EXPERIMENTAL NEUROLOGY 92, 186–196 (1986)

Rat Cerebral Cortical Estrogen Receptors: Male–Female, Right–Left

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Received August 8, 1985; revision received November 4, 1985

We determined the concentration of cytosol estrogen receptors in the postnatal, developing right and left cerebral cortices of Long–Evans male or female rats 2 to 3, 7 to 8, 14 to 15, and 25 to 26 days of age. Under anesthesia, the rats were gonadectomized and 24 h later they were killed by decapitation, and the dorsal right and left cerebral cortices were separated from the underlying white matter and placed on ice. Sephadex LH-20 gel filtration chromatography was used to dissociate the majority of alpha-fetoprotein-bound [^{3}H]estradiol while leaving the receptor [^{3}H]estradiol complex intact. The correction for the residual nonreceptor binding, including alpha-fetoprotein, was made using parallel incubation containing unlabeled diethylstilbesterol. The amount of residual nonreceptor binding was subtracted from [^{3}H]estradiol-bound protein to calculate high-affinity estradiol binding receptors. The results showed that in both sexes, estrogen receptor concentration was highest at postnatal days 2 to 3 in both the right and the left cerebral cortex and then decreased until 25 days of age. In the female, the right cerebral cortex, at postnatal day 2 to 3, had a higher estrogen receptor concentration than the left cortex ($P < 0.02$). In the male, the left cortex had a higher cytoplasmic estrogen receptor concentration ($P < 0.02$) than the right. Considering the reported growth-inhibiting effects of estrogen on the cerebral cortex, the results indicated that one determinant of cerebral dominance in both sexes may be the differential exposure to estrogen, in the case of the male testosterone converted to estrogen, during a critical period of development.

INTRODUCTION

Systematic right–left, male–female, cerebral cortical thickness measurements have been determined for the Long–Evans rat. Those data indicate that in general the right cortex is thicker than the left in the male, with the opposite being true in the female (10, 11).

Abbreviation: SDN-POA—sexually dimorphic nuclei in the preoptic area.

[1] The authors are extremely grateful to William Young of the Department of Zoology at U.C. Berkeley for supplying the animals, to Ray Chavez for his general assistance, and to E. Rosalie Greer for statistical assistance.

0014-4886/86 $3.00

In both male and female rats, the cortex in general grows very rapidly postnatally from birth to 10 days of age, then continues to grow but less rapidly until sometime between 26 and 41 days of age. At this time it begins to decrease in thickness and continues at a slow rate throughout the lifetime of the animal (6). The size of the neurons (5, 8), the extent of the dendritic branching (9, 14), the number and kind of dendritic spines (9, 13), the length of the synapse (7), as well as the number of glial cells (4), all contribute to the degree of cortical thickness.

Gonadal hormones have been found to alter cortical morphology. Gonadectomy of newborn male rats not only reduces brain weight (24), but partially reverses the right-greater-than-left cortical thickness pattern which develops 3 months after gonadectomy (12). Gonadectomy in the newborn female completely reverses its normal left-greater-than-right cortical thickness pattern (22). Types of postsynaptic thickenings, which have been postulated to represent more mature types (20), are found in the cerebral cortex of the ovariectomized female rat compared with an intact control. Ovariectomized rats receiving replacement estrogen (ethinylestradiol 0.1 μg/100 g body weight) have a thinner cortex and a greater neuronal packing density than an untreated control, suggesting that the neurons are smaller and possess less packing density (23).

In addition, it has been demonstrated that estrogen receptors are present in the cerebral cortex of both sexes for the first 3 weeks of postnatal life (2, 19).

With the knowledge that sex steroid hormonal concentrations can alter cortical structure, that the hemispheric asymmetry patterns are different in male and female rats, and that both males and females have estrogen receptors in their cortices during the first 3 weeks of life, the question was raised, "Do male and female rats have similar patterns of distribution of estrogen receptors in their developing right and left cortices?" The following experiment addresses that question.

