

Short communication

Adult ovary transfer counteracts the callosal enlargement resulting from prepubertal ovariectomy

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Abstract

The rat corpus callosum (CC) is larger in males than females, and is sensitive to hormone manipulations during development. Previous research found that, in rats, CC sensitivity to testosterone ended by postnatal day 8 (P8). In contrast, more recent findings demonstrated that CC responsiveness to ovarian hormones continued at least through P70. The current experiment extends these findings by showing that the female callosum is still sensitive to ovarian hormones as late as P130, well into adulthood. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Development and regeneration

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Our laboratory has performed ongoing research assessing gonadal hormone contributions to rat corpus callosum (CC) development [1,5,6,8–10,15–18]. The cross-sectional area of this fiber tract is larger in male than in female rats. In both sexes, gonadal hormones have been shown to exert organizational, or permanent, effects on callosal morphology. Androgen deprivation from embryonic day 17 (E17) through adulthood reduced (demasculinized) CC size in males [9]. In contrast, postnatal castration of male rats before or after the postnatal day 1 (P1) testosterone surge did not affect adult CC size [8,18]. Thus, prenatal exposure to testosterone is sufficient to masculinize permanently the male rat CC. However, if testosterone exposure does not occur prenatally, the CC is still sensitive to testosterone postnatally since handled female rats treated with testosterone on P4, but not P8, exhibited enlarged adult CC [8,10]. These data demonstrate that CC sensitivity to the organizing effects of testosterone extends beyond the

developmental time point where critical exposure normally occurs (E17) so that a normal-sized male CC is expressed in adulthood, and ends by P8.

In contrast to the early time frame for testosterone effects, female CC responsiveness to ovarian hormones extends later in development, well beyond the first postnatal week of life. Indeed, previous findings suggest that the female CC is organized by ovarian hormones sometime between P25 and P70, since P25 ovariectomy (Ovx) enlarged (defeminized) the adult callosum, while P70 Ovx had no effect, yielding a normal sized adult female CC [4]. Further, CC size did not fluctuate across the estrous cycle [17]. These findings, taken together, indicate that ovarian hormone effects on the female callosum are irreversible, not transient.

Although callosal feminization is not reversed by P70 ovarian hormone removal, a not-yet feminized CC is still responsive to P70 ovarian hormone replacement. P70 ovary transfer (OvT) or estradiol supplementation counteracted the enlarging effect of P25 Ovx, resulting in smaller (feminized) CC as assessed on P130 or P188 [3,4]. The current study was performed to determine if the CC of P25 Ovx females are still responsive to the organizing

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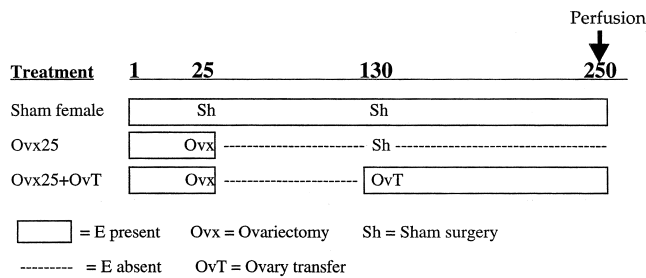


Fig. 1. Schematic depicting surgical procedures and estrogenic milieu for each treatment group.

influences of ovarian hormones if given OvT further into adulthood, as late as P130. The experimental design is shown in Fig. 1

Purdue–Wistar rats were bred in our closed colony. One day after birth (P1) 14 litters were culled to eight pups, with each litter containing a female:male ratio of 5:3 or 6:2. Following weaning on P21, animals were housed in same-treatment pairs until perfusion.

At least three females from each litter were used. Treatments were assigned within litters. On P25, two females from each litter received Ovx (Ovx25) and one female received sham surgery (Sham). On P130, the Sham and one Ovx25 female received sham surgery, and one Ovx25 female received an OvT (Ovx25+OvT) using a halved ovary from a non-experimental Purdue–Wistar female rat. Any extra females from a litter used in the study were randomly assigned to a treatment group. The resulting treatment groups were: Sham, Ovx25, and Ovx25+OvT.

