

# Lack of Activational Influence of Ovarian Hormones on the Size of the Female Rat's Corpus Callosum

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MACK, C. M., R. H. FITCH, L. A. HYDE, A. J. SEAMAN, H. A. BIMONTE, W. WEI AND V. H. DENENBERG. *Lack of activational influence of ovarian hormones on the size of the female rat's corpus callosum*. *PHYSIOL BEHAV* 60(2) 431–434, 1996.—The sex difference in the midsagittal area of the adult rat corpus callosum (CC) has been shown to be mediated, in part, by gonadal steroids in early development, with the sensitive period of hormone action in the female extending at least up to postnatal day 25. Given this prolonged sensitivity, the current study attempted to delineate organizational vs. activational influences of gonadal hormones on the female rat CC. In Experiment 1, callosal size was examined across the estrous cycle at 52 and 90 days of age. In Experiment 2, females were ovariectomized at 78 days and CC parameters assessed at 110 days. Last, in Experiment 3, females were ovariectomized at 78 days and sacrificed at 110 days; in addition, sham females were sacrificed during proestrus or estrus. Neither stage of estrous cycle nor adult ovariectomy affected midsagittal CC size. These results provide evidence for organizational effects of ovarian steroids on the female callosum, with the sensitive period of hormone action ending sometime between days 25 and 78.

Ovarian hormones    Corpus callosum    Female rat    Activational influence

THE midsagittal area of the rat corpus callosum (CC) is sexually dimorphic in the neonate and adult, with the male CC being larger in three different strains (2,4,6,8,9,14,18,26,27). Endogenous gonadal steroids in both sexes play a role in mediating this difference. In the male, androgen blockade concurring with the onset of the prenatal testosterone surge (22) and extending beyond the 2-h postnatal rise in testosterone levels (3) reduced CC size to that of the female (8). Because castration on day 1 following the postnatal surge is without effect (6), the organizational effect of androgens can be attributed to 1) the independent action of either rise in testosterone levels, or 2) an additive or interactive effect between the two surges.

In contrast, female CC responsiveness to ovarian steroids extends through a currently undefined postnatal period. Ovariectomy up to day 16 enlarged callosal area compared to sham controls (9). This effect can be attributed primarily to the absence of estrogenic inhibition, as chronic estrogen treatment beginning on day 25 prevented this increase (14). However, unlike the male, it is unclear whether estrogen is acting organizationally, as the extended postnatal sensitive period does not fit into the tra-

ditional dichotomy of steroid action on brain structure. The conventional assumptions are that organizational (permanent) effects occur during a restricted perinatal period whereas activational (transient) effects typically occur in adulthood [but see (1,23)]. Similar to our findings, investigators have reported ovarian steroid responsiveness in rat cortical architecture that extends well beyond the neonatal period, including puberty (17,19), and even adulthood (20). It is unclear, however, whether these are organizational effects because they may be dependent on the levels of gonadal steroids at the time of sacrifice. This appears to be the case in the hippocampus and hypothalamus, where dendritic morphological alterations have been found to occur across the estrous cycle (10,24).

Whether the gross size of a group of axons like those of the CC would manifest changes across the estrous cycle is unknown. The current study was therefore designed to distinguish between permanent vs. transient steroid-induced changes in female callosal size. First, the CC was examined in female rats sacrificed at different stages of the estrous cycle. Because alterations in neuronal morphology and protein synthesis occur within 2 h of

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TABLE 1  
CALLOSAL AREA (mm<sup>2</sup>) FOR FEMALES SACRIFICED ACROSS THE  
ESTROUS CYCLE

|         | Diestrus                | Proestrus               | Estrus                   | Metestrus               |
|---------|-------------------------|-------------------------|--------------------------|-------------------------|
| 52 days | 2.430<br>(0.168)<br>[3] | 2.654<br>(0.108)<br>[3] | 2.641<br>(0.117)<br>[7]  | 2.494<br>(0.060)<br>[4] |
| 90 days | 2.735<br>(0.108)<br>[3] | 3.022<br>(0.136)<br>[3] | 2.881<br>(0.096)<br>[11] | 2.424<br>—<br>[1]       |

Values are means with SEMs in parentheses. Numbers of subjects are in brackets.