METHODS

Long–Evans male or female rats were obtained from the Department of Zoology, University of California, Berkeley at postnatal ages of 2 to 3, 7 to 8, 14 to 15, and 25 to 26 days; the day of birth was considered day 1. Each age group was separated into males and females and housed with a foster mother with six to eight animals per cage (47 $\times$ 26 $\times$ 21 cm). All animals were maintained on water and Purina lab chow. Under anesthesia, (either hypothermia for the 2- to 3-day-old group, or ether for the 8- to 26-day groups), the rats were gonadectomized 24 h before their brains were removed for study. A dorsal approach was used to remove the ovaries and a ventral for the testes. After the wound was surgically sealed, the rats were placed for 3 h in a container

set into a water bath at 37°C, then returned to their mothers after their wounds were painted with a gentian violet solution consisting of 20 ml 10% nitrocellulose, 30 ml amylacetate, and 0.5 g gentian violet. The mother's nose was also painted with gentian violet to mask any foreign odor on the pups. At the indicated intervals, the animals were killed by decapitation, and the right and left cerebral cortices were separated from the underlying white matter and placed on ice. (See Fig. 1 for medial–lateral and anterior–posterior boundaries.)

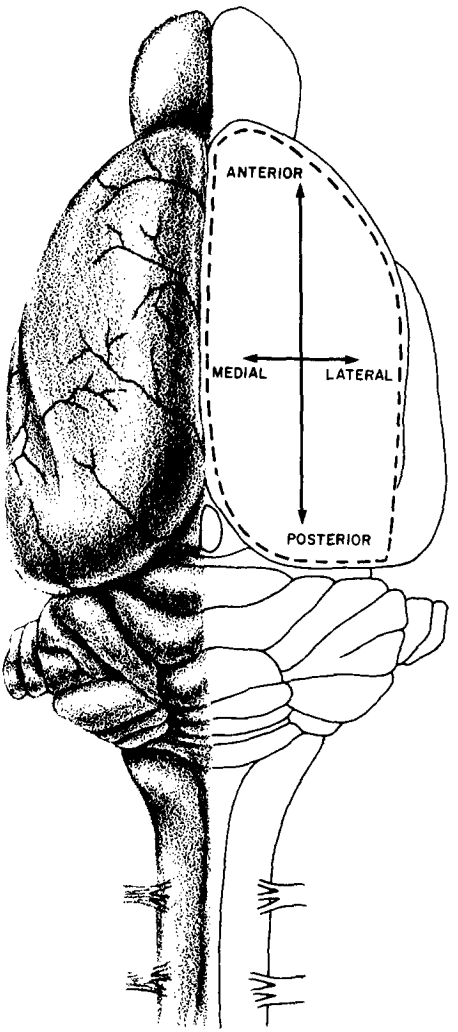

FIG. 1. The dotted line indicates anterior to posterior and medial to lateral boundaries where the right and left cerebral cortices were removed from Long-Evans male or female rat brains.

The brains, from which the cortex had been removed, were stored in 10% Formalin–saline and later transferred to 30% sugar–Formalin for 48 h before sectioning. Frozen, transverse, 20-μm sections were cut and stained with Windle's buffered thionine solution. These sections served to confirm the boundaries of the assayed tissues.

