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

CHRISTINE M. MACK,* ROSLYN H. FITCH,† LYNN A. HYDE,* AMY JO SEAMAN,* HEATHER A. BIMONTE,* WEI WEI* AND VICTOR H. DENENBERG‡

*Biobehavioral Sciences Graduate Degree Program, University of Connecticut, Storrs, CT 06269 USA, †Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102 USA and ‡Biobehavioral Sciences Graduate Degree Program and Department of Psychology, University of Connecticut, Storrs, CT 06269 USA

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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.

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 traditional 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 responsivity 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
TABLE 1

<table>
<thead>
<tr>
<th>CALLOSAL AREA (mm²) FOR FEMALES SACRIFICED ACROSS THE ESTROUS CYCLE</th>
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<tr>
<td></td>
</tr>
<tr>
<td>52 days</td>
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<tr>
<td></td>
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<tr>
<td>90 days</td>
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<td></td>
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</tbody>
</table>

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

Main effect of age: p < 0.05.

Table 1 shows the callosal area for females sacrificed across the estrous cycle. The data indicate that there are significant differences in the callosal area at 52 and 90 days. The values are presented as means with standard errors of the mean (SEM) in parentheses. The number of subjects is also given in brackets.

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 proestrous and estrous 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 estrous females having higher weights than proestrus females.

Table 2: Uterine Weight (g) for Females Sacrificed at Proestrus and Estrus

<table>
<thead>
<tr>
<th></th>
<th>Proestrus</th>
<th>Estrus</th>
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<tbody>
<tr>
<td>52 days</td>
<td>0.237</td>
<td>0.397</td>
</tr>
<tr>
<td></td>
<td>(0.020)</td>
<td>(0.038)</td>
</tr>
<tr>
<td></td>
<td>[3]</td>
<td>[7]</td>
</tr>
<tr>
<td>90 days</td>
<td>0.417</td>
<td>0.552</td>
</tr>
<tr>
<td></td>
<td>(0.034)</td>
<td>(0.037)</td>
</tr>
</tbody>
</table>

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

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