cage with arms radiating to their more solitary sleeping quarters. With this experimental design, the rats could voluntarily seek solitude or only one or two partners. Group living for the old animals might be more stressful than living in the standard cages, as indicated by the loss of the enriched animals earlier than the nonenriched. We will have to complete a new experiment to determine whether providing an escape from the enriched condition will prolong the lifetime of the very old animals. For the present, we are satisfied that with the conditions used in our experiment we have shown that the old brain has the potential to respond to increased stimulation.

Since nerve cells in the cortex do not divide shortly after birth, the animals must live for a lifetime with the same nerve cells, in our experiment, for 904 days. Therefore, it is essential to maintain these cells by providing a healthy support system, whether it be the glial cells, the cardiovascular system, the urinary system or other systems of the body. If properly maintained, supported and stimulated, the cortical nerve cell possesses a unique potential for adaptation at any age.

The results of this study demonstrating cortical plasticity in the very aged animal contain both caution and promise for our aging human population. They caution us against entering into inactive life styles that reduce the sensory stimuli reaching our brains, and they provide hope, if we continue to stimulate our brains, for healthy mental activity throughout a lifetime.

6

THE INTERACTION BETWEEN SEX HORMONES AND ENVIRONMENT

Investigators have considered the impact of both the environment and sex hormones on the brain since the nineteenth century. Even as early as 1819, the Italian anatomist Malacarne postulated that experience could alter brain structure (1). And in 1885, Gowers noted that epileptic seizures, which frequently originate in the cerebral cortex, varied with the phase of the menstrual cycle (2). In spite of these very early reports, no one has put together an experimental program dealing with the interaction of the sex hormones, the environment, and the cerebral cortex.

Most of our early studies dealt with male rats so they would not be influenced by any possible variables such as that of the estrous cycle on the female cerebral cortex. We were trying to determine what purely external environmental influences existed and wanted to have as few variables as possible. It was not until 1971 that we published the first results on the anatomy of the female brain exposed to our different environmental conditions (3). We had learned how responsive the female cortex was to both the experiential environment and the level of the sex steroid hormones.

Like many scientific discoveries, knowledge of the impact of the female hormones on the cortex came quite by accident. We discovered it at a time when we were interested in learning the effect of the enriched environment upon the next generation. But before examining the brains from F_1 pups, we wished to confirm that morphological changes had taken place in the brains of their parents, both enriched and impover-

ished. The usual effects of the environment on cortical thickness were confirmed in the male brain, but an unexpected finding was that pregnancy also modified the dimensions of the cerebral cortex. Of course, a great many metabolic changes occur during pregnancy, and these could be responsible for the differences we were finding in the brains of the pregnant rats. But we began to turn our attention to the interaction of female sex hormones and differential environments in affecting cortical structure.

The following study provided the clue that the female sex hormones brought about changes in the environmentally enriched or impoverished cerebral cortex. We examined the cortical thickness in the brains of the following groups of Long-Evans rats: 24 male rats from enriched and impoverished conditions, 24 postpartum females from enriched and impoverished conditions, and a control group of 24 nonpregnant enriched and impoverished females.

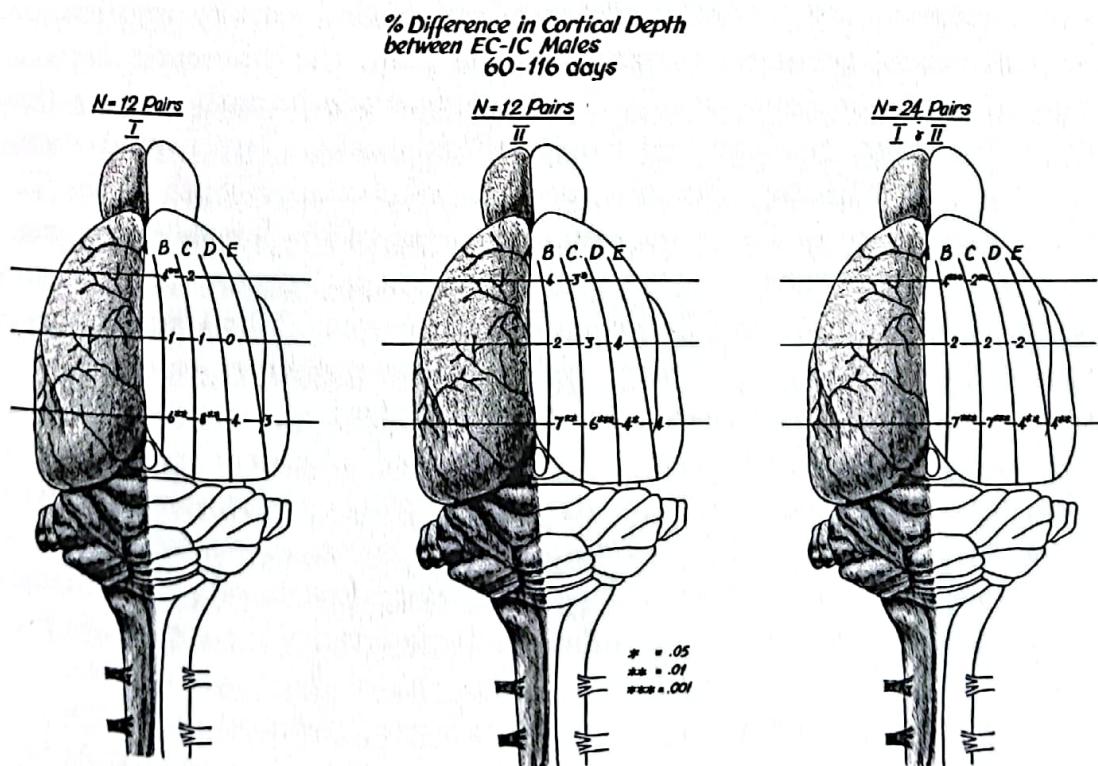
The experimental procedures for these animals are those that were reported in Chapter 5 where the environmental influences on the prenatal pups were discussed. They will be described again here briefly as a background for these results on adult males and females. At 60 days of age both males and females were separated into enriched or impoverished conditions. At 90 days of age, males and females from like environments were placed together in standard cages for mating. All animals were returned to their original conditions, either enriched or impoverished, for most of the 21-day gestation period. But one day before they were to give birth, the females from the enriched condition were housed individually in standard-sized cages. The females that were already in the impoverished conditions during gestation remained housed singly in their cages during parturition. The period the parents were in the experimental conditions was the age span from 60 days to 116 days, a total of 56 days. Maze experience was terminated after 15 days of enrichment.

The control group of females was similarly divided between the enriched and impoverished conditions. But this group was treated differently from the preceding group of females once the 30 days of differential living conditions had ended. Instead of housing an enriched female with an enriched male or an impoverished female with an impoverished male, we housed this group for 5 days accordingly, two enriched females or two impoverished females to a standard colony cage. (A better control might have been to use a castrated male with the female during this 5-day period, but here we might have had to deal with effects of pseudopregnancy on the cortex.) The brain and organ weights from the

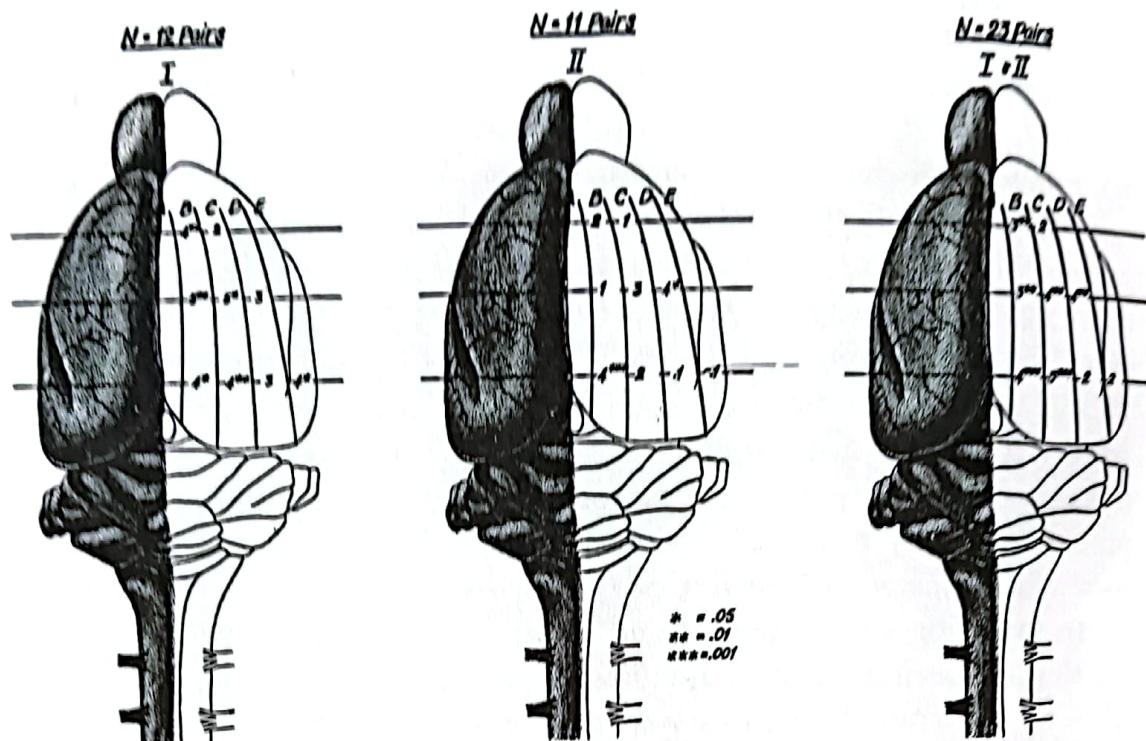
nonpregnant females were used for comparison with those from the postpartum females and the males.

Immediately after the birth of the pups, animals from all conditions were sacrificed, and the brains were removed for histological study. Figures 31a, b, and c illustrate the results of these experiments with males (a), nonpregnant females (b), and postpartum females (c). There was an initial (I) and replication (II) experiment for each group, with I and II summed for the final results. As seen in Figure 31a, the cortical thickness differences in the Long-Evans male parents were primarily in the occipital cortex, a 7% difference ($p < 0.001$) in area 18; the frontal cortex also showed a difference, but to a lesser extent. In the male rat, the somatosensory cortex did not respond significantly to the environmental conditions during this experimental period. These data suggest that males are primarily visually responsive animals because their visual cortex changed so much compared to the rest of the cortex.

In summing experiments I and II with the 24 pairs of nonpregnant females, the occipital cortex did differ between the enriched and impoverished rats, but the differences were only 4% ($p < 0.001$) in area 18 (see Figure 31b). This difference was significantly smaller than the difference in this region between enriched and impoverished males. However, in



% Difference in Cortical Depth
between EC/IC Non-pregnant Females
60-116 days



the somatosensory cortex, which receives general sensory information, such as touch, pressure, temperature and pain, the differences between the enriched and impoverished females were significantly greater than those between enriched and impoverished males. The frontal cortex showed approximately the same amount of change in both sexes. The marked response in the somatosensory cortex of the female to the environmental conditions suggests that she is more "general sensory" responsive rather than visually responsive as the male. However, in a separate experiment, using female rats in their respective environmental conditions in the age span from 60 to 90 days and a more challenging arrangement of the toys, we encountered a cortical response more like that of the male. Having the females climb over a pile of toys to reach their food caused the occipital cortex to change as much as 7%. The results showed that it was possible, with female rats at a different age, dealing with a more encumbering arrangement of the toys, to produce changes in the occipital cortex identical to those of the male.

It was the pregnant females which exhibited the most surprising cortical changes after living in enriched or impoverished conditions. At first we believed that no cortical thickness changes had occurred between enriched and impoverished pregnant females, because both the initial and

% Difference in Cortical Depth
between EC-IC Post-Partum Females
60-116 days

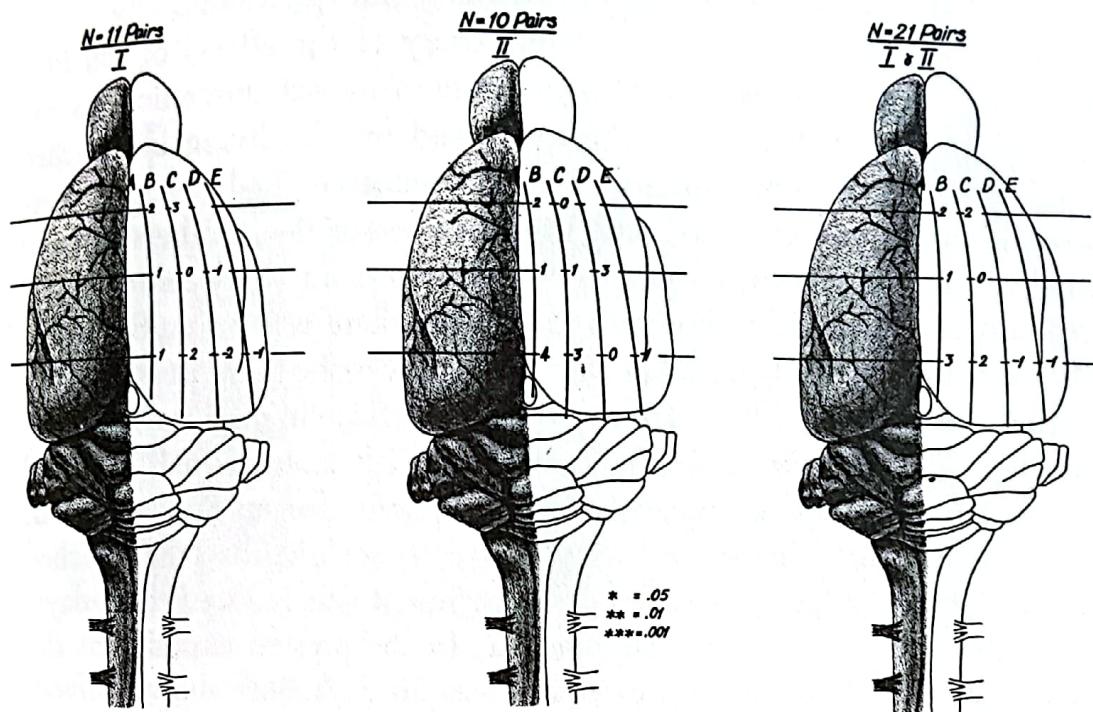


FIGURE 31. Percentage differences in cortical depth (thickness) between enriched (EC) and impoverished (IC) rats in their environments from 60 to 116 days, experiment I, experiment II, and experiments I + II: (a) males, (b) nonpregnant females, and (c) postpartum females.

replication experiments indicated no cortical differences between animals in the two experimental conditions (see Figure 31c). Upon careful examination, however, we noted that pregnancy had a marked effect upon the impoverished rat's cortex, actually increasing its dimensions to equal those of the enriched pregnant rat's cortex. In fact, with additional comparisons, in no instance did the cortical thickness measures of the impoverished postpartum female differ significantly from those of the impoverished male. The two had reached a comparable level of development.