The following technique, which was a modified assay adapted from Barley *et al.* (2) and MacLusky *et al.* (18) was utilized for quantifying cytosol estrogen receptors. Six to eight rats per age group were used for the receptor analysis. The cortical samples from each group of animals were homogenized in a glass Teflon homogenizer (with a clearance rate of 0.125 mm) with 3.5 ml TEGD buffer per gram cortical mass, at 2 to 3°C. The TEGD buffer consisted of 10 m*M* Tris (tris-(hydroxylmethyl)aminomethane), 1.5 m*M* disodium EDTA (ethylenediaminetetraacetate), 10% (vol/vol) glycerol, and 1 m*M* dithiothreitol (Sigma Chemical Co., St. Louis, Missouri) adjusted to *p*H 7.4 with hydrochloric acid (dithiothreitol was added immediately before use). The homogenates were centrifuged 1 h with an ultracentrifuge, using a Ti50 rotator in a model L3-50 ultracentrifuge (Beckman Instruments), at 100,000 *g* (average) and refrigerated at 0 to 2°C. The supernatant cytosol fraction was decanted and kept on ice before use. Samples of 200 μl supernatant at room temperature were added to triplicate assay tubes containing 100 μl [2,4,6,7-^{3}H]estradiol-17β (Amersham, Arlington Heights, Illinois; specific activity, 92 to 100 Ci/mmol) with a final concentration of 1 n*M* [^{3}H]estradiol. (Note: a saturation curve was obtained, and the final concentration used was well above the saturation point.) To control for low-affinity binding, 200-μl samples of supernatant, at room temperature, were added to triplicate assay tubes containing 100 μl unlabeled diethylstilbesterol (DES, Sigma) with a final concentration 5×10^{-7} *M*; the final reaction volume was 0.3 ml. The samples were incubated 30 min in a waterbath at 30°C, then were placed on ice before adding to gel filtration columns. The columns (6 × 0.7 cm) were packed with Sephadex LH-20 (Pharmacia Fine Chemical, Piscataway, New Jersey) and equilibrated with the TEGD buffer at 2 to 3°C. The samples were added to the columns, equilibrated 1 h at 2 to 3°C, and collected in liquid scintillation vials. To the eluted fractions, we added 9 ml hydrofluor, a scintillation fluid (National Diagnostical, Somerville, New Jersey) and counted with a Packard Tri-Carb liquid scintillation spectrometer with an efficiency of 32% and standard error of 2%.

Before each run, the integrity of the columns was tested as follows: to one column we added a mixture of 200 μl TEGD buffer and 100 μl [^{3}H]estradiol with a final concentration of 1 n*M*, and to the other column, 300 μl TEGD buffer alone. The eluted volume was counted, as described above, and we found only background counts per minute in both samples; therefore, we concluded the column system's integrity was intact. A total protein analysis,

as described by Lowry *et al.* (17) was made on each sample, using crystalline bovine serum albumin, to normalize any weight difference in the cerebral cortical samples from the right and left hemispheres.

The identification and quantification of estrogen receptors in the neonatal rat brain is made difficult because of the presence of alpha-fetoprotein in the blood and cerebrospinal fluid (25, 28). Because alpha-fetoprotein is present in enormous quantities relative to the concentration of receptors, it competes with the receptors for available free estrogen and interferes with detection of any estrogen receptor complex form. In this assay, Sephadex LH-20 gel filtration chromatography was used to dissociate the majority of alpha-fetoprotein-bound [^{3}H]estradiol, leaving the receptor [^{3}H]estradiol complex intact. The correction for the residual nonreceptor binding, including alpha-fetoprotein, was made using parallel incubations containing unlabeled diethylstilbesterol. In our final calculation, the amount of residual nonreceptor binding was subtracted from [^{3}H]estradiol bound protein to calculate high-affinity estradiol binding receptors. Both sexes were gonadectomized 24 h before use to minimize the competition for receptors from endogenous gonadal steroids. Dithiothreitol was added immediately before use to TEG buffer to prevent reduction of disulfide bonds of cystiene residues. This was done to preserve the structural and the charge integrity of the receptor molecules in their native form.

Statistical Analysis. Receptor concentration of both sex groups was analyzed using a two-way analysis of variance (four age groups by two hemispheres) with hemisphere as a repeated measure. A one-way analysis of variance was used to test for a decrease in estrogen receptor concentration in the right and left cerebral cortex with respect to age. A paired Student's t test determined significant differences between right and left estrogen receptor concentrations; P values of 0.05 or less were considered significant.

RESULTS

Results of quantification of the cytosol estrogen receptors for the female right and left cerebral cortex are presented in Fig. 2. Estrogen receptor concentration was greatest at postnatal days 2 to 3 in both the right and left cerebral cortex. Thereafter, until 25 days of age, receptor concentration decreased rapidly in both cortices. A one-way analysis of variance showed the decrease with age was statistically significant ($P < 0.005$) for both the right and left cerebral cortex. Furthermore, the decrease in the number of estrogen receptors per milligram protein was different in the right cerebral cortex from the left and varied with age in both the left and right sides (See Table 1). The rate of decline of estrogen receptors in the female from 2 to 3 days to 7 to 8 days of age was more rapid in the right cerebral cortex (60% reduction, P

