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Plasticity in the 904-Day-Old Male Rat Cerebral Cortex

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Ten pairs of male Long–Evans rats living in nonenriched environments (3 rats per small cage) were transferred to either enriched environments (10 rats per large cage plus "toys") or nonenriched environments (2 rats per small cage) at 766 days of age. One hundred and thirty-eight days later, at 904 days of age, the cerebral cortical thickness from these animals was measured on projected, 10-μm, thionine-stained, transverse sections. Although the thickness in the enriched rats was greater than in the nonenriched rats in all sections through the frontal, parietal, and occipital cortices, the 4 to 10% differences were statistically significantly different in only the frontal and occipital cortices. Right greater than left cortical thickness differences were not statistically significant in either the enriched or the nonenriched animals by 904 days of age. Neuron and glial counts were made on enlarged photographs of area 18 in the occipital cortex on 6-μm-thick, luxol fast blue-stained sections. No significant differences in cell counts were noted between the enriched and nonenriched animals. No significant differences in neuronal counts were found among 108-, 650- (from previous experiments), and the 904-day-old nonenriched rats. The notable findings were the plasticity of the extremely old, enriched rats' occipital cortex and the lack of the loss of neurons in cerebral cortical area 18, whether or not the environments were enriched. These results showed that the cerebral cortex remained structurally plastic throughout the lifetime of the organism.

INTRODUCTION

Evidence of continuous structural reorganization in the mammalian forebrain is becoming increasingly apparent. Not only does the cerebral cortical tissue alter its dimensions in response to the external (3–5, 10, 14) and internal environment (6, 15, 18), but right–left differences also change during the lifetime of the rat (9, 10). That the very young, preweaned animal's cerebral cortex can be enhanced with an enriched environment has been clearly demonstrated (17). Numerous reports have indicated that the young postweaned animal's cerebral cortex maintains structural adapt-

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ability to environmental alterations (3–6, 11, 15, 19, 20, 23, 24). Morphologic changes have been noted in adult animals (12, 22) and even in 650-day-old animals (2).

This paper will deal primarily with the very old rats, 904 days of age. Several questions were asked: (i) Can the structure of the very old rat cerebral cortex be altered by an enriched environment? (ii) Is the right cortex of 904-day-old rats significantly thicker than the left in both the enriched and nonenriched condition? (iii) Is there a significant right–left cerebral cortical thickness difference in nonenriched 904-day-old rats compared with younger nonenriched rats? (iv) Is there a difference in neuron–glial counts in rats enriched from 766 days to 904 days compared with nonenriched rats? (v) Is there a significant difference in neuron counts in area 18 in the cerebral cortex of nonenriched rats autopsied at 108, 650 (from previous experiments), and at 904 days of age?

METHODS

Ten pairs of Long–Evans male rats were placed into two separate environments at 766 days of age. Prior to that time, they had lived in groups of three in small cages (20 × 20 × 32 cm) after weaning at about 25 days of age. The idea of an enriched experimental condition was originally conceived by Hebb (13) and has been used continuously in conjunction with histological studies in the Berkeley laboratories since the early 1960s (3). This condition consisted of a large cage (70 × 70 × 46 cm) in which the rats had access to numerous, replaceable objects which were changed biweekly from a common pool of objects. The animals sniffed, climbed on and into, and generally explored these objects. The nonenriched condition, on the other hand, consisted (in these experiments) of two of the large old rats per small cage (20 × 20 × 32 cm). The rats in both conditions had free access to food and water and were exposed to a 12:12 light:dark cycle in the same room. The cages were cleaned twice weekly when the rats in the enriched condition are given a little tender care by being held. At about 900 days of age, one rat in the enriched condition died of unknown causes, and by 904 days, a total of three rats in the enriched condition had died, so the total experiment was terminated. As with many aging studies, it was the sturdy rats which survived, so we were comparing sturdy enriched survivors with the sturdy nonenriched survivors.