The results on the pregnant rats showed that the female cortex definitely was sensitive to the interaction of pregnancy and the special living conditions. Several questions immediately arose in response to these results. We wondered whether there was a "ceiling" to the effect created by these conditions beyond which the cortex would not increase in thickness. On the other hand, perhaps we could find a way to induce the first cortical fold by combining sex steroid hormones with enrichment; since the mechanism is not known for folding and the intracranial dimensions were identical in enriched and impoverished animals. What could an increasing cortex do in a confined space? We now began experiments to

examine some of the roles played by the sex hormones in the development of the cortex during varied environmental experience.

But before we continued with our study of the effects of sex hormones on the cortex, we found some interesting sex differences by examining the body weights of the males and females living in enriched and impoverished environments. No differences in body weight were noted between the enriched and impoverished males, or between the enriched and impoverished postpartum females; but between the non-pregnant enriched and impoverished females, there was a significant 6% difference in body weight in favor of the impoverished rats. It is of interest that this body weight difference should show up in only the nonpregnant females. In early experiments dealing with male animals that had been in enriched and impoverished environments for an 80-day period, the impoverished males were always of greater weight than the enriched males. But when the duration of the experiment was reduced to 30 days, the body weight differences disappeared. In the present experiment the time of exposure to the environments was 56 days, just about halfway between the 30- and 80-day periods, and only the nonpregnant female experimental group showed the weight gain. Unfortunately, no explanation is available why this one group showed a difference at this particular time period, but the information is presented because once again body weight responses to the environmental conditions can be quite different from those in the cortex.

We continued to attempt to understand how ovarian hormones interacted with environmental diversity to influence development in the cerebral cortex. We had already demonstrated that ovariectomy alone could increase cortical thickness. In a new set of experiments (4), twelve sets of quadruplet Long-Evans female rats were divided as follows: From each set of quadruplets two were ovariectomized and two were sham-operated at day 1. At 21 days of age, the animals were weaned and placed three or four to a cage. At 60 days of age, the rats were separated between enriched and impoverished conditions. In other words, 12 ovariectomized rats lived in one enriched cage and 12 sham-operated rats lived in another. In addition, 12 ovariectomized and 12 sham-operated were separated into impoverished conditions, one animal per standard cage.

As with the pregnant animals in the previous experiment, it was again the impoverished animals that produced the most unexpected results. The total thickness of the impoverished ovariectomized rats' cortices became much greater than that of their sham-operated impoverished littermates (see Figure 32). The percentage differences ranged from 3% ($p < 0.05$) to 10% ($p < 0.001$), with area 18 developing as much as a

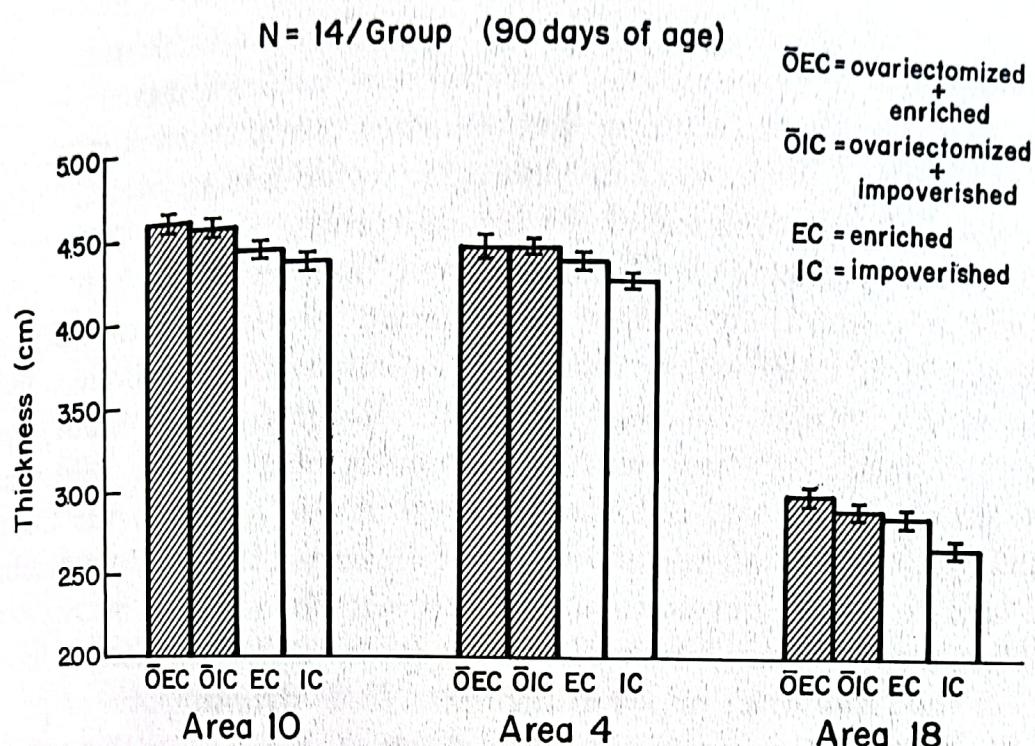


FIGURE 32. Cortical thickness in female Long-Evans littermate rats experiencing differential environments with or without ovaries, N = 14/group (ovariecomized day 1, autopsied 90 days of age).

10% difference ($p < 0.001$) in 13 out of 14 cases. There was no doubt that something unusual was taking place in the impoverished animals' cortices in response to the changes in ovarian hormone levels.

One might offer the hypothesis that the impoverished rats, living by themselves, were under stress and that the adrenal hormones related to stress were responding differently when the ovarian hormones were altered. That stress might be a factor has not been borne out by a comparison of adrenal gland weights between impoverished intact animals and enriched intact rats. In order to be certain that stress is not a major consideration and that adrenal hormones have not been altered, we will eventually have to measure the plasma levels of the corticosteroids (hormones secreted by the adrenal cortex) under the experimental conditions.

Between the ovariecomized enriched and the intact enriched rats, the only cortical regions which showed a significant difference in thickness were those from the frontal cortex (3%, $p < 0.05$, to 5%, $p < 0.01$); none of the differences in samples from the somatosensory cortex or occipital cortex reached a statistically significant level. Figure 32 shows clearly that ovariecomy increases cortical dimensions in both enriched and impoverished animals. It is known that ovariecomized animals take in more food, show increased protein intake, and do little voluntary

exercise (5). Protein intake is an important factor in contributing to cellular growth, whether it be dendritic length or cortical thickness (6, 7). Our results suggest that tissue synthesis could be increasing and producing larger neurons, accounting for the thicker cerebral cortex in the ovariectomized rats. If this were the case, one would find an overall cortical response, not one confined to the frontal cortex.

In these ovariectomized enriched and impoverished rats, we had another opportunity to look for a correlation between cortical thickness differences and body weight changes. We learned that the body weight of the ovariectomized enriched rats was 14% greater ($p < 0.001$) than that of the intact enriched rats. Yet the only significant cortical difference between the two groups of rats was seen in one region of the frontal cortex. On the other hand, when the whole cortex increased on account of a lack of ovarian hormones in the impoverished rats, the ovariectomized impoverished rats were also 14% ($p < 0.001$) heavier in body weight than the intact impoverished rats. These data support the previous findings with the pregnant enriched rats showing a negative correlation between body weight and cortical thickness, and the pregnant impoverished rats showing a positive correlation between body weight and cortical thickness compared with intact rats.

In examining differences and similarities between the sexes in the different environments, we also need to consider the effects of the interactions between males and females. Thus, we investigated whether the brain of the male increases more after living in the enriched condition with the female than when living only with other males. We planned an experiment in which 6 males and 6 females (the usual 12 animals) would live together in the enriched condition between the ages of 60 and 90 days. We chose this age and duration because we had previously learned that it is this age span with 12 rats of like sex living together that creates the most marked changes in the total cortical thickness. We knew that pregnancy would occur when males and females were placed together, whether or not toys were present, and the gestation period for pregnant female rats is only 21 days. We did not want the females to give birth in the enriched condition with all the other rats present, because six females with their litters plus the six males would undoubtedly cause problems, resulting in the loss of the pups and stress to the parents. Therefore we placed one group of six 60-day-old females with the six 60-day-old males for the first 15 days of the total 30-day period and then replaced these females (which we expected to be pregnant by now) with six new 60-day-old females for the last 15 days.

Pregnancy was confirmed, as anticipated, in all 12 females at the end

of their 15-day term in the experiment. Since we already knew that pregnancy compounded the changes in the cortex of enriched females, we primarily compared the brain changes in the enriched males living with males and those in the enriched males living with females.

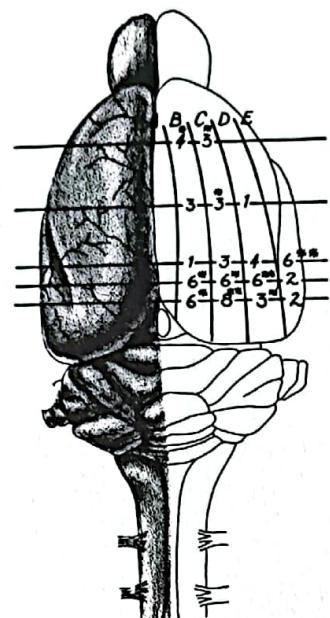
In both the right and left hemispheres, the frontal cortex was thicker in the enriched males living with females than in the males that had no female companionship. In the rest of the cortex, the trends were not as clearly delineated. The frontal lobe changes may have been caused by the close association of the rodent frontal lobes to the hypothalamus and its functions related to sexual behavior. A replication experiment is needed to confirm the trends of these results, since there were only six males in the enriched condition with the females, but these preliminary results do offer the first information indicating that living with females alters the structure of a male's cerebral cortex.

Our findings that pregnant and nonpregnant animals respond differently to the environmental conditions was our first clue that the sex steroid hormones could alter cortical structure. We then explored the role of the ovarian hormones and cortical morphology further, learning that removal of the ovaries at birth altered the cortical asymmetry pattern to resemble that of the male cortex. Removal of the testes at birth reversed the right-greater-than-left pattern in the frontal and somatosensory male cortex. This finding indicated that hormones from the testes can modify cortical structure. Now, we wondered what interaction testosterone might have with the different environmental conditions in their influence on structural changes in the cortex. We studied two groups of male animals, one castrated at birth, before the hypothalamus had adopted the characteristic male form, and one castrated at 30 days of age, after maleness was better established. After weaning, all animals lived three per standard colony cage until 60 days of age. Both castrated groups were divided between an enriched and an impoverished condition between 60 and 90 days of age. The resulting modifications in the thickness of the cerebral cortex were very similar to those found in the intact rats, whether the testes were removed at birth or at 30 days of age (see Figure 33a and b). Both the frontal and occipital cortex developed differences, of up to 4% and 8%, respectively, with the somatosensory cortex showing no significant changes. (See Figure 28 for the corresponding data for intact males.) Thus, it appears that the external environmental conditions rather than sex hormones are responsible for these cortical alterations in the male rat.

That removal of the gonads does not alter the cortex of the enriched or impoverished male but removal of the ovaries affects the female cortex

a.

Percent Differences in Cortical Depth between Neonate Castrates in Enriched and Impoverished Environments (N=11 pairs)



b.

Percent Differences in Cortical Depth between Rats Castrated at 30 Days of Age and placed in Enriched or Impoverished Environments (N=12 pairs)

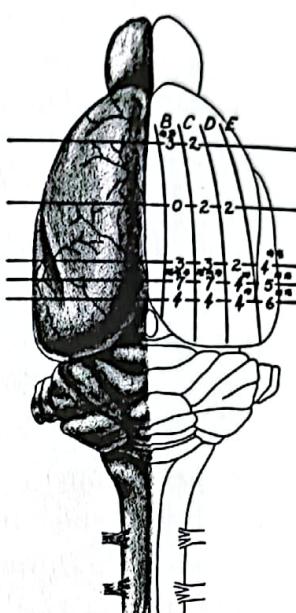


FIGURE 33. (a) *Percent differences in cortical depth (thickness) between neonate male castrates living in enriched and impoverished environments between 60 and 90 days of age (N = 11 pairs).* (b) *Percent differences in cortical depth between rats castrated at 30 days of age and placed in enriched or impoverished environments (N = 12 pairs).*

exposed to these environmental conditions enticed us to continue to study the female. After first learning that the female sex steroid hormones altered the structure of the cerebral cortex, we held a laboratory meeting to discuss the next step in our research. In the early 1970s over 50 million women in the world were on contraceptive hormones for birth control; we wondered whether these hormones could also be affecting cortical function. Undoubtedly, most people will agree that "the pill" is the most effective method of birth control, but we were absolutely ignorant about its effect on this quite plastic region of the brain, the cerebral cortex.

