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Effects of Pregnancy and Differential Environments on Rat Cerebral Cortical Depth[1]

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Pregnant and nonpregnant Long–Evans female rats were placed in enriched, standard colony, and impoverished environmental conditions (comparisons were made among sextuplets). Cerebral cortical depth measurements of the nonpregnant rats supported previous findings that the somesthetic and occipital areas of the environmentally enriched rat are thicker than those of the environmentally impoverished nonpregnant rat. Pregnancy altered the response to these differential environments by increasing the cerebral cortical depth of the pregnant animals, particularly those in the impoverished condition. However, a significant overall effect of an enriched environment occurred in the pregnant rats as well. The present experiment indicates that cerebral cortical chemistry and morphology can be altered depending on the environmental conditions, sex, and hormonal state of the animal. If the mechanisms which cause the brain to function are ever to be understood, all variables should be taken into consideration.

The importance of external environments and their influence on the central nervous system of animals during critical changes in the internal environment needs to be clarified. In the past decade, numerous investigators have shown that the cerebral cortex of male rats can be modified as a consequence of external environments. Male rats kept in enriched environmental conditions (EC) compared to littermates in impoverished environmental conditions (IC) have repeatedly been shown to have a thicker cortex (Diamond, Rosenzweig, Bennett, Lindner, and Lyon, 1972; Diamond, Ingham, Johnson, Bennett, and Rosenzweig, 1975b), a greater number of glia (Altman and Das, 1964; Diamond, Law, Rhodes, Lindner, Rosenzweig, Krech, and Bennett, 1966), under some conditions, larger neuronal nuclei and perikarya (Diamond, 1967; Diamond, Johnson, Ingham, Rosenzweig, and Bennett, 1975), increased dendritic branching (Holloway, 1966; Volkmar and Greenough, 1972; Uhlings, Diamond, and Veltman, 1976), changes in synaptic dimensions (Møllgaard, Diamond, Bennett, Rosenzweig, and Lindner, 1971; West and Greenough, 1972;

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Diamond, Lindner, Johnson, Bennett, and Rosenzweig, 1975a), and more total cerebral protein (Rosenzweig, Bennett, and Diamond, 1972).

In 1971, Diamond, Johnson, and Ingham reported that occipital cortical depth differences existed between nonpregnant female rats living in enriched and impoverished environments and that these differences varied from those of male rats under identical conditions. When cortical depth differences were compared between immediate postpartum rats living in enriched and impoverished environments, no cortical depth differences were found. Because of the unexpected results of the postpartum EC-IC rats, comparisons were also made between the postpartum and nonpregnant rats living in the experimental environments, although these animals were not littermates. These results implied that pregnancy had a greater effect on the cortex of the rat in the impoverished condition than on the cortex of the rat in the enriched condition. The present study was designed to confirm these previous findings with female rats, to add a standard colony environment (SC) to observe if the enriched effect or the impoverished effect was responsible for the cortical depth differences, and to compare the cortical depths of pregnant with nonpregnant littermate rats living in the three environments.

Initial and replication experiments were run. In each experiment, female sextuplet Long–Evans rats were obtained from the animal colony in the Physiology–Anatomy Department at the University of California. In order to duplicate the experimental conditions (Diamond *et al.*, 1971), all of the animals lived three to a standard colony cage after weaning at 25 days of age. At approximately 60 days of age, each rat was placed into its respective environmental condition for 30 days (no significant body weight differences were noted among the rats placed in their conditions at 60 days of age).

Experimental conditions and anatomical techniques have been published previously (Diamond *et al.*, 1972), but a brief description is given here. Three environmental conditions were used. The enriched condition consisted of 10 to 12 rats living together in a large wire cage (70 × 70 × 46 cm) with a collection of objects (e.g. ladders, small mazes, jars etc.) which were changed daily. The EC rats were allowed a half-hour of free exploration in a Hebb–Williams maze with the pattern of barriers changed daily. All of the pregnant EC animals lived separately from the nonpregnant EC animals. The standard colony condition was basically a continuation of the environment in which the animals had been living until 60 days of age. Three rats were housed together in a single standard U.C. Anatomy rat colony cage (34 × 20 × 20 cm). Wire screening covered the top and front of the cage, and the sides and back were lined with sheet metal. Three pregnant SC animals lived together in a single cage, and three nonpregnant SC animals lived in another identical cage. The impoverished condition consisted of rats living singly in the same type of

cage as the SC animals. All rats had access to food and water *ad libitum*. A fluorescent light, controlled by a timing device, provided 12 hr of light and 12 hr of darkness.