Ketaset–Rompun was used to anesthetize animals during surgical procedures. For Ovx, two dorsolateral incisions were made in the skin and peritoneum, and the ovaries and tips of the uterine horns were removed. Sham surgery consisted of skin incision only. For OvT [21], after skin and muscle incision, the ovary was transplanted into the kidney. This was done by making a small incision in the kidney capsule, creating a pocket using micro-forceps, and inserting a halved ovary into the pocket. The ovary was then gently probed until it was secured between the kidney capsule and kidney cortex, and the muscle and skin were sutured. Before and after P130 surgery, animals were vaginally smeared for several cycles to confirm proper hormonal status.

For perfusion, which occurred on P250, animals were overdosed with sodium pentobarbital and perfused through the ascending aorta with saline followed by a mixed aldehyde fixative. The brains were removed and stored in sucrose-formalin for cryoprotection. The olfactory bulbs, brain stem, and hindbrain were removed, and the brains were sagittally sectioned at 45 microns. The 12 sections closest to midline in each hemisphere were mounted onto glass slides in a gelatin-alcohol medium, stained with Cresyl violet, and cover-slipped. Using a projection micro-

scope, the CC of the right hemisphere section closest to midline that was intact was traced at a magnification of 23×. Each CC drawing was then traced 5 times onto a digitizing tablet connected to a computer. The software package *Stereology* yielded callosal parameters of area and 99 equidistant widths perpendicular to the longitudinal axis for each drawing [5]. The average of the 5 drawings was used to yield the callosal measurements for each subject. Callosal widths were divided into 7 region-specific factors based on previous factor analysis [5]. Beginning in the anterior, the 7 callosal width factors were: widths 1–5 (W1–5), W6–17, W24–38, W46–57, W62–72, W79–95 and W96–99. Since work from our laboratory has consistently shown that P70 OvT affects callosal size of P25 Ovx females in the midbody/posterior region [3,4], we also assessed W58–61 and W73–78. The final measures were callosal area and the nine width factors.

Prior to P130, all females receiving P25 Ovx exhibited consistent leukocytic vaginal smears while Sham females demonstrated cyclic smears. After P130 treatment, Ovx25 females exhibited leukocytic smears, whereas Sham and 14 of the 17 Ovx25+OvT females demonstrated cyclic smears. The females that did not cycle exhibited mainly leukocytic smears and were excluded from the study. The OvT groups had more variable smears than Shams, occasionally exhibiting a 2–3 day estrous phase. This has been seen before in females receiving OvT around P70 [21]. Together, these data confirm complete removal of ovarian tissue in Ovx females and cyclic ovarian hormone release in females receiving OvT.

ANOVA revealed no significant Litter effects for overall area. Therefore, subjects were treated as individual observations. For each treatment group, Table 1 shows the number of subjects and means±S.E. for callosal area, and Fig. 2 shows the means for each callosal width region.

Ovx25 females were compared to Shams using one-tailed *t*-tests since the direction of the effect was known. Ovx25 females had larger CC than Sham females in overall area, W1–5, W6–17, and W58–61 [*ts*(28)=2.21, 2.69, 3.21, 1.79; *ps*<0.05]; marginal effects were found in W24–38, W46–57, and W62–72 [*ts*(28)=1.68, 1.53, 1.32; *ps*<0.10].

Two-tailed *t*-tests determined that Ovx25 females had larger CC than Ovx25+OvT females for area, W46–57, W58–61, and W62–72 [*ts*(27)=3.55, 3.38, 3.37, 2.61; *ps*<0.005, 0.005, 0.005, 0.05]. Ovx25 females also had somewhat larger CC in W1–5, W6–17, and W24–38 [*ts*(27)=1.85, 1.81, 1.94; *ps*<0.10].