We designed an experiment to study one contraceptive hormone and its relationship to the cerebral cortex (8). We wished to ascertain whether norethynodrel, the progestin component of Enovid, at the time a widely used oral contraceptive, altered not only the thickness but also the electrolyte and water content of the cortex of rats exposed to different

experiential environments. We used samples from the occipital cortex, because this region responds most frequently to our environmental conditions. We were particularly interested in the water and electrolyte measures, because we know these change during pregnancy but we did not know whether they were responsible for some of the cortical increases that resulted from our hormonal alterations.

Norethynodrel did alter the response of the cortex to environmental enrichment. In the animals we treated with this hormone, some areas of the cortex were thinner than the corresponding areas in non-treated animals in the enriched condition. One area in the somatosensory cortex and one in the occipital cortex were found to be significantly thinner, and 15 out of 15 or all the other cortical areas measured showed a trend in this direction.

Not only did norethynodrel reduce the enlarging effect on the cortex, but it had previously been shown to reduce protein synthesis in the brain (9). Furthermore, norethynodrel has been reported to have a slight but significant anesthetic effect on the central nervous system (10). Thus, it was possible that norethynodrel was dampening the brain's excitability, preventing the animals from responding to the stimulation of an enriched environment, as reflected in the thinner cortex.

In addition to possessing cortical thickness differences, the norethynodrel-treated animals in the enriched condition had significantly less cortical sodium, a trend toward both less chloride and higher potassium. There were no differences in electrolytes and water between animals kept in the impoverished condition whether they received norethynodrel or served as controls. If norethynodrel was acting to dampen the response of the cortex to environmental stimulation, these results would be expected. The impoverished environment, by definition, is a low stimulation environment, and norethynodrel would be expected to have a minimal effect. The finding of no difference in cortical water between the experimental groups of animals is consistent with the work of Bennett et al. (11).

We also learned that the sodium concentration in the occipital cortical samples of rats kept in the enriched environment was greater than in the cortices of those animals living in the impoverished condition. It has been proposed that the concentration of intracellular sodium correlates positively with the excitability of the nervous system. It is thus possible that animals in the enriched environment may have a more excitable nervous system than the animals in the nonenriched condition. This hypothesis was supported by the work, reported in Chapter 4, of another

student, Lee Dorosz, who demonstrated a greater peak-to-peak amplitude in the electrically stimulated cortex of the enriched animals compared to the impoverished (12).

What we have learned of the relationship between sex steroid hormones and the asymmetry of the cerebral cortex opens new horizons. It suggests that neuronal and glial cell counts and thickness measurements in male and female human brains should be examined carefully to help us understand some of the biological basis for behavioral differences as well as similarities. The irregularities in the thickness of the human cortex will make the measurements more difficult than they are in the rat. In addition, the range of male and female behavior is extensive, and overlaps exist; but by accumulating data on human brains, scientists can obtain average values as we did for the rats. Very little knowledge is available on the effects of sex steroid hormones on the cerebral cortex during a lifetime, whether female or male.

In summary, the studies reported in this chapter clearly indicate that the cerebral cortical structure responds not only to the sex hormones, but it responds differently depending upon the environmental conditions. If male and female rats experience similar enriched or impoverished environments during the same period of adulthood, the male occipital cortex responds significantly more than that of the female; whereas, the female somatosensory cortex responds more than that of the male. However, if the female is further challenged in her enriched environment, her occipital cortex responds as much as does that of the male. Male rats living with female rats show a greater frontal cortical response than do males living only with males. Pregnancy increases the dimensions of the adult cerebral cortex, as does ovariectomy at birth. Apparently, the cerebral cortex is very susceptible to changes in the concentration of the sex steroid hormones and sometimes in most unpredictable ways. Teasing apart the roles played by the ovarian hormones from those played by the hormones of the adrenal gland will be a challenge for the future.

7

OVERCOMING DEPRIVATION AND STRESS

A few studies have illustrated that an enriched environment can overcome either deprivation or possible stress to the brain. Malnutrition, one form of deprivation, was examined in a recent collaborative investigation on brain development. In this study enriched diets and enriched living conditions were provided in an attempt to help the brain compensate for the detrimental effects of a protein deficient diet during pregnancy and lactation (1). Nutrition plays a critical role in human brain development and thereby in intellectual prowess because food supplies the nutrients that catalyze the chemical reactions underlying mental activity. But we are surprisingly ignorant about how this process operates.

In the late 1960s, Winick and Noble reported that malnutrition during the "critical period" of brain growth resulted in a permanent reduction of brain cells (2). This frightening news focused attention on the potential of the prenatal brain to grow during several stages of its development, not only the very early "critical period." Many subsequent human and animal investigations have dealt with behavioral measures following nutritional restrictions, but such measures were difficult to reproduce because the findings were often inconsistent. Human studies suffer from the same limitations in interpretation as do animal studies when the behavioral testing is done on the very young. With the young child it is difficult to use the results of any test as an index of future performance. In the early 1980s studies on both animals and humans demonstrated the apparent effectiveness of enriched living conditions in alleviating some of the behavioral deficits brought about by early nutritional deficiencies. Though the behavioral manifestations are an ulti-

mate concern, our present attention is focused on neuroanatomical changes related to malnutrition and subsequent nutritional and environmental enrichment.

Such an anatomical study, conducted in collaboration with a Peruvian graduate student in Berkeley's Department of Nutrition, Arianna Carugh, revealed that rehabilitation through enriched diets and enriched living conditions could overcome some of the deficiencies in the brains of offspring whose mothers were exposed to protein deficits during pregnancy and lactation (1).

Carugh's experimental design was as follows. Pregnant Sprague-Dawley rats were fed a 17% protein semipurified diet from day 13 to day 17 of the 21-day gestation period. At 17 days, the pregnant rats were paired by weight and randomly assigned to one of two groups: (1) controls, receiving 17% protein diets, and (2) protein-restricted females, receiving 8% protein diets. These two dietary conditions were continued throughout the rest of the pregnancy and the following 21-day lactation period, during which the litters were reduced to 8 pups per mother.

At weaning, the male pups from the control litters were placed either 2 to a standard cage or 12 in the enriched cage, and all of them had access to the 17% protein diet. The pups from the protein-restricted mothers were paired by weight and randomly assigned to one of four groups, with groups 1 and 2 as the protein rehabilitated animals and groups 3 and 4 as the low protein animals: (1) a group maintained on a 17% protein diet while being housed in a standard condition, (2) a group maintained on a 17% protein diet and housed in an enriched condition (in these experiments, the enriched condition consisted of the usual 12 rats with "toys," but the toys were changed daily instead of two times each week as with the other experiments), (3) a group retained on a 6% protein diet and housed in the standard condition, and (4) a group retained on a 6% protein diet and housed in an enriched condition. The standard colony animals were housed two per cage rather than the usual three as in our other experiments. After the animals had lived for 30 days (from 21 days after birth to 51 days) in their respective environments, the brains were collected for measurement of either cortical thickness or dendritic branching. The dendritic measurements were taken from pyramidal cells in layers II and III, where, as previously mentioned, the greatest cellular changes due to environmental enrichment had been demonstrated.

As anticipated, the control animals, benefiting continuously from the 17% protein diet, were significantly heavier than the protein-rehabilitated animals, which in turn were significantly heavier than those retained

on a 6% protein diet. Differential rearing did not significantly affect any of the body weights. But the cerebral cortices of the animals in the 6 groups did show differences, quite marked at times. In the frontal cortex, the standard colony controls were thinner than the environmentally enriched controls by 4%, the standard colony protein-rehabilitated animals were thinner than the enriched protein-rehabilitated by 6%, and the low-protein animals always had the lowest values in cortical thickness. The environmentally enriched animals also had significantly thicker somatosensory and occipital cortices than the standard animals. For example, in the occipital cortex, this difference amounted to 3% between the standard colony 17% protein group and the enriched-condition 17% protein group. As much as a 9% cortical thickness difference was seen between the standard colony rehabilitated and the enriched rehabilitated animals.

The dendritic branching patterns also showed some differences among the groups. The enriched-condition rehabilitated animals had significantly more high-order dendrites than those animals in the standard colony 17% protein group or the standard colony 6% protein group. When comparing the animals rehabilitated with the 17% protein diet, those in the enriched cages had 17% more dendrites than those in the standard colony cages. In fact, the protein-rehabilitated animals in the enriched condition had a greater number of dendrites than the standard colony controls, whose mothers, as well as they, had always had access to the 17% protein diet. The protein-rehabilitated animals in the enriched condition had only a slightly smaller number of dendrites than the enriched control. No cell counts were made in these studies, but dendritic branching was greater in the protein-rich environmentally enriched group than in the protein-rich standard colony group. In fact, in all areas of the cerebral cortex measured, it was the enriched environment plus the 17% protein diet which created the largest changes in thickness.

These experiments dealing with different types of protein deficiencies with both dietary and environmental rehabilitation clearly indicated that the type of environment in which the protein-deprived animal lives is very important in determining the development of cortical structure. The data clearly show that a protein-rich diet is beneficial for the healthy development of the cerebral cortex, but if for some reason this is not possible, then an enriched diet plus an enriched experiential environment will improve cortical development if provided during the early postnatal period. Postnatal environmental enrichment does play an important role in overcoming certain cortical structural deficiencies caused by dietary inadequacies during pregnancy and lactation.

Having dealt with the importance of protein in the diet and experien-

tial environments to enhance cerebral cortical development, we can turn to another dietary factor significant to brain function, namely glucose. Whereas other tissues such as muscles can rely on alternate fuels like fatty acids, the brain depends almost exclusively on glucose for its energy. The brain has a critical need for energy and oxygen to maintain its high metabolic rate necessary to form and propagate substances for synaptic transmission and to maintain proper ionic gradients. Synapses use a great deal of energy, and glucose supplies this energy.

Different parts of the brain use glucose at different rates. In order to learn what parts of the brain were most active in our enriched living conditions, we studied glucose uptake (3, 4). We hypothesized that the enriched animals would have a more active glucose uptake than the standard colony animals, because the enriched brains have larger neurons with a greater dendritic surface for interaction with other neurons. Therefore, one would anticipate greater metabolic activity in the enriched brains compared to the standard.

For this study, 24 male Long-Evans rats were separated at 57 days of age so that half entered the enriched condition and the other half, the standard colony condition. After 30 days in these respective environments, a pulse of radioactive deoxyglucose was administered intravenously, and concentrations of radioactive deoxyglucose and glucose in the arterial plasma were monitored for a preset time between 30 and 45 minutes. At a prescribed time the brain was removed and prepared for autoradiography to measure the localization of glucose utilization in 30 brain regions. Eventually, we had to consider this only a pilot study, because final data were available on only four enriched and six non-enriched rats.

The 30 areas (including both right and left hemispheres) which were compared were as follows: prefrontal cortex, frontal cortex, anterior cingulate cortex, nucleus accumbens, septal nuclei, caudate putamen, diagonal band of Broca, parietal cortex, corpus callosum, globus pallidus, lateral thalamus, ventral thalamus, medial habenula, lateral habenula, hippocampal fimbria, CA₁ hippocampus, CA₁ hippocampus anterior, dentate gyrus, amygdala, occipital cortex, medial geniculate, lateral geniculate, entorhinal cortex, superior colliculi, inferior colliculi, reticular formation, auditory cortex, substantia nigra (two separate samples), and area 17 of the visual cortex.

The results were the exact opposite from our prediction. Examination of all of these regions revealed that only the frontal and parietal (somatosensory) cortex were significantly different ($p < 0.05$) between the en-

riched and standard control rats. In both the frontal and parietal cortices, rates of glucose utilization were 13% lower in the enriched rats than in the standard controls. Whether or not these effects were random events or were actually due to the experimental conditions can only be determined by a second series of experiments with larger samples of rats. These statistically significant glucose uptake changes did occur in parts of the cerebral cortex where thickness changes due to our environmental conditions had been found previously. One might suppose that the differences were related to the number and size of blood vessels in the frontal and parietal cortices. Other regions of the brain reportedly have richer blood supplies than the cortex, so vascular distribution is not the only factor to account for these uptake differences. However, both the frontal and parietal cortices have more blood vessels than the occipital region, for example, which did not show a significantly decreased uptake in the enriched animals. Yet, the occipital cortex possesses larger blood vessels in the enriched than in the nonenriched. Therefore, we do not believe that the vascular supply alone is the major factor involved in these glucose uptake differences.

Though we did not obtain the results we were expecting, i.e., an increased glucose uptake in the most active regions of the enriched animals' brains, we did learn that it was the cerebral cortex which was the most involved part of the brain in the enriched and nonenriched animals under these experimental conditions. In an earlier study we dissected the brains of the enriched and impoverished animals into 15 different areas, and only the cerebral cortex showed significant wet-weight differences. Therefore, whether one studies the brains by measuring wet weight or by utilizing radioactive glucose, the areas showing the greatest changes between the experimental environmental groups are in the cerebral cortex.

The fact that the enriched animals had decreased rather than increased rates of glucose utilization was unexpected. In every region measured, with but one single exception, the nonenriched rats had a greater glucose uptake than the enriched. The exception was the corpus callosum, where the values were equal. It is conceivable that the differences in "adaptability" between the enriched and nonenriched animals to the experimental procedures contributed to these surprising results. In order to inject the radioactive glucose to measure glucose uptake, it was essential to immobilize the rat. If this type of experimental procedure produced stress for the animal, then the meaning of our results could be that it was the enriched rat that could withstand the stressful situation more than the

nonenriched. The enriched animal might be more "adaptable" to the confined condition; the nonenriched animals would then be using the additional glucose to combat the effects of stress on their metabolism.