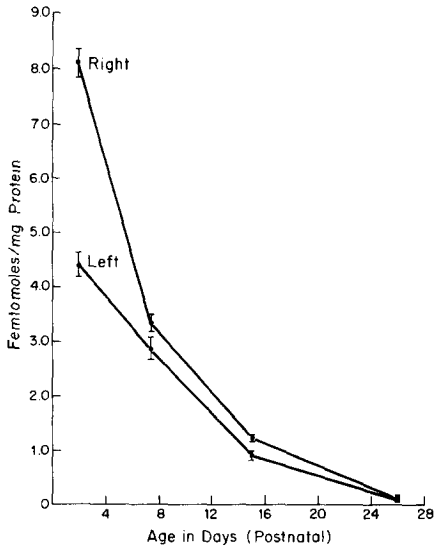

FIG. 2. Postnatal development of cytosolic estrogen receptors in Long–Evans female rats in the left and right cerebral cortex. Each point represents $\bar{x} \pm$ SE of three observations on pooled material from six to eight animals.

< 0.001) than in the left cortex (33%, $P < 0.001$). Thereafter, until 25 days of age, the rate of decrease in receptors was similar in both cortices. In the left cortex, there was a rapid rate of decline from 2 to 3 days to 14 to 15 days of age, and then the decrease was slower (see Fig. 2).

The female right cerebral cortex at postnatal day 2 to 3 had a higher estrogen receptor concentration than the left cortex ($P < 0.02$). During this time, the receptor concentration difference between the right and left cortex was 80% (R–L/L). From postnatal days 7 to 8 and onward, the receptor concentration was consistently greater in the right cortex than in the left, but this difference was statistically nonsignificant.

For the male rats, the receptor concentrations are presented in Fig. 3. As in the female, these concentrations were highest during early development (postnatal days 2 to 3). Between 2 to 3 and 7 to 8 days of age, the male left cerebral cortex showed a greater decrease in estrogen receptors (45% reduction, $P < 0.001$) compared with the right cortex (21% reduction, NS). Thereafter, until 25 days of age, this rate of decrease was parallel in both cortices. In the left cortex the rate of decrease was very rapid from 2 to 3 days to 14 to 15 days of age and then slowed from 14 to 15 days onward (Table 2). In contrast, in the right cortex, the rate of decrease was slow (21% reduction, NS) from 2 to 3 days to 7 to 8 days of age, then became very rapid from 7 to 8 days to

TABLE 1

Decrease in Cytosolic Receptor Concentration in the Right and Left Cerebral Cortex of Female Rat Brain[a]

Hemisphere	Age (days)	Estrogen receptor (fmol/mg protein)	% Reduction	P
Right	2–3	8.035 ± 0.416		
	7–8	3.254 ± 0.264	59	≤0.001
	14–15	1.167 ± 0.054	64	≤0.001
	24–25	0.027 ± 0.003	98	≤0.001
Left	2–3	4.375 ± 0.343		
	7–8	2.940 ± 0.201	33	≤0.001
	14–15	0.984 ± 0.020	67	≤0.001
	24–25	0.019 ± 0.003	98	≤0.001

[a] The receptor concentration is $\bar{x}$ ± SE of three determinations at each age group of pooled cerebral cortex from six to eight animals.

14 to 15 days of age (92% reduction, $P < 0.001$). Thereafter, the rate of estrogen receptor decrease was slow.

In the male, the left cerebral cortex had higher cytoplasmic estrogen receptor concentrations ($P < 0.02$) than the right cortex. This difference was statistically significant at all age groups, except at 14 to 15 days of age.

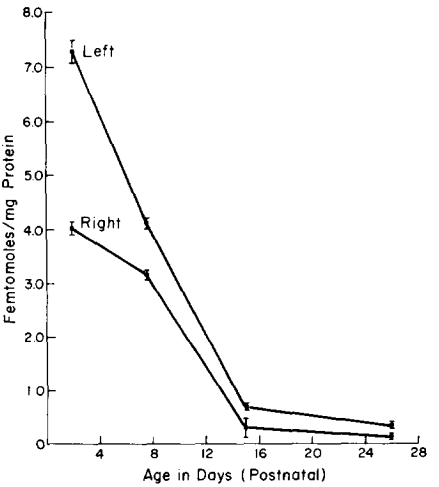

FIG. 3. Postnatal development of cytosolic estrogen receptors in Long–Evans male rats in their left and right cerebral cortex. Each point represents $\bar{x}$ ± SE of three observations on pooled material from six to eight animals.