At approximately 90 days of age, one rat from each environmental pair was placed in an SC cage with a Long–Evans breeding male. Each female was housed with the male for 5 to 6 days. The nonpregnant littermate from each condition stayed in another SC cage with another nonpregnant female for the same period of time. A vaginal smear indicating sperm determined the day of conception. Even with positive sperm checks, some of the animals failed to conceive. In such a situation, the results from that animal were not used, thus, accounting for the unequal number of pairs in various comparisons. After mating, each female was returned to her previous experimental condition until the day before parturition. At approximately 115 days, the pregnant rats were put into individual SC cages to allow the births to occur separately.

Immediately after parturition, the mothers were taken from the pups and coded so their previous experimental conditions were unknown until all measurements were completed. The mothers were anesthetized with ethyl ether and perfused with formol saline. The brains were carefully removed, placed in formol saline for several days, rinsed with distilled water, and put in a 30% sucrose solution for 2 days. Twenty-micrometer coronal sections were cut on a Reichert freezing microtome. Three different areas of the cortex were studied, using the plates from Konig and Klippel (1963) as a reference for defining the following cortical areas. For the divisions into cortical segments see Diamond *et al.* (1975b). The cortical areas studied were: (1) M Area (motor cortex divided into b and c segments), immediately before the anterior crossing of the corpus callosum (plate 12a). (2) S Area (somesthetic cortex with b, c, and d segments), the continuity of the anterior commissure across the midline (plate 18a). (3) V Area (occipital cortex with b, c, d, and e segments), at the posterior commissure (plate 42a).

All tissues were stained with Windle's thionin stain (Windle, Rhines, and Rankin, 1943). A Bausch and Lomb microslide projector magnified the tissues 22.5× so that an outline of the brain and the corpus callosum could be clearly traced. Layer I was omitted because previous experiments had shown no difference due to the experimental conditions in this area (Diamond *et al.*, 1972). Percentage differences in cortical depth were obtained by subtracting the control measurement from the experimental measurement, dividing by the control, and multiplying by 100. The SC was the control relative to the EC. The IC was the control relative to the EC and SC. Previous investigations by this laboratory on cortical depth measurements have been reported as percentage differences between littermates using the Student's *t*-test as in Table 1. One of the objectives of this project was to see if previous results on female rats could be

TABLE 1
Effects of Environment and Pregnancy on the Cerebral Cortical Depth of Rats