In order to test whether stress is part of the explanation for the results of these deoxyglucose uptake studies, a future experiment might purposely restrain the rats by putting them in a confined enclosure after they had experienced the enriched or nonenriched conditions. Then the corticosteroids, the hormones produced by the adrenal cortex in response to stress, could be quantified to determine the degree of stress. If the nonenriched animals did show the higher concentrations of the steroids, then our interpretation of the deoxyglucose studies would be reasonable.

To continue with this line of reasoning, if immobilization was a stressful experience for the rats in these glucose uptake studies, then the immune system may be playing some role in the circle of events which follow stress, beginning with the adrenal cortex and its hormonal action on lymphocytes. We know that stress affects the immune system by decreasing the number of white blood cells which defend the body through acquired immune responses. It will be seen shortly that in our experiments with the nude mouse, an animal deficient in white blood cells called thymic T cells, it was the frontal and lateral regions of the parietal cortex which showed a decrease in cortical thickness measurements in comparison with a "normal" mouse used as a control. These were the very areas which showed the only significant changes in the glucose uptake studies in the nonenriched rats. This similarity in regions for these two quite different types of experiments may be only coincidental, but these findings should be mentioned in the event that such information may be useful as we learn more about the interaction among the cerebral cortex, stress, and the immune system.

The field which integrates these subjects called psychoneuroimmunology is yet in its infancy. But the correlation between the well being of the psyche (cerebral function) and the well-being of the body has been recognized since the time of Hippocrates. The hypothesis that regions of the cerebral cortex that are partially under the influence of voluntary control interact with the immune system is only now gaining acceptance. The results to be presented next suggest that specific areas of the cerebral cortex are associated with immune functions, which in turn can be affected by stress.

Renoux et al. (5), Bardos et al. (6), and Renoux (7) studied the effects of lesions in both the right or left cerebral cortices on the activity of the natural killer white blood cells. Their results suggest that the natural killer responses of mouse spleen T-cells are controlled primarily

by the left neocortex with a modifying influence by the right neocortex. We were interested in refining the experiments of Renoux et al. and Bardos et al., who produced quite extensive cortical lesions in the dorsal and lateral parts of the frontal, parietal, and occipital cortex without penetrating the corpus callosum. Their later lesions destroyed only the frontal and parietal cortex.

We wondered if one cortical region more than another was responsible for the reported immune deficiencies in the lesioned animals. For about one year, we attempted to correlate various cortical lesions with T cell activity and mammary tumor growth (8). Having no success with such approaches, we decided to attack the problem from another direction and examine histologically the cerebral cortex of an immune-deficient animal: the nude mouse, a mouse with a greatly reduced functional thymus gland.

Our experiment was designed to explore differences in cortical morphology between the nude mouse of the BALB/c strain and the normal BALB/c mouse. We already knew that the nude mouse has endocrine problems such as a deficiency in gonadal hormones (9, 10), as well as some neurological problems, such as a reduction in cerebellar size (11). Yet, in spite of its known deficiencies, we learned that many cerebral cortical areas in the nude mouse did not differ significantly from those in the normal mouse.

Not only were we interested in identifying the areas of the cortex which might be deficient in the nude mouse, but we were also interested in any differences in cortical cell populations between the two groups of mice. Since antigen markers have been localized on glial cells, we wished to learn about glial cell populations in the cortices of these thymus-deficient mice. Some investigators had found glial cell differences in the nervous system of nude mice. For example, Kerns and Frank (12) made cell counts in the lumbar ventral gray matter of the spinal cord in homozygous and heterozygous nude mice. They reported a 29% decrease in oligodendrocytes and a 52% increase in astrocytes in homozygous mice compared with heterozygous mice. Another link between the immune system and glial cells has been shown more recently by Merrill et al. (13). They proposed that the T cells participating in inflammatory reactions catalyzed a process that induced proliferation and maturation of astrocytes and oligodendrocytes. In addition, Belokrylov (14) from the Soviet Union homogenized cerebral cortical tissue and found that it contained components which reconstituted the T cell population in the spleen of thymectomized animals, whereas white matter was less active and muscle tissue showed no activity at all. (Belokrylov did not specify whether the

tissue samples were from the right or left cortex or from a particular cortical area.)

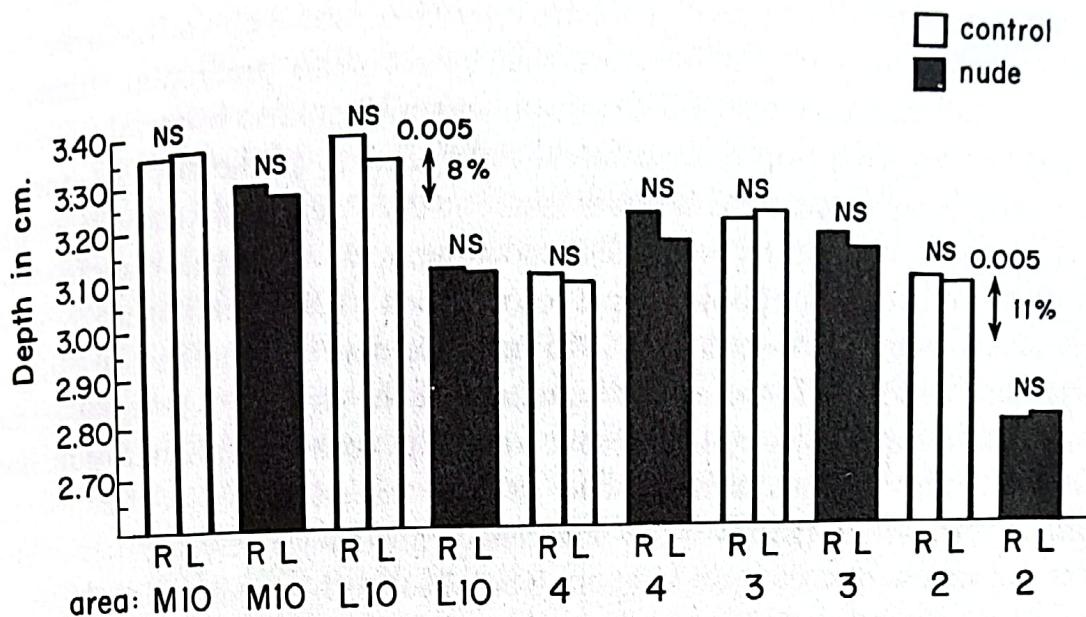
In light of these observations indicating that the cells in the central nervous system were related to immune function, we decided to compare the cortical thickness and differential cell counts in the immune deficient nude mouse with a control. First, thickness measurements of the frontal, parietal, and occipital cortex were made on transverse histological sections from female nude BALB/c mice and normal BALB/c mice. Second, neurons and glial cells were counted in the left area 18 (medial occipital cortex) in both groups of animals. Since the left area 18 cortex was statistically significantly thinner than the right in the nude mouse, this area was chosen for the cell counts in both the nude mice and the control mice.

The results from the BALB/c normal mouse disclosed that the right cortex was thicker than the left in 4 out of 9 cortical areas measured and so there was no distinct trend in asymmetry. However, in the female nude mouse, the right cortex was thicker than the left in 7 areas out of 9, with area 18 reaching a statistically significant difference. Thus, in general, the female nude mouse did have a thinner left cortex than right.

The cell counts in the left area 18 indicated that the nude BALB/c mouse had fewer neurons and glial cells per microscopic field than did the normal BALB/c mouse. However, the differences become statistically significant only in the oligodendrocyte counts, where the nude mouse had 25% ($p < 0.02$) fewer oligodendrocytes per field than did the normal BALB/c animal.

In these experiments, only the lateral frontal and lateral somatosensory cortex were significantly smaller in the female nude mouse than in the normal female BALB/c mouse (see Figure 34). At first glance these results appeared unusual, because when body size is examined, the normal mouse is considerably larger than the nude mouse. But, on the other hand, we had mentioned previously that in the enriched and impoverished rats, there was often a negative correlation between cerebral cortical dimensions and body weight, and so the fact that the nude had a smaller body weight than the control, yet had only two cortical regions which were significantly smaller, was not too surprising. In the investigations of Bardos et al. (6), lesions in the dorsal and lateral left cortex resulted in a 50% reduction in the number of splenic T cells and a severe depression of T cell-mediated responses. Our results suggest that the lateral frontal lobe might have a role in modifying immune deficiencies, since it is so much smaller in the nude mouse than in the normal.

In our experiment, however, it is both the right and left frontal lobes



Cortical area and condition

FIGURE 34. Right-left cortical (frontal and parietal or somatosensory) thickness differences between BALB/c mothered and BALB/c nude mice.

which are deficient in the nude mouse. In their earlier work, Renoux et al. did not show a reduction in T cells with a right hemispherectomy, but their more recent reports indicate that the right hemisphere as well as the left play a role in immune function. Renoux (7) stated that partial ablation of the left neocortex did indeed inhibit T cell-mediated responses and natural killer cell activity and that a similar lesion in the right hemisphere caused a small but reproducible increase in these activities. Thus, there appears to be some interaction between both hemispheres in these immune responses. That the left hemisphere of the nude mouse had fewer oligodendrocytes than the normal BALB/c mouse supports the data of Kerns and Frank (12), who reported a decrease in oligodendrocytes in the central nervous system of the nude mouse. We have not counted oligodendrocytes in the right cortex in these mice.

The smaller size of the lateral somatosensory cortex could signify one of at least two things or possibly both. Since the nude mouse has no body hair, it could result from smaller amounts of sensory input from the body surface in this mouse. Or it may be that the lateral somatosensory area is another cortical region involved in the control of the immune system. Only further experiments in this field will help to clarify these results.

If the frontal cortex does closely monitor the immune system, then one might predict that people given frontal lobotomies would demonstrate immune deficiencies. There are only two, not well-controlled clinical cases reported in the literature that bear on this prediction. First, in 1939, Messimy (15) reported that monkeys subjected to bilateral prefrontal lobectomy developed an unusual enlargement of the thymus and of the lymphatic system. This work has not been repeated, and the result may have been due to an undetected infection, since the responses to surgery affected lymphoid tissue other than that within the thymus gland. Second, in 1961, Ascengi (16) presented an unusual case of a nineteen-year-old male in whom enlargement of the thymus was noted three months following frontal lobe severance due to a self-inflicted gunshot wound. The thymus actually had an appearance similar to that of an infant. All other tissues were apparently normal. These are not well-defined cases, but they do suggest frontal lobe-thymus relationships.

Now how does this knowledge that the frontal lobe and the oligodendrocytes are reduced in the nude mouse, which is known to possess a deficient immune system, become important to us as we study the effects of experiential environments? We wish to learn whether we can produce measurable changes in the immune system as a consequence of our enriched and impoverished environmental conditions. We have demonstrated that both the right and left frontal lobes are altered when rats are placed in these conditions. Also, we have found that animals in the enriched condition have more oligodendrocytes than do those in the impoverished condition. Thus, differential environments can alter the number of oligodendrocytes and the frontal cortical thickness. In the future we will take the next step of investigating whether we can produce measurable changes in the immune system as a consequence of our environmental conditions. Why is it important to identify more precisely the mechanisms responsible for cerebral-cortical control over the immune system? At least one advantage is obvious. If the cortex does have mechanisms that regulate antibody-antigen activities in response to environmental input, then self-regulation of one's health becomes a responsibility. Evidence of cortical responsivity to environmental input would provide substantial support for the use of such cortical control processes as meditation and biofeedback.

In continuing our attempt to understand the relationships between the structure of the cerebral cortex and possible stressful situations, we investigated the effects of crowding on rats. Crowding is considered stressful under conditions where competition for space or food is likely. The usual number of rats which were housed together in our enriched

conditions was 12. We hypothesized that by increasing this number we would be creating an overcrowded condition which might have deleterious or stressful effects on cortical development. Over the years we have purposely kept the same basic enrichment paradigm, with 12 animals past the age of weaning, to allow for as much consistency as possible in the amount of sensory input. If one of the main objectives of our investigation was to study the effects of enriched environments throughout the lifetime of the animals, then similar conditions had to be maintained for the entire period of study.

But the idea to change the number of animals in the enriched cage surfaced when my husband and I were invited to give lectures in China in the fall of 1985. Before going, I wanted to plan an experiment that might prove of interest to our hosts. The Chinese were already feeding 1 billion people. Nutrition would still be of interest as a subject for investigation, but I thought that the Chinese might now be more interested in the problems created by their crowded living conditions. We did not know the effect of overcrowding on brain development. Here was an appropriate moment to attempt an experiment by placing a larger number of animals together in the enriched cage than the usual 12 animals.

Overcrowding, defined as increasing the number of individuals in a confined space, has been considered both detrimental and beneficial. Deleterious effects, caused by too many rodents in a confined space, have been reported where competition for food and mates exists. Calhoun (17), working with mice, noted increased hostility, destructive behavior of adults toward the young, emotional instability, space encroachment, retardation of sexual maturity, and increased infant mortality. However, certain aspects of Calhoun's experimental conditions may have been more responsible for these outcomes than crowding per se. The cages in which the mice were confined were cleaned only every two weeks, and the dead mice were not removed daily. Anyone who has worked with mice knows how important it is to keep the cages clean because of the overpowering odors from urine, to say nothing of dead mice.

In contrast to the detrimental effects reported by Calhoun from his studies on mice, Ross (18) reported that crowding by itself did not seem a sufficient cause of aberrations in human behavior, noting that some communities thrive in close quarters. Needless to say, the factors affecting responses to crowding are complex, and we sought to illuminate at least some of them by examining the effects of crowding on brain development in our enriched rats.