TABLE 2

Decrease in Cytosolic Receptor Concentration in the Right and Left Cerebral Cortex of Male Rat Brain[a]

Hemisphere	Age (days)	Estrogen receptor (fmol/mg protein)	% Reduction	P
Right	2–3	7.126 ± 0.414		
	7–8	4.038 ± 0.165	44	≤0.001
	14–15	0.562 ± 0.114	86	≤0.001
	24–25	0.312 ± 0.172	44	NS
Left	2–3	4.000 ± 0.143		
	7–8	3.140 ± 0.049	21	NS
	14–15	0.244 ± 0.172	93	≤0.001
	24–25	0.118 ± 0.078	52	≤0.001

[a] The receptor concentration is $\bar{x}$ ± SE of three determinations at each age group of pooled cerebral cortex from six to eight animals.

DISCUSSION

This experiment demonstrates that cytosolic estrogen receptors are differentially localized in the right and left cerebral cortices of both sexes, and that they are in a dynamic state of flux from birth until almost 1 month postnatally. In the female rat, during postnatal days 2 to 3, both cortices have a maximum estrogen receptor concentration compared with the other periods measured. This finding is in agreement with the results of MacLusky *et al.* (19) They reported the mean estrogen receptor concentration, from pooled right and left cerebral cortical samples to be highest at 2 to 3 days postnatally and to decrease rapidly thereafter. However, in the present investigation, during early postnatal life (2 to 3 days, earlier days were not studied), left–right cortical estrogen receptor differences are found. There are greater numbers of cytosolic estrogen receptors (80%, $P < 0.02$) in the female right cortex compared with the left. In the male, the picture is just the opposite with 80% more cytosolic storage receptors in the left cortex compared with the right. The first 5 to 6 days of postnatal life in the rat are considered to be a critical period of rapid growth and steroid-induced maturation of the central nervous system. Gonadal steroids act during the critical period to (i) promote sexual differentiation of synapse patterns (27), (ii) increase the volume of the sexually dimorphic nucleus in the preoptic area (SDN-POA) (1), and (iii) alter the number of neurons in the spinal nucleus of the bulbocavernosus which effects sexual differentiation (3). Rapid cortical thickness growth also occurs during this period (6).

How is the development of morphologic asymmetry in both sexes related to the differential localization of estrogen receptors? In the female, the left

cerebral cortex is thicker than the right in the majority of measurements, with the opposite being true for the male. Estrogen has several effects on the cerebral cortex: (i) it decreases the cortical thickness (23), (ii) decreases neuronal volume (23), (iii) inhibits amino acid incorporation into proteins in the neurons (16), (iv) decreases the incorporation of [^{3}H]thymidine into DNA and [^{14}C]orotic acid into RNA (29), (v) increases neuronal packing density (23), and (vi) alters synaptic anatomy (20).

In the female rat, the right cerebral cortex has a greater number of estrogen receptors than the left cortex. Considering the reported growth-inhibiting effects of estrogen, we postulate that the left cerebral cortical growth can be enhanced by decreased responsiveness to estrogen as reflected by the fewer estrogen receptors in the left cerebral cortex compared with the right. Data from the male rat cerebral cortex also lend support to this idea that an increased estrogen binding during development results in diminished cortical growth in the left cortex compared with the right.

Therefore, results from our experiment indicate that one determinant of cerebral dominance in both sexes may be the differential exposure to estrogen during a critical period of development. At this time another question we might ask is "Are estrogen receptors asymmetrically placed in other regions of the brain?"

Several investigators show that there are sex differences in estrogen receptor binding in other brain regions which are undoubtedly important in determining sexual behavior. Rainbow *et al.* (26) report that there are higher cytoplasmic estrogen receptor concentrations in the medial portion of the preoptic area of female than in male rats. Furthermore, Nordeen *et al.* (21) report that with systemic administration of [^{3}H]estradiol, the female rats retain more steroid than do males in both the preoptic and medial basal hypothalamus. Those investigators also show that exposure of the left hypothalamus to estrogen (by unilaterally implanting estrogen pellets), results in defeminization, and that unilateral estrogen exposure of the right hypothalamus results in masculine development. They concluded, therefore, that sexual differentiation is affected by differential exposure of estrogen to the developing diencephalon.