Area	EC vs IC (n = 21 pairs) Difference (%)	EC vs IC (n = 21 pairs) Student P	EC vs SC (n = 23 pairs) Difference (%)	EC vs SC (n = 23 pairs) Student P	SC vs IC (n = 21 pairs) Difference (%)	SC vs IC (n = 21 pairs) Student P
A. Effect of environment on cerebral cortical depth of nonpregnant rats						
Mb	1.98	NS	0.29	NS	2.70	≤0.05
Mc	1.32	NS	0.23	NS	1.70	NS
Sb	4.43	≤0.01	2.28	NS	2.71	NS
Sc	3.72	≤0.01	2.67	NS	1.57	NS
Sd	1.57	NS	1.76	NS	0.60	NS
Vb	3.94	≤0.05	3.64	≤0.05	0.53	NS
Vc	3.96	≤0.05	3.44	≤0.05	1.32	NS
Vd	1.67	NS	2.72	NS	−0.13	NS
Ve	1.22	NS	2.10	NS	0.16	NS
	PEC vs PIC (n = 16 pairs)		PEC vs PSC (n = 18 pairs)		PSC vs PIC (n = 18 pairs)	
B. Effect of environment on the cerebral cortical depth of pregnant rats						
Mb	0.05	NS	−0.02	NS	0.38	NS
Mc	0.35	NS	0.68	NS	0.16	NS
Sb	0.71	NS	2.71	≤0.05	−2.07	≤0.05
Sc	3.05	NS	1.46	NS	2.07	NS
Sd	3.07	≤0.05	1.70	NS	2.01	NS
Vb	3.53	NS	3.58	NS	0.08	NS
Vc	2.99	NS	2.41	NS	0.40	NS
Vd	1.75	NS	2.48	NS	−1.18	NS
Ve	2.06	NS	2.36	NS	−0.45	NS
	PEC vs EC (n = 19 pairs)		PSC vs SC (n = 21 pairs)		PIC vs IC (n = 18 pairs)	
C. Effect of pregnancy on the cerebral cortical depth of rats in differential environments						
Mb	1.62	NS	1.92	NS	3.67	≤0.01
Mc	1.11	NS	0.70	NS	2.25	NS
Sb	0.51	NS	0.88	NS	4.73	≤0.01
Sc	1.43	NS	3.21	NS	2.67	NS
Sd	2.13	≤0.05	2.47	NS	1.54	NS
Vb	0.66	NS	1.95	NS	1.94	NS
Vc	0.54	NS	2.21	NS	2.77	NS
Vd	1.08	NS	1.05	NS	2.21	NS
Ve	0.67	NS	−0.33	NS	1.22	NS

replicated. Application of the Student's t-test in this investigation indicates that the present findings are almost identical to previous t-test evaluations (Diamond *et al.*, 1972).

The percentage differences and the Student's t-analysis of the cortical

depth (Table 1a) of the nonpregnant rats indicate that the cerebral cortex of the female rat, like that of the male rat, responds to an enriched environment. In the V area, the cortex of the enriched rat is 3–4% ($P \leq 0.001$) thicker than the cortex of the impoverished rat. These percentage differences are not as large as those found between male littermates under similar conditions (Diamond *et al.*, 1971). Significant differences between the EC and the SC cortices indicate that the enriched environment is responsible for the EC versus IC effect in the V area. In contrast to the cortical depth differences in the female, the S area in the Long–Evans male shows no significant cortical depth change in response to environmental manipulation (Diamond *et al.*, 1971).

The percentage differences and the Student's *t*-analysis of the cortical depth of pregnant rats (indicated as PEC, PSC, or PIC) also replicates previous findings in this laboratory (Diamond *et al.*, 1971). In the M and V areas, no significant cortical depth differences are found between any of the conditions. The significant differences in the S area of the pregnant rat are not easily explained, but may further suggest that this area demonstrates more plasticity in the female than in the male. Overall, the inconsistency in the area differences and the nonsignificant values suggest limited experiential differences in brain measurement, at best, among pregnant rats living in the three environments (Table 1b).

The percentage differences and the Student's *t*-analysis on the cortical depth between pregnant and nonpregnant pairs indicate significant increases in cortical depth in some S and M area comparisons of the IC pregnant animal, but no difference in the V area. These results are similar to the nonlittermate comparisons made by Diamond *et al.* (1971). In addition, recent results by Pappas (1976) indicate that the S area is more responsive to ovariectomy than are the V or M areas (Table 1c).

Because the large number of Student's *t*-tests could have resulted in some significant values by chance alone, an analysis of variance was performed on the data. Unlike multiple *t*-tests, the components of the analysis of variance are statistically independent. The analysis of variance model includes in one system the linear equation of all factors thought to be important determinations of cerebral cortical depth. Table 2 lists a single general mean for each experiment and the experimental condition mean for the conditions, environment, and pregnancy. The integers in brackets show their numerical relationship to each other in increasing order. The second half of Table 2 gives the probability of the main effects.