For the initial experiment, we wished to place 60-day-old male rats

in three conditions: (1) 12 rats separated into 3 per cage ($32 \times 20 \times 20$ cm), a standard colony condition with 213 cm^2 per rat; (2) 12 rats altogether in a large enrichment cage ($70 \times 70 \times 45$ cm with "toys," the usual enriched condition, with 408 cm^2 per rat; and (3) a "crowded" enriched condition. But we were not certain what would constitute "crowding." At first, we placed 24 rats into the enrichment cage, but the rats did not "look" crowded. Even when we increased the number to 30, we agreed that no obvious crowding existed, but by the time 36 rats were placed in the cage, there was a consensus that this condition was now considerably more crowded (even though the space per rat was well within the required limits stated by the Berkeley Office of Laboratory Animal Care). So 36 rats in a large enrichment cage with toys constituted the crowded enriched condition, 136 cm^2 per rat. The toys were matched in the second and third conditions in the cages and were changed from a common pool of clean toys two times each week during cage cleaning. The animals remained in their conditions for a 30-day period, because we had previously learned that most of the cortex responds to the experimental conditions during the age span from 60 days to 90 days.

The results indicated that whether there were 12 or 36 rats in the enrichment cage, the thickness of the medial occipital cortex increased significantly by 4 to 6% compared with the standard colony rats (see Figures 35 and 36). In addition, the crowded enriched group showed a significant 4% increase in area 39 that was not so clearly demonstrated in the enriched rats in the uncrowded condition. Since this region is one where multisensory integration takes place, it is possible that the additional crowding constituted a more stimulating sensory environment.

The environmentalist, Calhoun, offered some ideas that might be important in interpreting the results of our experiment (17). He postulated that escape factors allow human beings to tolerate, or to adapt to, high population densities, even under conditions where there is an overload of stimuli. Strain may occur early only in the subordinates in a group with no escape factors; with time, however, the effects of over-crowding may become manifest throughout the whole group. We hypothesized that interaction with the toys might be diverting the rats' attention or entertaining them sufficiently to mitigate the stress of the crowded condition.

Contrary to other reports on the effects of crowding, we observed no fighting among the 36 males in the enrichment cage. In fact, they seemed to be living in harmony for the duration of the experiment, to be finding sufficient space to be comfortable, and to be interacting well

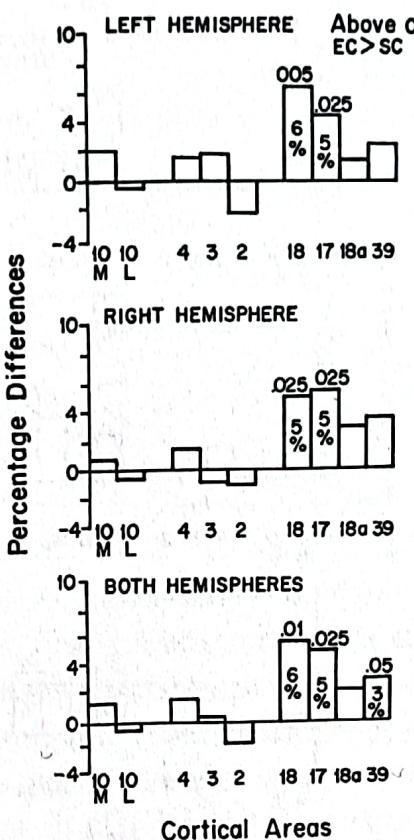


FIGURE 35. Percentage differences in cortical thickness between standard colony (12) and enriched condition (11) male Long-Evans rats.

with the toys. There was no apparent stress in this type of living arrangement.

It would be interesting to see whether males living in the same crowded situation would fight one another if females were introduced into their midst. In the experiment described in Chapter 6 in which six males and six females were housed in the enriched cage, no obvious fighting took place. If a female or group of females lived in a cage near the crowded cage or if a single female or several were introduced briefly into the crowded cage, I wonder if we would have made a similar observation.

In summary, the results in this section have shown that environmental enrichment can mitigate the impact of a range of deprivations and stressful situations. The enriched condition in conjunction with an enriched diet enhanced cortical development in animals subjected to early dietary insufficiency. The enriched environment appeared to have a beneficial effect on rats experiencing restraint during glucose administration.

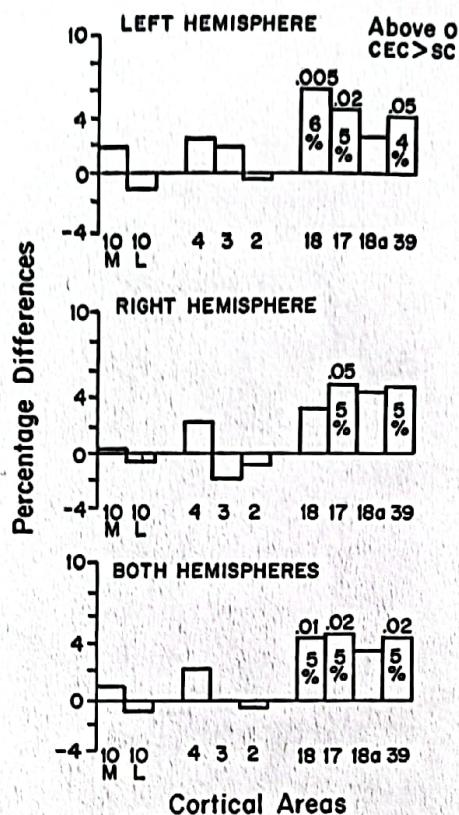


FIGURE 36. Percentage differences in cortical thickness between crowded enriched (16) and standard colony (12) male Long-Evans rats.

The enriched condition increased the cortical dimensions by a similar or even slightly larger amount whether 12 or 36 animals lived in an enrichment cage. Thus, the beneficial effects of enriched living conditions are found using many experimental variables. Evidence exists showing that the effects of stress and the immune system are related to the cerebral cortex. The lateral frontal and parietal cortices are suggested areas for further study in attempts to gain further knowledge on how the cortex can regulate immune functions.

8

THE IMPACT OF AIR IONS

The possibility that physical environmental stimuli, such as electromagnetic waves, may have effects on the brain has already received some attention by other investigators. That the balance of positive and negative ions in the atmosphere may be another such stimulus is suggested by the early work of Sulman et al. (1). These investigators studied the responses of groups of "weather-sensitive" individuals to changes in the ionization of air. Though the relationship between air ions and weather-sensitive people is not well established, scientists are attempting to gain a better understanding of human sensitivity to weather and of the biological effects of atmospheric ions in general. According to Sulman, only about 30% of the population is weather-sensitive.

Being one of these weather-sensitive people, I found this developing field to be of great interest. As a girl, walking 2 miles home from school uphill through the sagebrush, I could always predict an oncoming thunderstorm by the presence of painful headaches. How could the weather cause my head to hurt? How could something in the atmosphere alter something within the skull? I like the passage in *Notes from the Underground* in which Dostoevsky carries out a discussion with his readers on whether "science itself will teach man that he never had any will of his own, . . . that everything he does is not done by his willing it, but is done by itself, by the laws of nature" (2). The laws of nature do sometimes act in unexpected ways, as we shall learn in this chapter by studying the responses of the cerebral cortex to the levels of air ions.

The word "ion" was coined by Michael Faraday because ions migrate. Ionization occurs in the air from collisions between particles. Air ions form when energy from radioactive compounds in the soil or from cosmic rays acts upon a gas molecule and causes it to eject an electron.

The molecule stripped of its electron becomes a positive ion, and the displaced electron then attaches to a neighboring molecule, which becomes a negative ion. Each molecular ion immediately attracts nonionized molecules to form a cluster of 8 to 12 molecules—an air ion. Negative ions can be produced naturally around falling fresh water, e.g., waterfalls or showers. In normal, clean air over land there are about 1500 to 4000 ions per cubic centimeter, but in polluted air in cities or in stale air in buildings, the ion content can be considerably lower. The ion content in the building where I work, the Life Sciences Building, was measured to have fewer than 100 positive ions per cubic centimeter.

In 1960 Krueger and Smith suggested that the known physiological and biochemical effects of air ions may be due to their ability to alter the metabolism of biogenic amines, of which the neurotransmitter serotonin is one (3). There is evidence that the cyclic nucleotides participate in some of the metabolic events underlying synaptic transmission within the brain and that changes in the brain content of compounds such as adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP) may reflect interactions between some neurotransmitters and their synaptic receptors. We wondered whether air ions could influence serotonin, cyclic AMP, as well as cyclic GMP in our rat brains and, if so, were these changes related to behavior?

The neurotransmitter serotonin has been implicated in changes in mood. A well-controlled study correlating air ions, serotonin concentration in the urine, and mood was performed by Sigel at the University of California in San Francisco (4). She asked each of 33 men to spend 2 hours in a small room containing high levels of either positive or negative ions; later, each man spent 2 hours in a room with the other kind of ion. Sigel found that both types of ions reduced serotonin and made the men feel good. The results indicated that ions clearly influence behavior, but in a complex way that can only be understood one step at a time.

We decided to take one such step in our laboratory. Since no single investigator was adequately prepared to produce and monitor the ions, to design the behavioral paradigm, and to quantify the chemical changes, we formed a team of scientists: Elaine Orenberg, Ph.D., a neurochemist at Stanford University; Michael Yost, a graduate student in public health at Berkeley; Professor Albert Krueger, our specialist in the field of air ions; James R. Connor, a graduate student in the physiology and anatomy department at Berkeley; Michael Bissell, M.D., a neuropathologist at the Veterans Administration hospital in Martinez, California; and myself.

Our experiments were divided into three groups. The first study,

which used pups as subjects, was undertaken to investigate whether the effects of negative air ions depended on whether the animals lived in enriched or impoverished environments; serotonin and cerebral cortical weight were measured.

In the first experiment (5), groups of male Long-Evans rats were housed in enriched or impoverished environments, with and without increased levels of negative air ions. Nine littermate pairs of 6-day-old pups were distributed among six mothers into two experimental environments: a multifamily enriched condition and a unifamily impoverished condition. (These behavioral conditions were similar to those reported in Chapter 5 in connection with our study of the effects of differential environments on the developing brain in animals not yet weaned.) In the multifamily enriched condition, three mothers, each with three pups, were housed in one large cage filled with toys. In the unifamily condition, one mother was housed with her three pups in a standard colony sized cage with no toys. There were three cages housing the unifamily rats.

To serve as controls animals lived in atmospheric conditions, i.e., received air delivered by the building ventilation system and contained fewer than 100 positive ions per cubic centimeter of air. These animals were grouped in a similar fashion and were housed in either a wire-mesh enrichment cage or standard laboratory cages (18 pups in the atmospheric-condition groups), but in a separate room. All pups lived in their respective housing from the age of 6 days to the age of 26 days.

Both the enriched and the nonenriched animals exposed to negative air ions lived in Lucite cages, with Lucite toys in the enrichment cage. A grounded wire-mesh floor was suspended over the sawdust waste collection tray at the cage bottom. A fan supplied continually moving air to each cage, and a filter was used to free the air of particulates.

An air ion density of 1×10^5 negative ions per cubic centimeter was maintained in both the large and small cages. Negative air ions were generated by corona discharge from Amcor Modulion power supplies and regulated in each cage by adjusting the ionization potential with separate variable transformers on the ac lines of each generator. The ion density of the air in each cage was measured with the aid of a Royco volumetric counter and was correlated with the flow of current from the wire-mesh floor to an earth ground. Daily checks of the current flow to ground were made to ensure proper operation of the ionization equipment.

Before brain samples were dissected for chemical and wet weight measures, all animals were coded to prevent experimental bias. Uniform

samples of somatosensory and occipital cortices were surgically removed from both hemispheres, weighed, and frozen. All procedures were accomplished within 4 minutes.

The results of these experiments were most revealing, informing us of the many ways the ion content of the air can affect the cerebral cortex. First, in both the initial and the replication experiments the wet weights obtained before the chemical assays from the samples of the somatosensory and occipital cortices were heavier in rats receiving negative ions than in those living in atmospheric conditions. Here was evidence that exposure of rats to high levels of negative ions increased the weight of the outer layers of the brain.

The chemical changes were equally consistent. We learned that the enriched rats living in a negative ion atmosphere had significantly less serotonin (61%, $p < 0.01$) and cyclic AMP (45%, $p < 0.05$) in the somatosensory cortex than the enriched rats living in atmospheric conditions. Cyclic AMP is a second messenger; i.e., it translates extracellular messages into an intracellular response. Serotonin and cyclic AMP concentrations in the occipital cortex were also significantly less (45%, $p < 0.05$, and 35%, $p < 0.05$, respectively) in the enriched rats receiving negative ions than in the enriched rats living in atmospheric conditions. It appears that in the enriched condition, but not in the impoverished condition, negative ions prevent the increases in serotonin and cyclic AMP concentrations that occur in atmospheric conditions. The cyclic GMP levels increased slightly, though not significantly, in the somatosensory cortex and in the occipital cortex of both the enriched and impoverished rats receiving negative ions in comparison with their counterparts living in atmospheric conditions.

Our data on serotonin are consistent with other reports showing that negative air ions decrease brain serotonin. Gilbert (6) used negative ions to reduce emotionality caused by isolation; the reduction in emotionality paralleled a decrease in serotonin. Olivereau (7) found that brief exposure of rats to 1.5×10^5 negative ions per cubic centimeter of air modified their ability to adapt to a stressful situation. He considered the effect to be due to a reduction in serotonin caused by the action of air ions.

In most instances, the pattern of change in the content of cyclic AMP was in the same direction as the change in serotonin. If changes in cyclic AMP are serotonin-dependent, cyclic AMP might reflect the metabolic alteration in serotonin resulting from negative air ions, as well as from the living situation.

The content of cyclic GMP was relatively unchanged by either the atmospheric or the environmental state. This may indicate that the neu-

ronal cells selectively control steady-state tissue levels of the two cyclic nucleotides by independent regulatory mechanisms.