Main effect of age:  $p < 0.05$ .

estrogen treatment (15), and estrous phase effects on neuronal architecture occur within 24 h of proestrus (10,24), one would predict decreased CC size at proestrus compared to diestrus and/or estrus if estrogen is acting activationally. Secondly, female rats were ovariectomized in adulthood and compared to sham controls. If estrogen is exerting activation effects, the ovariectomized group should exhibit significantly larger CCs, as has been seen in neonatal OVX rats (9,14). Conversely, if estrogen is exerting organizational effects within a sensitive window that ends at or before puberty, adult OVX should be without effect.

#### METHOD

Subjects for all experiments were female Purdue–Wistar rats bred in our closed colony. All litters were culled to eight pups at birth and weaned on day 21. In Experiment 1, the rats were control animals from a past experiment (7). In that study, intact control females had received either a single SC injection of oil (0.05 ml), or sham ovariectomy on day 12. Surgery consisted of two dorsolateral skin incisions performed under ether anesthesia. Following neonatal treatment, pups were returned to their home cage and subsequently treated in an identical fashion. Following weaning, animals were housed in same-treatment pairs. Animals were sacrificed at 52 days ( $n = 17$ ; 10 oil treated and 7 sham operated) or 90 days ( $n = 18$ ; 7 oil treated and 11 sham operated) for CC morphometric analysis. On the day of sacrifice, vaginal smears were taken, the phase of estrus determined (25), and the uterus weighed.

In Experiment 2, animals were weaned and group housed (five to six per cage) with littermates. At 78 days of age, two females from each litter were randomly chosen for analysis. Under ether anesthesia, one female was ovariectomized ( $n = 7$ ) while the second female concurrently received sham surgery ( $n = 7$ ). Two dorsolateral incisions were made in the skin and peritoneum, and the ovaries and the tips of the uterine horn were removed. Sham surgery consisted of a skin incision only. Animals were singly housed for 4 days following surgery and then returned to their original group-housed condition.

In Experiment 3, animals were reared identically to those in Experiment 2, and also received OVX ( $n = 21$ ) or sham surgery ( $n = 15$ ) at 78 days of age. Additionally, sham females were sacrificed at either the proestrus ( $n = 10$ ) or estrus ( $n = 5$ ) phase of their cycle.

At 110 days of age, all animals were anesthetized and perfused with 0.9% saline followed by a 4% formaldehyde/0.1% glutaraldehyde fixative. The brains were removed and stored in sucrose formalin for cryoprotection. The olfactory bulbs and hindbrain were removed, the brain weighed, and sagittal sections

(45  $\mu$ m) obtained. The 12 sections closest to midline in each hemisphere were mounted and stained with cresyl violet. Using a projection microscope, the closest intact callosum to midline from the right hemisphere was traced at a magnification of 23 $\times$ . Each drawing was then traced five times onto a digitizing tablet connected to a Macintosh computer and the average taken to yield one callosal tracing for each subject. Callosal area was obtained using the software package Stereology (4).

#### RESULTS

##### Experiment 1

Group differences in overall CC area were examined using a  $2 \times 4$  (Age  $\times$  Phase) analysis of variance (ANOVA). The means and SEMs for all groups are shown in Table 1. A significant main effect of age showed 90-day-old females to have larger CCs than 52-day-old animals,  $F(1, 27) = 3.32$ ,  $p < 0.05$  (one-tailed test). There was no main effect of Phase, nor an Age  $\times$  Phase interaction.

To test our classification of animals into the four estrous groups, a  $2 \times 2$  (Age  $\times$  Phase) ANOVA was used to compare the uterine weights of proestrus and estrus females (Table 2). Results showed significant main effects of Age,  $F(1, 20) = 11.19$ ,  $p < 0.01$ , and Phase,  $F(1, 20) = 8.74$ ,  $p < 0.01$ , with 90-day-old females having higher uterine weights than 52-day-old animals, and estrus females having higher weights than proestrus females.

##### Experiment 2

Treatment assignments were randomly made within litters and thus repeated-measures ANOVAs (one-tailed tests) were used to test for group differences. Ovariectomy at 78 days of age had no effect on callosal area, but did increase brain weight,  $F(1, 6) = 45.36$ ,  $p < 0.01$  (Table 3, left half).