At 904 days of age, the remaining rats were anesthetized with sodium pentobarbital and perfused first with normal saline followed by 10% Formalin–saline. The brains were removed and placed in fixative until being embedded in celloidin. Both 6- and 10-μm transverse sections were cut providing representative cortical samples from the frontal (areas 10M

medial, and 10L lateral), parietal (areas 4, 3, 2), and occipital lobes (areas 18, 17, 18a, and 39). [König and Klippel, Figs. 12, 18, and 42, respectively (16)]. The thinner sections were used for cell counts and the thicker ones for cortical thickness measurements. Only cell counts from the occipital lobe are reported here. Due to technical difficulties, two enriched and two nonenriched brains did not fulfill our standards for histologic measurements and were discarded.

Our usual methods for measuring cortical thickness and neuron–glial counts were used (4, 5). The thickness of the right and left cerebral cortex was measured on projected images of the brain slices stained with thionin (9). The neuron and glial counts were made on enlarged photomicrographs (×640) from sections stained with luxol fast blue–cresyl echt violet. The photographic method has been presented elsewhere (8). The cells were marked on a Plexiglas acrylic plastic sheet secured over the enlarged photographs composed of four strips, each made from single microscopic fields extending from the pial surface to the corpus callosum beginning at the medial elevation of the corpus callosum. The standards for marking the cells included the following criteria: Nuclei of neurons had to have distinct nucleoli, the nuclei of astrocytes had to be close to standard light color and size, the nuclei of oligodendrocytes darker in color and a standard size. The peripheries of the nuclei had to be as distinct as those on a standard photograph. After the first counts were made, they were repeated by a second individual without knowledge of the information obtained by the first individual. The two independent counts were compared by the superimposition of the plastic sheets. The average of the two adjusted counts was taken as the final value. As the investigators collected the data, they were unaware of the specific experimental conditions to which each animal had been previously exposed.

RESULTS

Table 1 offers the thickness measurements, the percentage differences, and their statistical significance values as obtained from a Mann–Whitney *U* test. It was evident that although all cortical areas showed enlargement with enrichment, some regions were affected more than others. It is of interest to point out that the greatest differences were found in the occipital cortex, with areas 18, 17, and 18a indicating significant changes in response to the stimulating environment. None of the most lateral areas, 10L, 2, or 39, was responsive to environmental stimulation at this very old age. Also none of the areas measured from the parietal cortex, areas 4, 3 or 2, were statistically significantly different in the enriched compared with the nonenriched animals. At all ages in which we have measured the cortical

TABLE 1

Percentage Cortical Thickness Differences between 904-Day-Old Male Long-Evans Rats Exposed to Enriched or Nonenriched Environments[a]

	Area	Enriched ($N = 5$)[b]	Nonenriched ($N = 8$)	%	P
Frontal cortex	10M	2.69 ± 0.187*	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 ± 0.194	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

[a] Values are $\bar{x} \pm$ SD. Micrometers can be obtained by multiplying by 444.
[b] Exposure to the enriched conditions was from 766 days of age.

dimensions after exposing animals to enriched environments, it is the occipital cortex which most consistently responds. The frontal cortex is always more variable than the rest of the cortex. Figure 1 illustrates the thickness differences in graphic form and these differences are clearly evident in these very old rats.

There were no significant differences between the thickness of the right and left hemispheres in either the nonenriched or the enriched animals at 904 days of age. In the nonenriched, in nine cortical areas measured, the right was thicker than the left in eight areas in the eight animals studied.

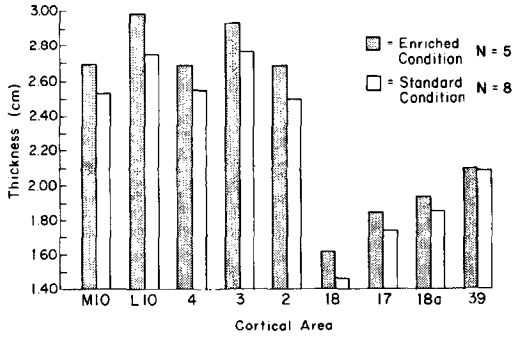

FIG. 1. Environmental influences on thickness of the cerebral cortex of rats exposed between 766 and 904 days of age to enriched conditions.