We chose 6-day-old rats as experimental animals in this study in order to identify potential effects of air ions on neural development. There appears to be a serotonin-dependent adenylate cyclase system which participates in some of the metabolic events underlying synaptic transmission in very young animals, and which decreases in sensitivity with age. Our results showing that serotonin and cyclic AMP are similar in their response patterns to our environmental conditions may be due to this coupling. It is conceivable, however, that negative air ions could have shifted the apparent developmental stage of the rats in this study by changing the concentration of some hormones, such as prolactin, sex hormones, or serotonin-derived melatonin. A direct effect of air ions on prolactin levels was proposed by Olivereau in his study of the effects of air ions on the spontaneous movements of amphibian larvae. A direct hormonal effect of this kind in our system may have caused the increased cortical weights and decreased cortical levels of serotonin and the second messenger cyclic AMP reported here. A further study comparing the effects of negative and positive ions and different environments on various neurotransmitters would help clarify how the responses change the developmental stage of the animal brain. Undoubtedly, the direction of change of one transmitter does not indicate how other transmitters are altered.

Having shown these effects of negative ions on weight and neurotransmitter concentration in the young, developing cortex, we turn to our second study, one measuring the effects of elevated levels of negative ions in older brains. For this experiment we were interested in learning whether brains from animals living for an extended period of time in conditions with high levels of negative ions aged more rapidly than those from animals in atmospheric conditions. It seemed of importance to understand the effects of long-term exposure to these ions because of evidence which suggests that negative ions increase metabolic activity. For example, it has been shown that negative ions increase ciliary motility in the respiratory tract. In addition, as was found in the first experiment described in this chapter, the weight of the developing cerebral cortex increases with high levels of negative ions. With these two pieces of information, it appeared to us that negative ions might have the effect of accelerating maturation and aging by somehow increasing metabolic processes. If this were the case, could the ions affect the aging process of nerve cells? In order to answer this question, we planned an experiment with Professor George Ellman in the psychiatry department at the

University of California in San Francisco to learn whether the aging pigment called lipofuscin, found in nerve and glial cells and thought to be a metabolic biproduct, could be measurably altered with prolonged use of negative ions.

For this experiment, female Long-Evans rats, 7 months of age, were separated into one of four environmental conditions: (1) an enriched condition with the addition of 1×10^5 negative air ions per cubic centimeter (8 rats in a large cage, with the toys changed every other day excluding weekends); (2) a nonenriched condition with 1×10^5 negative air ions per cubic centimeter (2 rats per small cage, providing a total of 6 rats in 3 small cages); (3) an enriched condition with atmospheric air with the rats caged as in the first group; and (4) a nonenriched condition with atmospheric air, with the rats caged as in the second group. At 14 months of age, 7 months later, the somatosensory and occipital cortices of the left hemisphere were removed, weighed, and frozen in order to assay for the lipofuscin (8).

Since the results were statistically nonsignificant, they are mentioned only to show trends. Quite unexpectedly we learned that the lipofuscin concentration was less in the enriched animals than in the nonenriched, whether the animals were exposed to excess ions or not. The percentage differences in lipofuscin concentration between the enriched and nonenriched were quite large. With the negative ions, the differences were 16%, and in atmospheric conditions, 9%. The standard errors of the mean were much larger than those seen in the wet weights of the same tissues, for example. These data indicate that there is great variability in lipofuscin accumulation in individual rats during aging. They also suggest that animals living in an enriched condition do not accumulate lipofuscin at the same rate as animals living in a nonenriched condition. As has been mentioned previously, enriched rats have more large capillaries in their cortices than nonenriched rats. It is possible that the more efficient vascular system transports precursors of lipofuscin away from the cell. If so, then we still do not know the answer to our original question, Does lipofuscin accumulate faster in enriched or in nonenriched brains? Whichever way the process does work, the data suggest there is less of the pigment in the more active enriched brains when the animals have lived in their respective conditions for as long as 7 months during early adulthood. Since it is thought that an accumulation of lipofuscin in the cytoplasm of the cell can hinder its function, less pigment in the enriched cells could be interpreted as beneficial.

Though our primary interest in air ions was their effect on the brain, it was relatively easy to take blood samples and examine the effects of

high negative ion concentrations on white blood cell counts, as well as on brain sections taken from the same animals. If we could find changes in the white blood cells, some of which are related to antibody-antigen responses, then we would be encouraged to utilize the brain slices to make the more tedious counts of glial cells, which also have antigen markers. In an experiment using mice in three separate age groups, we found that white blood cell counts were altered with high levels of negative ions. The design of this third experiment was as follows. One-month-old mice lived in standard colony mouse cages with 25 mice per cage. One cage, placed on an electrically grounded floor, was supplied with negative ions (2×10^3 ions per cubic centimeter), and the other cage had no electric field. The animals were exposed to these conditions for different time periods: 3 weeks, 2 months, and $3\frac{1}{2}$ months. For the two groups of mice and for the three different age groups, the lymphocyte counts were greater by 11% ($p < 0.01$) to 18% ($p < 0.05$) in the ion-rich condition than in the animals living in the grounded cages. The neutrophil count was in the opposite direction: 25% ($p < 0.01$) to 36% ($p < 0.05$) lower in the ion-rich atmosphere than in the other group. These results clearly indicate that the number of white blood cells can be changed with high levels of negative ions. With these results we will some day return to our slides of brain sections to examine the ion effect on glial cell populations.

These experiments have informed us how sensitive nerve and blood cells are to different levels of atmospheric ions. In the brain cells, the strength of the effects depends on whether the animals are living in enriched or impoverished conditions. The high levels of negative ions decreased both serotonin and cyclic AMP in the enriched but not in the impoverished animals' brains. The ion studies have been used primarily to indicate how subtle changes in the ion content of the air can alter brain structure and chemistry, not to endorse or discriminate against the use of ions. Undoubtedly, further research will demonstrate that many other structures are equally sensitive to the ion content in the air.

9

LEARNING AND BEHAVIOR

The anatomical changes documented in the preceding chapters are important in themselves as indicators of the brain's plasticity, but their broader significance lies in their implications for learning and behavior. Many behavioral studies have evolved out of Hebb's original hypothesis (1) that enriching experiences early in life lead to permanent brain changes that enhance problem-solving capabilities.

Psychologists have known for a long time that early experience influences the adult performance of an animal. In early experiments in the 1950s (2-6), investigators were interested in such matters as how much experience in complex environments was necessary to produce a highly intelligent adult animal and when, specifically, during early life this experience had to occur.

Forgays and Reid (7) investigated the second of these questions and attempted to learn when during the short maturation period a limited amount of exposure to the complex environment had to occur in order to effect superior problem-solving ability in the adult animal. Their animals lived in a large box ($124 \times 71 \times 28$ cm) with "playthings" and were compared with animals living in regular laboratory cages. Five groups were given the enriched experience, each during a different age period—0 to 21 days, 22 to 43 days, 44 to 65 days, 66 to 87 days, and 88 to 109 days—and a sixth group had no enriched experience. There were differences in error scores in the Hebb-Williams maze test among the six groups in this study when they were tested two weeks after their enrichment experience. For example, the findings from four groups are mentioned. The group exposed for 3 weeks immediately after weaning, from day 22 to day 43, made the fewest errors compared with those exposed during the first 3 weeks of life (0 to 21 days) and with those

exposed at about the time of maturity (88 to 109 days). As anticipated, the nonenriched animals made the most errors; all of the enriched animals were better problem solvers than those with no enrichment. There did not appear to be a critical period for learning which would designate that only at one time can a specific influence be effective. The results from this experiment of Forgays and Reid are consistent with the evidence from our anatomical measures showing that the cerebral cortex can be altered in structure as a consequence of enrichment at any age, but at some ages more than at others.

Later, in 1968, Denenberg et al. (8) noted that virtually all of the studies that had tested Hebb's original theory found that exposure to an enriched condition soon after weaning enhanced problem-solving and perceptual abilities. But they pointed out that the experimenters had been testing the animals soon after removing them from the different environmental conditions and consequently they were not really testing Hebb's hypothesis that the induced changes were relatively permanent. The data from Forgays and Reid's experiment (7), in which the animals were tested about 2 weeks after experiencing enrichment, did support Hebb's theory. Denenberg et al. (1968), however, allowed an extensive period to intervene, about 1 year, between termination of the environmental conditions and testing. Between birth and 21 days of age, 38 rats were raised in maternity cages or in enriched free environments (one square meter of floor space with toys). At 21 to 50 days of age, the animals were placed either in laboratory cages or in free environments. Then all rats were kept in laboratory cages until testing at 371 days of age. They found that either preweaning enrichment (from birth to 21 days) or post-weaning enrichment (21 to 50 days) had a significant effect on performance.

The smallest error scores on the Hebb-Williams testing maze were found in those animals that had received the free-environment experience both before and after weaning, when compared with the animals which had free-environment experience only before weaning. Though different error scores were obtained for the different groups, the data allowed the investigators to conclude that early enrichment brought about permanent brain changes as measured by enhanced learning experience after a period of one year.

These types of behavioral tests are continuing in the 1980s. Ivinskis and Homewood (9) studied the effects of preweaning environmental enrichment on later problem-solving behavior in rats. In essence the results they found through behavioral studies supported the findings from our own anatomical work, reported in 1971, that enrichment during pre-

weaning significantly increased cortical thickness, whether or not the enrichment had taken place before the eyes first opened (10). Ivinskis and Homewood found that exposure to the enriched environment for a period of 7 days before the eyes opened improved rats' later problem-solving behavior above that of the controls, and even raised it to the level of rats that had received such experience after the eyes had opened. These investigators suggested that the presence of the mother in the enriched condition might result in additional stimulation to the pups during the preweaning period.

Warren et al. (11) chose to study middle-aged (600- to 750-day-old) enriched mice with matched controls on four learning problems and an activity test. This was fortunate because we had anatomical data on rats within this age group. Warren et al. found that the enriched mice were significantly superior on incidental learning and on a food-seeking task, but did not differ significantly from the controls on a brightness discrimination task, on the Lashley III maze, or on an activity test. The enriched mice also had more cerebrocortical cells with very high levels of RNA, leading the authors to conclude that the mammalian brain appears to have the capacity to remain responsive to environmental enrichment well into advanced age. These results support our morphological findings for this middle-aged group very nicely, but we still do not have any behavioral data from others to compare with our anatomical data in our very old (904-day-old) enriched rats.

Most investigators studying correlations among behavior, enrichment, and anatomy examine the difference between the means of their results. Watson and Livesey (1982) introduced an important consideration regarding the evaluation of data by pointing out that scientists should examine the variability of the experimental animals (12). These investigators found in comparing enriched and isolated rats which had lived in their experimental conditions for periods ranging from 10 to 51 days (in four experiments) that the enriched groups were more similar in their maze performance than were the isolated animals. These results suggest that enriched group living provides an environment for some biological processes to become more synchronized. This has been shown to be true in certain human living situations; for example, females living together have been known to develop synchronized menstrual cycles. The isolated rats appear to develop their own idiosyncratic behavior, as demonstrated by their more varied maze performance.

These examples of behavioral data, collected from the early 1950s to the 1980s, indicate that environmental enrichment facilitates learning, memory, and problem solving as tested by maze performance. However,

one is still faced with the question whether the greater learning ability results because an enriched condition promotes better encoding or faster encoding or the encoding of more information, or whether a larger nerve cell caused by enrichment can just process information more efficiently. Any of these possibilities either singly or in combination may be partially responsible for the enriched rat's ability to perform better in a maze than the nonenriched rat.

In order to tease apart what cortical areas and neural structures are involved in enabling the enriched rat to be a better maze runner than the nonenriched animal, Rosalie Greer decided to study an abnormal rat, one with marked learning deficiencies. Its cortex could be compared with that of an animal without such deficiencies, a similar approach to that employed in the experiments examining the cerebral cortex of the immune-deficient nude mouse. She studied the cortex of the Brattleboro rat, which not only shows abnormalities in learning and memory but also possesses a defect in the synthesis and release of the hormone vasopressin, sometimes called the antidiuretic hormone. The question then surfaced whether an animal without vasopressin and with difficulties in learning and memory would develop changes in the cerebral cortex in response to stimulating environments. To answer this question, two types of rats were raised: (1) a heterozygous Brattleboro rat with an abnormal vasopressin concentration in certain brain regions and (2) a homozygous Brattleboro rat with a total absence of vasopressin. Greer compared the cortical thickness and dendritic patterns of these two types of Brattleboro rats (13-15).

In order to carry out this series of experiments, these special rats had to be ordered from the National Institute of Health and then bred in the Berkeley laboratories. The offspring from the animals were housed singly in standard cages for 48 hours at some time between 33 and 40 days of age to diagnose the level of the antidiuretic hormone by volume of water intake, in other words, to determine which were homozygous and which heterozygous. In our laboratories, enough animals were produced to form three groups of male homozygous and heterozygous rats. In the first group, 5 homozygous 60-day-old rats and 9 heterozygous 60-day-old rats living in standard colony conditions were sacrificed to serve as a baseline. In the second group, 5 homozygous 90-day-old rats and 5 heterozygous 90-day-old rats living in standard colony conditions were sacrificed. In the third group, 12 homozygous and 12 heterozygous rats which had lived since 60 days of age in enriched conditions were sacrificed at 90 days of age.

The brains from these rats were processed to reveal the effects of age

and enrichment on the dendrites of pyramidal neurons in layers II and III in the occipital cortex, as well as on the cortical thickness. Cortical thickness increases in response to enrichment in both types of Brattleboro rat were greater and more widespread than in normal Long-Evans rats. Whereas the maximum cortical thickness response in normal rats has been in the medial occipital cortex, area 18, the maximum response in both types of Brattleboro rats was in the sample of the lateral posterior cortical sample, area 39. The location of the dendritic response within the occipital cortex was different for the homozygous and the heterozygous animals, with the homozygous responding more in the lateral cortex. In addition, in the Brattleboro strains many subcortical regions, such as the hippocampus and the region containing the thalamus and hypothalamus, showed increases in response to enrichment, something not seen in the other strains studied.