In the cerebral cortices of both sexes, the distribution of estrogen receptors is a dynamic and complex process. Why are estrogen receptors present in high concentration in both cortices at early postnatal life, and virtually absent after the 1st month in both sexes? One consideration is that estrogen concentrations in the cerebrospinal fluid control the gene expression of its receptors. During early postnatal life, there are high concentrations of alpha-fetoprotein, which by binding to steroids, produce less free estrogen in the cerebrospinal fluid. To increase sensitivity to small amounts of estrogen, neurons may increase receptor synthesis. A similar mechanism is seen in the down-regulation

of insulin receptors. A second consideration is the migration of receptor-containing neurons from one brain region to another (15). The third possibility is the further differentiation or death of a class of estrogen-responsive neurons. In the case of the diencephalic nuclei (SDN-POA), the end of the critical period may coincide with a loss of the potential to make afferent or efferent connections. The exact mechanism is not known at this time.

It now seems evident that the differential localization of gonadal steroids during a critical period of development can exert profound effects on morphogenesis in the central nervous system. This may include regulating the number of neurons, neuronal size, extent of dendritic branching, and synaptogenesis. Our investigation indicates that in both sexes, the cerebral cortex that has increased estrogen binding has decreased cortical growth, and the cerebral cortex that has lower estrogen binding has increased cortical growth. Differential growth of the two hemispheres in both sexes is undoubtedly important in determining male and female behavior. Although a unifying mechanism that integrates morphologic, biochemical, and behavioral asymmetries is not known, our investigation does shed some light in understanding this complex phenomenon.

REFERENCES

1. ARNOLD, A. P., AND R. A. GORSKI. 1984. Gonadal steroid induction of structural sex differences in CNS. *Annu. Rev. Neurosci.* **7**: 413–442.
2. BARLEY, J. M., GINSBURG, B. GREENSTEIN, N. J. MACLUSKY, AND P. J. THOMAS. 1974. A receptor mediating sexual differentiation? *Nature* **258**: 259–260.
3. BREEDLOVE, S. M., AND A. P. ARNOLD. 1983. Hormonal control of a developing neuromuscular system. II. Sensitive periods for androgen induced masculinization of the rat spinal nucleus of the bulbocavernosus. *J. Neurosci.* **3**: 424–432.
4. DIAMOND, M. C., F. LAW, B. LINDNER, M. R. ROSENZWEIG, D. KRECH, AND E. L. BENNETT. 1960. Increases in cortical depth and glial numbers in rats subjected to enriched environment. *J. Comp. Neurol.* **128**: 117–128.
5. DIAMOND, M. C. 1967. Extensive cortical depth measurement and neuron size increase in cortex of environmentally enriched rats. *J. Comp. Neurol.* **131**: 357–364.
6. DIAMOND, M. C., R. E. JOHNSON, AND C. INGHAM. 1975. Morphological changes in young, adult and aging rat cerebral cortex, hippocampus and diencephalon. *Behav. Biol.* **14**: 163–174.
7. DIAMOND, M. C., B. LINDNER, R. E. JOHNSON, E. L. BENNETT, AND M. R. ROSENZWEIG. 1975. Differences in occipital cortical synapses from environmentally enriched, impoverished, and standard colony rats. *J. Neurosci. Res.* **1**: 109–119.
8. DIAMOND, M. C., R. E. JOHNSON, C. INGHAM, M. R. ROSENZWEIG, AND E. L. BENNETT. 1975. Effect of differential experience on neuronal nuclear and perikarya dimension in the rat cerebral cortex. *Behav. Biol.* **15**: 107–111.
9. DIAMOND, M. C., AND J. R. CONNOR, JR. 1981. A search for the potential of the aging brain. Pages 43–58 in S. J. ENNA, T. SAMORAJSKI, AND B. BEER, Eds., *Brain Neurotransmitters and Receptors in Aging and Age-Related Disorders*, Aging, Vol. 17. Raven Press, New York.