##### Experiment 3

In this study, individual treatment groups contained littermates. Littermate correlations revealed no significant litter effects for CC area and thus subjects were treated as independent observations. Because our main interest was to determine whether the phase of estrous cycle affected CC size, the Sham/proestrus and the Sham/estrous groups were compared. As can be seen in Table 3 (right half), no significant difference was found for callosal area. These two groups were thus combined (Sham P + E group in Table 3) and subsequently compared to the OVX group. Identical to our findings from Experiment 2, sham and OVX

TABLE 2  
UTERINE WEIGHT (g) FOR FEMALES SACRIFICED AT  
PROESTRUS AND ESTRUS

|         | Proestrus               | Estrus                   |
|---------|-------------------------|--------------------------|
| 52 days | 0.237<br>(0.020)<br>[3] | 0.397<br>(0.038)<br>[7]  |
| 90 days | 0.417<br>(0.034)<br>[3] | 0.552<br>(0.037)<br>[11] |

Values are means with SEMs in parentheses. Numbers of subjects are in brackets.

Main effect of age:  $p < 0.01$ ; and phase:  $p < 0.01$ .

TABLE 3  
CALLOSAL AREA (mm<sup>2</sup>) AND BRAIN WEIGHT (g) FOR 110-DAY-OLD FEMALE RATS THAT WERE OVARIECTOMIZED (OVX) OR SHAM OPERATED AT 78 DAYS OF AGE

|              | Experiment 2     |                    | Experiment 3     |                  |                  |                   |
|--------------|------------------|--------------------|------------------|------------------|------------------|-------------------|
|              | Sham             | OVX                | Sham P           | Sham E           | Sham P + E       | OVX               |
| CC area      | 3.734<br>(0.136) | 3.781<br>(0.058)   | 3.506<br>(0.061) | 3.678<br>(0.128) | 3.563<br>(0.059) | 3.569<br>(0.059)  |
| Brain weight | 1.417<br>(0.020) | 1.550**<br>(0.021) | 1.320<br>(0.016) | 1.302<br>(0.007) | 1.314<br>(0.011) | 1.357*<br>(0.015) |
| n            | 7                | 7                  | 10               | 5                | 15               | 21                |

In Experiment 3, sham females were sacrificed at the proestrous (P) or estrous (E) stage of the estrous cycle. Compared to Sham or Sham P + E group in each experiment: \*  $p < 0.05$ , \*\*  $p < 0.01$ .

females showed no callosal area difference, but the OVX rats had significantly heavier brains than the shams,  $F(1, 34) = 4.54$ ,  $p < 0.05$ .

#### DISCUSSION

The present results found no evidence for an activational role of ovarian hormones on the size of the female callosum. No differences were observed for CC area across the estrous cycle in either 52- or 90-day-old animals. Also, the CC values of 78-day-old sham females sacrificed at proestrus and estrus did not differ from each other, nor from OVX females. Lastly, there was no effect of day 78 ovariectomy on CC area. Uterine weight was increased at estrus compared to proestrus, which is consistent with the reported time course of estrogenic effects on this variable (17).

Our data do not rule out, however, possible activational influences on the CC at the cellular level, as recent evidence has shown structural plasticity across the estrous cycle in subcortical brain regions (10,24). It should also be noted that postpubertal OVX females did have significantly heavier brains, confirming our prior findings that changes in CC size do not solely reflect changes in overall brain size (2,4,6,9,14).

The callosal values in the 110-day-old animals were much larger than those obtained in our prior prepubertal OVX studies

(9,14). Upon reflection, this is likely the result of postweaning housing conditions. Whereas the present animals had been reared in groups of five to six, the rats from the two previous studies were pair housed. Indeed, a study on the effects of early experience on the CC has shown females rats that are group reared (12 per cage) in an enriched environment from days 25–55 to have significantly larger callosa than isolation-housed animals (13).

Taken together, these negative findings point to an organizational role of ovarian steroids on female CC development. Several researchers have made similar conclusions concerning gonadal hormone-dependent processes in the CNS in the female (5,11,12,21). Because chronic estrogen treatment beginning on day 25 reverses the enlarging effect of ovariectomy (14), the sensitive period of ovarian hormone organization must end sometime between days 25 and 78. An important event during this time frame that may render the brain insensitive to further steroid organization is the onset of puberty. This appears to be the case for the ability to display sexual behavior in adulthood, which has been shown to rely largely on the presence of prepubertal vs. postpubertal ovarian secretions (11).

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