These results shed additional light on the response of the brain to enrichment, suggesting a two-component response: first, a generalized reaction consistent with the arousal hypothesis offered by Walsh and Cummins (16), i.e., the animal is actively alert in the enriched environment, and second, a more specific, localized response in regions of greater neuron impulse traffic, as suggested by Szeligo and LeBlond (17). In the normal rat, greater neuron impulse traffic plays a larger role as indicated by the localized changes. Whereas, in the Brattleboro rat, the generalized reaction predominates. In both types of Brattleboro rats, a predominance of the generalized reaction is suggested by the widespread increases in both cortical and subcortical brain dimensions and is consistent with their prolonged arousal (18). The increases seen in cortical thickness and dendritic branching may result from excessive arousal, and any associated memory storage is probably maladaptive.

In normal rats there is evidence that vasopressin is released into certain limbic regions which include the hippocampus. In these regions vasopressin activates the neurotransmitter epinephrine and enhances consolidation of emotionally significant information. Evidence suggests that the hippocampus might be activated to facilitate the consolidation process and to impose a temporal limitation on this process via habituation.

Abnormally persistent memory in the homozygous rats, those completely lacking in vasopressin, was demonstrated by Celestian et al. (19). It is possible that the homozygous Brattleboro rat, lacking vasopressin, cannot normally facilitate norepinephrine release in critical limbic regions during emotional stress; thus, both consolidation and habituation are impaired. However, in situations involving chronic stimulation, such as the enriched environmental conditions or classical conditioning, consol-

idation can occur. But if hippocampal function is abnormal, then habituation is defective, arousal is prolonged, and consolidation is excessive. Thus, learning and memory in the homozygous rat may be either absent or abnormally persistent.

In summary, the dendritic branching and cortical thickening response to enrichment in the homozygous Brattleboro rat may be a correlate of maladaptive consolidation. Behavioral testing after environmental enrichment is needed in this strain with increased cortical dimensions to shed more light on their learning and memory abilities.

As we pursue our search to understand the effects of enrichment and the resulting brain changes on the ability of rats to learn mazes, it is important to compare these results with brain changes due to learning alone. For, we wish to know whether maze training will change the brain in a manner similar to the alterations induced by an enriched environment.

To answer this question we examined cortical responses to the specific learning conditions established by Kay Kerker in the late 1960s (20). She started an experiment with a very simple maze pattern in the Hebb-Williams maze and gradually increased the complexity of the learning conditions. Ten pairs of female Long-Evans rats at 71 days of age were used for this experiment. One rat from each littermate pair was given formal maze training while the remaining littermate was placed in an identical maze for the same length of time, with an unchanged simple barrier pattern and without food reward. The initial simple pattern for both animals consisted of one barrier between the start and the goal at the opposite corner of the box.

The maze training consisted of two parts: 10 days of pretraining and 22 days of formal training. For the first four days of pretraining, each maze animal was fed wet mash in the goal box and allowed to explore the empty maze for 30 minutes each day. For the next six days of pre-training, each experimental animal was put in the start box and required to find the goal box through a very simple barrier pattern for 30 minutes each day. The complexity of the patterns and the number of trials increased from day 5 to day 10. During the 22 days of formal testing, a series of barrier patterns, progressively increasing in complexity, were given, one each day. The first 12 patterns were those used in formal Hebb-Williams maze training, and the last 7 were more complex patterns (for the most complex, see Figure 37). On the last 3 patterns a second trial was given to check retention on the following day.

For Kerker's experiments, the original Hebb-Williams maze running experimental design was somewhat modified, ranging from 45 to 50

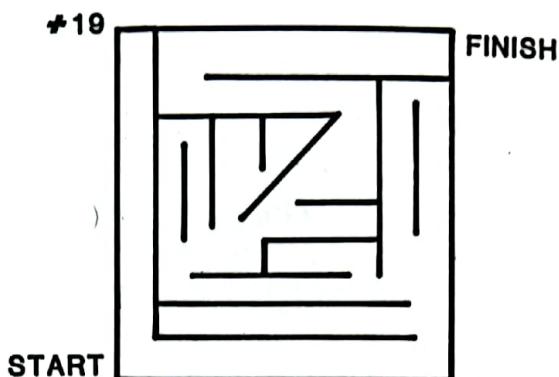


FIGURE 37. A floor plan of the most complicated maze, maze 19, used for testing brain changes with learning.

minutes in the maze, originally to 15 to 20 minutes, and in the goal box, from 20 seconds originally down to 10 seconds in the final patterns.

The running times and errors did not show a significant decrease with increasing trials for the first 12 barrier patterns, the Hebb-Williams training patterns. This was undoubtedly because they were so simple that the rats had no difficulty running the maze without errors on the first trial. Barrier patterns 13 to 19 were purposely made more difficult than the original Hebb-Williams patterns so that the rats could not solve them without errors on the first trial; also the feeding time was shortened.

In examining the cortical thickness differences between the brains of the maze-trained rats and those of the untrained littermates, Kerker found that only the lateral region of the posterior cortical section, area 39, showed a significant difference, 6% ($p < 0.025$). In addition to the cortical thickness in area 39 being greater in the trained animals, the neuronal nuclear diameter and nuclear area were 8% ($p < 0.05$) and 17% ($p < 0.03$) greater, respectively, in this region in the trained than in the nontrained.

No significant changes were found in the medial occipital cortex, the region which is usually modified in the normal enriched animals, or in the somatosensory regions. The responsive region was very localized in area 39, where multisensory integration is thought to take place.

To my knowledge, this preliminary investigation was the first to observe a morphological response of the cerebral cortex to problem solving in a maze. Over 10 years later, Greenough et al. (21) published their results showing the effects of formal training on dendritic branching in the occipital cortex. Adult rats were trained in two different types of procedures—a two-way alternative visual discrimination task and a Hebb-Williams maze. The experimenters found that neither procedure comparing the trained with untrained, induced the large increases in den-

dritic branchings from stellate neurons or in basilar dendritic branching from the pyramidal cells that were seen to result from comparing animals from enriched and impoverished conditions. Instead, the oblique or terminal branches from the apical shafts of pyramidal cells in layers IV or V were longer in the trained animals compared to the untrained.

We did not continue to measure apical or oblique branches in our enriched-impoveryed experiments after we learned that it was primarily the basal dendrites which responded to this kind of input. Greenough et al. supported our initial, unpublished findings of measurable cortical morphological changes in response to formal learning in contrast to those found as a consequence of the enriched living arrangement. However, they did not specify which region of their occipital cortical sample showed the changes—whether medial or lateral. If lateral, then our results and theirs do support each other very nicely.

Now, we can return to compare the results showing brain changes due to learning with those due to enriched environmental conditions. In order to compare these findings it is necessary to use experimental conditions that are as similar as possible. The trained animals were 71 days of age at the beginning of the experiment and were trained for 32 days (10 days of pretraining and 22 days of formal training). The closest enrichment experiment that we have is one where the animals were 60 days of age at the start of the experiment and received 30 days of enrichment. With maze learning, only area 39 in the cortex showed significant increases; with enrichment, areas medial 10, 4, 18, 17 and 18a developed significant increases (Figure 29). The results bear out what one might anticipate. Since enrichment provides a general multisensory input, more of the cortex changes. Whereas, learning to run a maze requires the animal's focused attention, and consequently, only one area showed measurable alterations. Since enrichment most consistently alters the neuronal structure in area 18, and now we have the information that learning to solve a maze modifies the structure of area 39, we can now focus our attention on these two areas. More refined chemical and molecular studies of these regions may provide a fuller understanding of learning and memory processing in the cerebral cortex.

10

THE SIGNIFICANCE OF ENRICHMENT

How much more do we know about how the brain works than we knew before we conducted our enriched and impoverished studies? We have learned a great deal about the interaction of the external and internal environment with the structure of the brain. We have learned that different regions of the cortex increase in size as the duration of exposure to the stimulating conditions is extended. We have learned that every layer of cortical neurons in area 18, the area responsible for visual integration, responds to our experiential environment, with the outer layers, II and III, showing the greatest changes. The neurons in the cerebral cortex exhibit an impressive amount of plasticity. We have learned that every part of the nerve cell from soma to synapse alters its dimensions in response to the environment.

The enlarged nerve cells with their more numerous glial support cells are apparently utilized by the rat to solve maze problems more effectively than rats without such modified cells. The mechanism by which the enlarged nerve cells improve learning ability is not yet known, but these findings clearly demonstrate brain enlargement as a result of brain use.

One often wonders how we can hold a train of thought for hours or record a memory for an extended period of 90 years or more if the flexibility of the cortical structures is so great. Obviously, some molecular configurations must remain stable at the same time that others exhibit change.

Just as the cortical neurons become larger in a stimulating environment, they decrease in size when there is less input from the millions of sensory receptors reporting from the body surface and the internal organs. It is just as important to stress the fact that decreased stimulation will diminish a nerve cell's dendrites as it is to stress that increased stim-

ulation will enlarge the dendritic tree. We have seen how readily the cortical thickness diminishes with an impoverished environment, and at times, the effects of impoverishment are greater than those brought about by a comparable period of enrichment.

These cellular changes that we have measured in the brain provide us with a better understanding of how the environment interacting with an hereditary base possibly influenced the brains of higher organisms, including the human brain. Those members of species which happened—by genetic happenstance—to have free extremities, a tendency to explore, and/or bigger brains, were better able to survive and pass on those genes. The upright human, with free upper extremities, continuously sought new challenges, new enriched conditions, and in turn could alter the dimensions of his brain. It is the interaction of the environment with heredity which has changed the brain over millions of years.

Perhaps the single most valuable piece of information learned from all our studies is that structural differences can be detected in the cerebral cortices of animals exposed at any age to different levels of stimulation in the environment. First, we found that young animals placed in enriched environments just after weaning developed measurable changes in cortical morphology. Then, we worked backward in age to the animals not yet weaned and found such changes, and we even found measurable effects of prenatal enrichment. Later, we moved forward in age to learn that the enriched young adult demonstrated an increase in dendritic growth, not only above that found in his impoverished mates, but even above the level of the standard colony animal. In the very old animal, with the cortex following its normal decline with aging, we again found the enriched cortex significantly thicker than the nonenriched. In fact at every age studied, we have shown anatomical effects due to enrichment or impoverishment. The results from enriched animals provide a degree of optimism about the potential of the brain in elderly human beings, just as the effects of impoverishment warn us of the deleterious consequences of inactivity.

Our ultimate goal in studying the aging animal brain is to bring as much dignity as possible to the aging human being, to indicate the potential of aging cerebral cortical cells, and to challenge the myths regarding the aging brain by critically evaluating them. For example, one of the most prevalent popular beliefs is that once we reach adulthood our brain cells are dying by the hundreds each day and therefore our mental capacities must be diminishing as well. The belief received support in 1958, when Benedict Burns (1) calculated from Brody's data (2) and Leboucq's data (3) that during every day of our adult life more than 100,000 neu-

rons die. These depressing data were derived in the following manner. Brody's estimation of neuron loss in the human cortex between 20 and 80 years of age was 30%, and Leboucq found a decrease in surface area of the brain between the ages of 20 and 76 years amounting to some 10%. Burns's estimated daily cell loss has been frequently quoted. More recently, however, Brody noted the prominence of this information in the lay literature and rejected it as scientifically inaccurate. The original studies included too few samples, and inadequate information was available about the living conditions of the brain donors prior to autopsy. Furthermore, Brody has since reported that some areas of the brain do not lose cells at all with aging, a finding similar to our own (4). Apparently, the loss of cells varies from region to region. For example, the locus ceruleus in the hindbrain and the nucleus of Meynert in the forebrain do lose nerve cells with aging; whereas, several of the cranial nerve nuclei and a nucleus in the hindbrain called the inferior olivary nucleus do not lose nerve cells throughout the lifetime of the individual.

There is some evidence that the decrease in brain weight and the degree of cortical atrophy in healthy old subjects who have no brain pathology is relatively slight. The brains of such individuals are within normal weight ranges for young adults and have cerebral hemispheres exhibiting no apparent cortical atrophy. Evidence does indicate that the number and size of the spines on cerebral cortical nerve cells are reduced in old individuals. But even spines can still be present in active old nerve cells; at least they are clearly present in animals two-thirds of the way through a lifespan. In studying the brains of old human beings it is important to be aware of the lifestyle prior to death, something scientists have been taking more seriously in recent studies. With such considerations, some medical texts now state that in many respects the healthy old brain is similar to the healthy young brain. The experiential environment is a major factor in maintaining the healthy old brain. A few of the myths about the deterioration of functioning during aging are slowly being replaced as scientific knowledge is beginning to offer some contrary evidence.

Such information stimulates us to adopt new attitudes toward aging and encourages us to plan for an active life in old age. Of course, many bright, energetic individuals have always done this; the knowledge of the potential of the brain was not a necessary inducement. Many people have looked to their grandparents who lived a long full life and concluded that they too could follow a similar path. There is no doubt that one's genetic background is important, but our studies suggest that the use of our nerve cells is critical to their continued health. Interviews with some

active elderly supported this view. For example, the 89 year old California wine taster still had his acute taste buds as well as a keen olfactory sense for sniffing good wines. The perspective developed in this book suggests that his continuous attention to his senses of taste and smell enabled them to remain acute during aging. The university chemist active at 98, was still publishing and reading without his glasses. His alert 92 year old wife continued to read out loud to him. We all know older people like these whose lives illustrate what we have learned about the potential of the cortical nerve cells to respond to the information coming in from the environment.