10. DIAMOND, M. C., G. A. DOWLING, AND R. E. JOHNSON. 1981. Morphological cerebral cortical asymmetry in male and female rats. *Exp. Neurol.* **71:** 261–268.
11. DIAMOND, M. C., R. E. JOHNSON, D. YOUNG, AND S. S. SINGH. 1983. Age related morphologic differences in the rat cerebral cortex and hippocampus: male–female, right–left. *Exp. Neurol.* **81:** 1–13.
12. DIAMOND, M. C. 1984. Age, sex and environmental influences, Pages 134–146 in N. GESCHWIND AND A. M. GALABURDA, Eds., *Cerebral Dominance—The Biological Foundations.* Harvard Univ. Press, Cambridge, Massachusetts.
13. GLOBUS, A., M. R. ROSENZWEIG, E. L. BENNETT, AND M. C. DIAMOND. 1973. Effects of differential experience on the dendritic spine count in the rat cerebral cortex. *J. Comp. Physiol. Psychol.* **82:** 175–181.
14. HOLLOWAY, R. L. 1966. Increased dendritic branching in layer II stellate neurons of occipital cortex as compared to deprived littermates. *Brain Res.* **2:** 393–396.
15. LEVI-MONTALCINI, R. 1964. Events in the developing nervous system. *Prog. Brain Res.* **4:** 1–29.
16. LITTERIA, M., AND M. W. THORNER. 1976. Inhibitory action of neonatal estrogenization of incorporation of ^{3}H-lysine into cortical neuroproteins. *Brain Res.* **103:** 584–587.
17. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with Folin-phenol reagent. *J. Biol. Chem.* **193:** 265–273.
18. MACLUSKY, N. J., I. LIEBERGER, AND B. S. MCEWEN. 1979. The development of estrogen receptor systems in the rat brain: perinatal development. *Brain Res.* **178:** 129–142.
19. MACLUSKY, N. J., C. CHAPTAL, AND B. S. MCEWEN. 1979. The development of estrogen receptor systems in the rat brain: postnatal development. *Brain Res.* **178:** 143–160.
20. MEDOSCH, C. M., AND M. C. DIAMOND. 1982. Rat occipital cortical synapse after ovariectomy. *Exp. Neurol.* **75:** 120–132.
21. NORDEEN, E. J., AND P. YAHR. 1982. Hemispheric asymmetries in the behavioral and hormone effects of sexually differentiating mammalian brain. *Science* **218:** 391–394.
22. PAPPAS, C. T. E., M. C. DIAMOND, AND R. E. JOHNSON. 1978. Effects of ovariectomy and differential experience on the rat cerebral cortical morphology. *Brain Res.* **154:** 53–60.
23. PAPPAS, C. T. E., M. C. DIAMOND, AND R. E. JOHNSON. 1979. Morphological changes in the cerebral cortex of rats with altered levels of ovarian hormones. *Behav. Biol.* **26:** 298–310.
24. PFAFF, D. W. 1966. Morphological changes in the brain of adult male rats after neonatal castration. *J. Endocrinol.* **36:** 415–416.
25. PLAPINGER, L., B. S. MCEWEN, AND L. E. CLEMENS. 1973. Ontogeny of estradiol-binding sites in rat brain. II. Characteristics of a neonatal binding macromolecule. *Endocrinology* **93:** 1129–1139.
26. RAINBOW, T. C., B. PARSONS, AND B. S. MCEWEN. 1982. Sex differences in the rat brain oestrogen and progestin receptors. *Nature* **300:** 648–649.
27. RAISMAN, G., AND P. M. FIELD. 1971. Sexual dimorphism in the preoptic area of the rat. *Science* **73:** 731–733.
28. RAYNAUD, J. P. 1973. Influence of rat estradiol binding plasma protein (EBP) on uterotrophic activity. *Steroids* **21:** 249–258.
29. VERTES, Z., M. VERTES, AND S. KOVACS. 1973. Effects of postnatal extradiol treatment of DNA and RNA synthesis in the brain. *Acta Physiol. Acad. Sci. Hung.* **44:** 428.