However, in the five enriched animals, the right was greater than the left in five and one-half of nine cortical areas measured.

Figure 2 presents the right versus left cortical thickness data in our development and aging study (7, 10), utilizing a one-tailed Student's t test to derive the levels of significance. The 904-day animal data were added to the graph. In general, the significant laterality differences were reduced by the time the nonenriched or control male attained 904 days of age. The number of animals in the 904-day group was smaller than in the other groups and, thus, the power for the test of significance is low. However, the mean difference was in the same direction for all nine cortical areas measured. (Also, the strong right > left differences in the hippocampus (10) in younger animals were greatly reduced with aging and were nonsignificant by 904 days of age.) In previous laterality studies in which we had small numbers of rats initially and later augmented the number of samples, we have not found a change in the direction of the laterality pattern or in the level of significance with a larger sample (10).

Neither the neuron number nor the glial number in these old animals was significantly different when the enriched animals were compared with

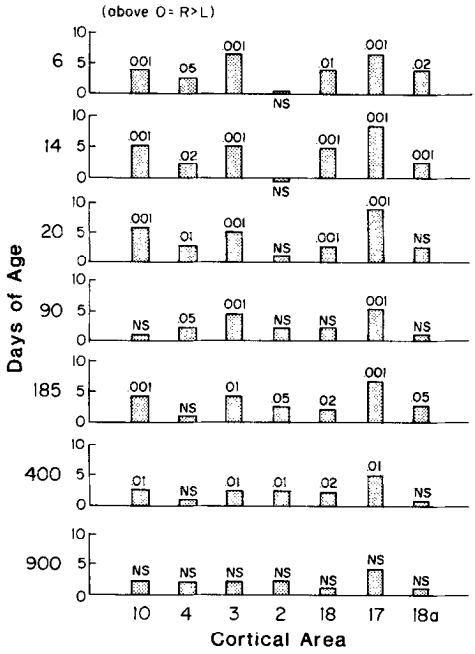

FIG. 2. Percentage difference between right and left cerebral cortical thickness in young, adult, and old aged Long–Evans male rats (nonenriched). P values are shown above the bars.

the nonenriched; the enriched rats had 10% (NS) more neurons per unit area than did the nonenriched. The glial counts were less by 10% (NS) in the enriched compared with the nonenriched rats.

Figure 3 shows the data from cell counts from only nonenriched rats at different ages. The cell data to 650 days of age were taken from a longitudinal development and aging study of the Long–Evans rats' cerebral cortex which were published elsewhere (8). The counts from 904-day-old rats were added for comparison. It is clear from this graph that the rat occipital cortex did not lose a significant number of nerve cells after 100 days of age as shown by a one-tailed Student's t test. The counts were supervised by the same technician, even though the 650-day-old rats' brains were counted in 1977 (8) and those in the 904-day-old brains in 1983. When the counts were placed side by side, they were surprisingly similar. Figure 3 also illustrates a decrease in neurons per unit area in the early part of the animal's life before 108 days of age. This decrease could possibly be due to either a continuation of the loss of neurons which occurred before birth or due to a change in the amount of neuropil creating fewer soma per unit area.

DISCUSSION

These results have positive implications for the potential of the aging mammalian brain. Cortical thickness increases in response to a stimulating environment and the lack of a significant loss of occipital cortical neurons with age, whether the animals live in enriched or nonenriched environments, are encouraging results. The cortical thickness changes are even more impressive when one considers that the animals lived in nonenriched environments for the major part of their lives (from weaning to 766 days of age) before being transferred to the more stimulating conditions. The

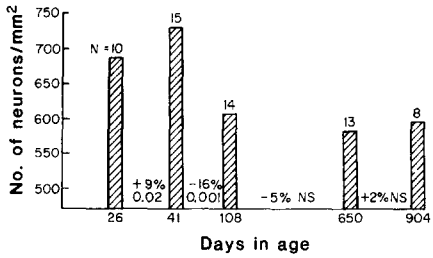

FIG. 3. Neuronal counts with aging in occipital cortex of male Long–Evans rats in standard colony (nonenriched) conditions. Percentage differences (and P values) from the preceding age are shown.

relatively old brain which had earlier adapted to a constant, nonenriched condition showed morphological adaptability when introduced to a new enriched condition in later life.