Table 1
Mean callosal area for each treatment group

Treatment	N	CC Area±S.E.
Sham	15	3.473±0.083
Ovx25	15	3.779±0.083
Ovx25+OvT	14	3.356±0.086

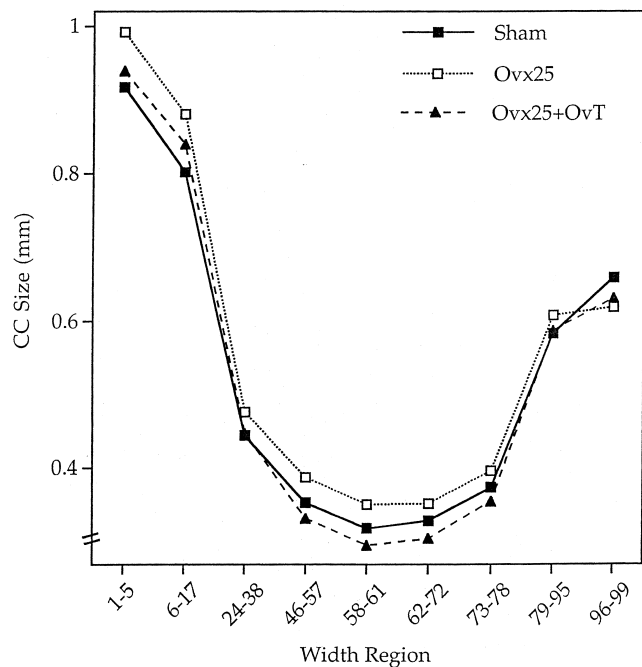


Fig. 2. Mean callosal size for each width region for each treatment group.

Ovx25+OvT females did not differ from Sham females for area or any width region.

Prior work demonstrated that P70 Ovx did not affect callosal size so that a normal-sized female CC was expressed in adulthood [4]. Although an already feminized callosum is not affected by removal of ovarian hormones on P70, we previously showed that a not-yet feminized callosum is still responsive to *replacement* of ovarian hormones on P70 [3,4]. The current report extends these findings, and demonstrates that callosal sensitivity to ovarian hormones continues even further, at least through P130. This finding shows that the female CC is still sensitive to estrogen at least 60 days beyond the developmental window of estrogen exposure that normally provides irreversible CC feminization (i.e., ending about P70). Thus, ovarian hormone stimulation starting on P130 can compensate for the lack of previous ovarian hormone exposure necessary for the expression of a normal-sized adult female CC.

These results suggest that there is flexibility in the window of callosal sensitivity to ovarian hormones. There is also evidence that there is flexibility in the window of callosal sensitivity to testosterone, since early exposure to postnatal testosterone can compensate for lack of exposure to prenatal testosterone [8,9]. It therefore appears that ovarian and testicular hormone effects on the CC both follow the general principle that sensitivity extends beyond the point that critical hormone exposure normally occurs, resulting in a feminized or masculinized callosum. However, there are marked differences in the temporal parameters characterizing this sensitive window; callosal responsiveness to testosterone ends by P8 while responsiveness to

ovarian hormones extends at least through P130, well into adulthood.

The central tenet of hormone action on brain organization has been that it occurs early in development (for review see [7]). This supposition is based upon overwhelming data showing that there is a limited neonatal sensitive window of organizational effects by androgen, combined with the findings that many neural systems (e.g., the hippocampus) and behaviors (e.g., spatial abilities) respond to adult ovarian hormone exposure in a transient manner [2,11–14,19,23,24]. However, our findings build upon accumulating evidence that the female brain is also sensitive to organizing actions of ovarian hormones during a time frame that extends far beyond the neonatal period.

Similar late organizational effects of ovarian hormones have been reported by others. For example, estrogen altered morphology of the rat arcuate nucleus as late as P25, an effect that was not transient across days [22]. Also, ethinylestradiol and progesterone administered P40 through P90 affected cortical thickness of P1 Ovx females, demonstrating that the female rat cortex is sensitive to ovarian hormone input beyond P40 [20]. Together, the data suggest that the female brain is remarkably flexible, and may be sensitive to the organizing effects of ovarian hormones throughout life.

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