But what about the millions of human beings who are discouraged and do not continue to stimulate their brains? Many people attend school for a dozen or so years and then find a job only to provide an income until retirement. Their living pattern usually moves toward slowing down until they finally fade away. The generally accepted knowledge about the brain is that it starts "going downhill" fairly early in life (which is true) and that after that, there is little one can do about changing this pattern (which is not true). As mentioned in Chapter 2, recent studies on the developing human brain have shown that the size of the cerebral cortex is already decreasing after the age of ten. In fact, the patterns of an increasing and subsequently decreasing curve were very similar for rats and humans during the early postnatal period. If we take advantage of our more recent knowledge regarding the plasticity of a lower mammalian cortex at any age, then we can offer encouragement to counteract the downhill slope in human beings. A different outlook emerges toward lifestyle, as a whole, and toward learning, in particular.

Opportunities for learning should be encouraged from shortly after conception and continued until death. The data from a Japanese laboratory and from ours showed the beneficial effects of stimulating environments during intrauterine life: improved maze behavior and increases in cortical structure in the animals after birth. Though the western world is only recently becoming aware of such a practice, for centuries Asian people have encouraged the pregnant mother to enrich her developing fetus by having pleasant thoughts and avoiding angry, disturbing behavior. At the same time, one is made aware of other beneficial factors in aiding the development of the fetus such as good diets and plenty of exercise after which the dendritic trees in cortical nerve cells are richly developed.

On the other hand, mothers need to be alerted to the negative effects on fetal development of such substances as alcohol. Alcohol administered to pregnant rats (5% alcohol in a protein-rich diet throughout gestation

from day 2) has been reported to cause a decrease in the body size of cortical pyramidal cells and in their number of dendrites in the brains of the offspring. Other results have shown that the nerve cells adjacent to the ventricles in the brain are also defective in rats exposed to alcohol before birth. Thus, the prenatal brain has been shown to be sensitive to negative influences like alcohol and malnutrition as well as to the positive influence of enrichment.

We still do not know whether an enriched condition during pregnancy can prevent some of the massive nerve cell loss, as much as 50% to 65% of the total population of cells, which occurs during the development of the fetus. It is apparent that overproduction of neurons occurs in the fetus because most neurons do not reproduce themselves after being formed: an excess number is needed as a safety factor. Therefore, those that are not involved in the early neuronal processing are "weeded out." At the present time it is believed that the limits of cell number are set by the genetic constitution. As mentioned in Chapter 1, investigators found the same number of cells in a single column of cortical cells, in rats, cats, dogs, monkeys and human beings (5). The genetic regulation of these cells appears to transcend species. Understanding the causes of this constancy in number is a complex process, for even fluctuations in body temperature can influence brain cell number. The body temperature of the pregnant female has a marked influence on the number of neurons that survive in the fetus. If the temperature is increased in the female guinea pig by 3 to 4°C for 1 hour in the latter part of gestation, the fetal brain weight is reduced by 10 percent. This reduction in brain weight is due to a loss of brain cells. Hyperthermia has not yet been established as a cause of human fetal brain damage and mental retardation, but we should be alerted to this possibility whether studying animals or man.

Though enriched experiential environments have not been shown to alter the number of nerve cells, our results have indicated that variation in the experiential environment can readily alter the size of the preexisting nerve cells in the cerebral cortex, whether in the cell body or in its rich membrane extensions, the dendrites, or in synapses. The importance of stimulation for the well-being of the nerve cells has been demonstrated in many species. But of equally weighty significance is the possible detrimental effect of too much stimulation. The eternal question arises, When is enough enough or too much too much? The reputed pediatrician, T. Berry Brazelton, points out that infants exposed to too much stimulation respond either by crying, by extending their periods of sleep, or by developing colic or withdrawing from any new approaches. In providing in-

creased stimulation for the young, the adult, or the old, one always has to keep in mind the need for adequate time at each phase of information processing: input, assimilation, and output. The integration of the input is essential before we can anticipate a meaningful output. As adults, we frequently say, "Let me think things over." It is essential to give the infant the same opportunity.

We have learned from our results that the nervous system possesses not just a "morning" of plasticity, but an "afternoon" and an "evening" as well. It is essential not to force a continuous stream of information into the developing brain but to allow for periods of consolidation and assimilation in between. I often tell the overworked student to go out and just lie on the lawn and watch the clouds drift slowly by.

We do not yet know the true capacity or potential of the brain. Our data at present suggest that nerve cells benefit from "moderate" sources of stimulation, allowing for new connections to be formed, and thus providing the substrate for more options. We have yet to try too much stimulation. Will the stimulated brain continue to increase or will its reticular formation sift out the excess stimulation?

To date we do not know whether there is a "ceiling" effect on brain growth beyond which no further expansion will occur. In our rats in the preweaning stage, one area (area 39) differed as much as 16% between the brains from the enriched rats and those from the nonenriched. A Swiss group (6), using super-enriched conditions, including additional space and toys, were also able to produce 16% differences in rats past the age of weaning. Does this mean that 16% cortical thickness differences represent the maximum change we can induce with environmental enrichment? I hesitate to accept such a premise at this time. Undoubtedly, with more imaginative experimental designs, utilizing additional creativity, we will find greater responses in the future. Of course, quantity of brain tissue is not our only goal. Quality is the ultimate objective. So far it has been shown that the thicker cortex is positively correlated with a better maze performance. Only further studies will provide information on the actual potential of the cerebral cortex to alter its structure with increased stimulation.

I recently uncovered a small book published in 1901 by the Macmillan Company called *The Education of the Nervous System* by Reuben Halleck (7). In essence, its message was that the best education we can provide the developing nervous system is one of stimulating all the five known senses. Halleck wrote that a person who has only one or even two senses properly trained is at best a pitiful fraction of a human being. He points out that recalled images of sensations we receive from the

world around us are powerful and necessary aids in further modifying and developing the sensory cells; not images of sight alone, but of every sense. What does lilac smell like? How do the tastes of cinnamon and nutmeg differ? It is possible for us to conscientiously train our senses, all of them, at any time in our lives. If we fine-tune the primary sensory areas early, the association cortices might then respond to more subtle differences in a greater variety of ways. Creative ideas could arise from a broader experiential base. The finding of more widespread changes in the brains of enriched rats than in those of rats trained to learn a specific task supports the claims of numerous educators, from Dewey on (8), that providing a wide variety of experiences to the growing child enhances intellectual development.

While all sensory input facilitates learning, the visual association cortex was the first to be responsive to enrichment in our experiments. This may be related to the fact that cortical association areas are the last areas to develop embryologically and the most recent phylogenetically. Thus, it is reasonable for the visual association area to show morphological changes in response to stimulation in a learning circuit. As far back as 1901, Fleschig (9) proposed that learning took place by impulses first entering a primary sensory cortical area, then going to the secondary or association cortex, and then into the limbic system. For visual input in Fleschig's model, the primary sensory cortex would be area 17 and the secondary or association cortex would be area 18, and then continuing to the limbic system. Within this pathway we might anticipate area 18 to be the region most likely to show change. And we find that it does respond in the shortest period of time to our experimental conditions. With a longer duration of exposure to the environmental conditions, area 17, the primary visual cortex, also demonstrates cellular changes. On the other hand, one part of the limbic system we have measured, the male hippocampus, has not demonstrated the same degree of plasticity as has the occipital cortex. However, some investigators have shown small amounts of hippocampal increases with enriched environments using female rats. Our findings offer support to our hypothesis that neural activity within the visual cortex is important for the initial information processing that facilitates learning. Our results indicate that it is the posterior part of the cortex rather than the frontal cortex which possesses the most plasticity. Future studies on the biochemistry of learning and memory in the mammalian cortex might therefore be most appropriately focused on this posterior region.

Though we have demonstrated the plasticity of the cerebral cortex, we are very much aware that the brain does not work by itself. Healthy

support systems, i.e., the cardiovascular, respiratory, urinary, and digestive systems, are essential for the maintenance of the healthy brain. The heart and its accompanying blood vessels need to be maintained through balanced diet and exercise. With exercise, the connective tissue surrounding the skeletal muscles and blood vessels can remain strong and aid with efficient circulation of the blood. The lungs should be free of disease, such as emphysema which can be caused by smoking or breathing air contaminated with pollutants. The body needs to take in adequate fluids to keep the kidneys working efficiently; these, in turn, keep the blood free of concentrated waste products. The digestive system needs to benefit from strong teeth that can break down food for efficient digestion, and from a fibrous diet to maintain the well-being of the large intestine. All of this is nothing new. It was Plato who said, back in 400 B.C., that a healthy body promotes a healthy brain and a healthy brain, a healthy body.

Not only is the brain dependent upon other systems, but each part of the brain interacts with another. The cortex, with its more refined intellectual functions, attempts to coordinate with the limbic system, with its more emotional functions. One without the other is only half an experience. In Nathaniel Hawthorne's story *Ethan Brand* (10), his main character is searching for the unpardonable sin. He concentrates to such an extent on his intellectual pursuit that he becomes emotionally starved. He eventually becomes dismayed and throws himself into his fiery kiln. When others discover the remains, all that is left is his charred rib cage enclosing a cold marble heart. He had discovered the unpardonable sin by neglecting to integrate the warm, emotional heart, in a metaphorical sense, with his intellectual pursuit.

Satisfying emotional needs is essential at any age. As we learned from our studies on aging rats, by giving our old rats a little tender loving care, we were able to increase their lifespan; those rats that received additional attention lived longer than those that did not. These results imply that the two regions of the brain, the limbic system and the cortex, need to work together efficiently for the well-being of the whole individual. Thus, it is important to stimulate the portion of the brain that initiates emotional expression, which encompasses the connections between the cerebral cortex and the limbic system, including the hippocampus, amygdala and hypothalamus. In our studies it was the cortex which responded more readily to the environmental conditions and not those parts of the limbic system which we measured, such as the hippocampus and amygdala. The fact that these structures are less adaptable to a varied environment implies that they are more basic to the survival of the indi-

vidual, suggesting that emotional well-being may be more essential for survival than intellectual. Other kinds of stimulation besides mental challenges, e.g., considerable personal attention and other forms of emotional involvement, may be essential to create changes in limbic structures. If this is so, how much more effort should we be making toward giving attention and care to each other? And how important it is for the intellectual components of the brain to be taught ways to guide the emotional ones.

Several of our measurements have indicated that even the deprived brain can adapt by changing in structure as a result of enriched living conditions. Such changes were discovered in both prenatally and postnatally deprived animals. If mother rats were protein-deprived during pregnancy and lactation and their newborn pups were given both protein-rich diets and enriched living conditions after birth, the pups' brains grew more than those of standard colony rats that were only nutritionally rehabilitated (11). In the experiment dealing with postnatal deprivation, the cerebral cortices were lesioned or damaged during infancy, and then the animals were placed in enriched living conditions (12). Upon measuring the length of these animals' cortices after enrichment, the investigators found that the length had increased to compensate for the previous damage. Thus, we must not give up on people who begin life under unfavorable conditions. Environmental enrichment has the potential to enhance their brain development too, depending upon the degree of severity of the insult.

In this limitless field of possibilities for future study, a few specific research avenues beckon most immediately. We wish to gain a better understanding about what elements are responsible for the growth of the cerebral cortex. In Chapter 1 the normal cortical growth pattern was presented, but we do not know what causes the rapidly growing cortex to reverse its direction shortly after birth. At present we are studying the role of opioid blockers, substances which block the endogenous or natural opiates (opioids) in the brain, because some investigators have demonstrated that various regions of the brain increase in size and cellular content when opioid receptors are blocked. Our initial results support these findings. In addition we wish to learn more about nerve growth factor and its role in cortical development. For years, nerve growth factor was thought to be confined to specific regions such as the sympathetic ganglia, but more recently it has been found in the hippocampus and cerebral cortex. We are particularly interested in its role and when it is active in the cerebral cortex. In light of the large number of people who are taking drugs for therapeutic reasons for long periods of time, it is

important to learn more about how the cerebral cortex responds under such medication. Thus, one specific question I would like to pursue now is whether the enriched condition favorably alters the brains of animals on antiepileptic drugs. For example, it is possible to obtain a type of rodent, a gerbil, which has spontaneous seizures. If this animal is given a seizure-reducing drug, will the enriched environment still increase the cortical dimension?

In addition, we wish to pursue a study to determine what agents play a role in creating the enlarged cortex in the offspring, the F¹ and F² generations, from the enriched parents. The high level of progesterone during pregnancy was suggested as one possible responsible factor. This suggestion has to be tested as well as others.

The ultimate goal of all of our studies has been to gain a better understanding of human behavior by examining its source, the brain. The simple enriched environmental paradigm allowing rats to interact with toys in their cages produced anatomical changes in the cerebral cortex. Now how do we apply this knowledge for the benefit of people? Since no two human brains are exactly alike, no enriched environment will completely satisfy any two individuals for an extended period of time. The range of enriched environments for human beings is endless. For some, interacting with objects is gratifying; for others, obtaining information is rewarding; and for still others, working with creative ideas is most enjoyable. But no matter what form enrichment takes, it is the challenge to the nerve cells which is important. In one experiment where the rats could watch other rats "play" with their toys but could not play themselves, the brains of the observer rats did not show measurable changes. These data indicate that passive observation is not enough; one must interact with the environment. One way to be certain of continued enrichment is to maintain curiosity throughout a lifetime. Always asking questions of yourself or others and in turn seeking out the answers provides continual challenge to nerve cells.

Finally, now that we have begun to appreciate the plasticity of our cerebral cortex, the seat of the intellectual functioning that distinguishes us as human beings, we must learn to use this knowledge. It must stimulate and guide our efforts to work toward enriching heredity through enriching the environment . . . for everyone . . . at any age.

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4 The Effects of Enrichment and Impoverishment

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7 Overcoming Deprivation and Stress

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