When measuring differences between littermates in the initial and replication experiments, as was done in the Student's *t*-evaluations, the results are relative and can be included together as in Table 1. Histograms of the two experiments showed the absolute numbers representing cortical depth in one experiment to be consistently higher than the other

TABLE 2
Analysis of Variance

Condition	Experimental condition mean	
	Expt 1	Expt 2
EC	3.640[a] (4)[b]	3.406 (4)
PEC	3.698 (6)	3.462 (5)
SC	3.569 (2)	3.342 (2)
PSC	3.616 (3)	3.388 (3)
IC	3.530 (1)	3.315 (1)
PIC	3.658 (5)	3.907 (6)
General Mean	3.618	3.377
Main effects	Probability	
Environment	$\leq .001$	$\leq .001$
Pregnancy	$\leq .001$	$\leq .001$
Area	$\leq .001$	$\leq .001$

[a] This is the interaction term mean of the three areas as determined in the analysis of variance.

[b] The numbers in the parentheses represent the increasing order of cortical depth thickness (1→6).

(compare experimental condition means between Expts 1 and 2 in Table 2). A variation in the measuring technique, such as a slight alteration in the height of the microslide projector, might reasonably be expected to cause this. Therefore, a separate analysis of variance was performed on the two experiments since absolute numbers (not relative numbers) are used.

For both experiments, the three-way analysis of variance showed that the main effects of environment, pregnancy, and area were significant. Considering the environmental effects, for the cortical depth, Table 2 shows the experimental condition mean EC > SC > IC for both experiments. This has been the general trend for the experiments in this laboratory involving differential environments. Considering the effects of pregnancy, the mean cortical depth of the PSC is always smaller than either that of the PEC or PIC. In experiment one, the experimental condition mean of the PIC almost equals the PEC, and, in the other experiment, the PIC is actually greater than the PEC (see Table 2). Considering the effects of pregnancy in the different environments, the mean cortical depth of the pregnant rat was always larger than that of the nonpregnant rat in both experiments. The comparisons involving pregnancy suggest that there is an overall increase in cortical depth caused by pregnancy, but that this increase due to pregnancy is more evident when the animal lives in an impoverished environment.

Both statistical tests show that the enriched environment increases the thickness of the cerebral cortical depth in the nonpregnant rat. This same

conclusion has been shown many times in this laboratory. The significance of these results is that females also respond to environmental stimulation and that their response is different than that of the male. The lack of significant differences by the *t*-test between pregnant animals in the three environments implies that some process during pregnancy alters the cortical depth response. While an overall effect of enrichment is evident, both statistical tests suggest that it is the IC cortex which is most affected by pregnancy and environment. This confirms previous findings by Diamond *et al.* (1971).

Because some of the changes in cortical depth might be due to alterations in the water or electrolyte content which occur during pregnancy, a preliminary investigation (Hamilton, 1976) into these parameters was performed. The increased cortical depth of the pregnant rat in the impoverished condition is accompanied by an increase in K and Na levels of the cerebral cortex when compared to the nonpregnant animal in the impoverished condition. No changes in water content of the cerebral cortex were found in any of the comparisons. The lack of a significant increase in the adrenal weight during pregnancy in the female rat living in the impoverished condition also implicates the adrenal gland and its hormones in altering the brain's response during pregnancy. More evidence was shown for the environmental response being altered by hormones when Hoover and Diamond (1976) gave norethynodrel (the progestin component of Enovid) to female rats living in enriched and impoverished environments. Preliminary evidence here indicated that the Na level in the occipital area was higher in the control animals living in the enriched environment when compared to the norethynodrel-treated rat living in the enriched environment.

If pregnancy or synthetic hormones can change cortical depth and alter (or mask) the response to environment, especially in the IC condition, these effects should be more thoroughly investigated because they may have some clinical significance. The cortical depth differences noted between male rats living in the enriched and impoverished conditions are associated with increased numbers of glia, larger neurons, more dendritic spines, etc. These types of studies have yet to be done with female rats. This investigation will undoubtedly alert others to the morphological and biochemical changes that occur in the cerebral cortex as a function of the environment, sex, and hormonal state of the animal.

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