It is evident that although all cortical areas show enlargement with enrichment, the effect is greater in some regions than in others. The occipital cortex responds more consistently to those enriched conditions than do other regions of the cortex. That the somatosensory cortex does not show a statistically significant difference in the Long–Evans male rat is not too surprising, for enrichment from 112 to 142 days of age did not alter the dimensions of the male somatosensory cortex either (6). Most of our more detailed histological studies have been confined to the medial occipital cortex, area 18, because throughout the years it has proven to be most responsive to these environmental conditions.

Even though the enriched animals proved to have thicker cortices than the nonenriched, it is not clear from these data whether the former animals actually grew larger cortices or maintained the size of the cortex which was present at 766 days, while the nonenriched cortex decreased its dimensions. In a previous experiment (22) we included a baseline group which was killed at the onset of the experimental conditions. In that experiment, the enriched rats did have thicker cortices at the termination of the experiment than both the standard colony controls (the nonenriched killed at the same time as the enriched) and the baseline controls. In those animals (22) which were 142 days of age at the time of autopsy after 30 days of enrichment, enrichment increased the cortical dimensions and did not just prevent the cortex from decreasing. Those data suggest the same could be happening in these older animals, but baseline controls would have to be included in future old age experiments to prove this point.

The stability of the occipital cortical neuron numbers in healthy animals was remarkable. Other studies have shown the stability of the somesthetic cortical neuronal populations in old rats (1), and our new data offer additional support to this lack of cortical neuronal loss with aging. However, a study by Stein and Firl (21) reported a decrease in frontal cortical neurons in 575-day-old rats compared with 130-day-old rats, but several questions arise in that study. Cells were counted in layer 2 in only two rats, one 575 and one 130 days old. It was not clear how cell types were determined and how cortical layer boundaries were determined on those nonperfused tissues. Before definite conclusions can be made on aging and neuronal populations in the frontal cortex, a more thorough study needs to be completed. It is very possible that different regions of the cortex may be aging at different rates, but that study did not provide sufficient evidence on which to make such a statement. (We have frontal, somatosensory, and occipital sections from all our developing and aging rat brains from 6 to

904 days of age so it will be possible to make future neuronal–glial counts on these celloidin embedded sections at all ages.)

It is not clear why the rats in the enriched environment began to die before those in the less stimulating condition. Previous studies indicate that stress is not a major factor in young animals grouped in the stimulating enriched environment (24). Perhaps, with aging, solitary living is more desirable, and group living, less tolerable. There are many experimental procedures that might shed light on this problem. The presence of the objects, not the social living is the main factor in increasing the brain dimensions in these experiments. Perhaps, fewer animals living together with the stimulus objects will prove to be the ideal combination for old animals. Optional group living might be another consideration with separate chambers available for sleeping and grooming and a common "dining room" combined with the stimulus objects combined.

CONCLUSION

In conclusion we can answer our five original questions. (i) Yes, the 904-day-old rats from an enriched environment had thicker cortices than those in nonenriched environments. (ii) No, there was no significant difference between the thickness of the right and left cortices in either the enriched or nonenriched 904-day-old rats. (iii) Yes, the right hemisphere was thicker than the left in the nonenriched male rat at 904 days, but the difference was not statistically significant as it is in the younger animals. (iv) No, there was no significant difference in neuron or glial counts per unit in area 18 of the occipital cortex between the enriched and nonenriched 904-day-old Long–Evans rats. (v) No, there was no significant difference in neuronal counts per unit area in area 18 of the occipital cortex in nonenriched Long–Evans rats between 108, 650, and 904 days of age.

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