

CORPUS CALLOSUM: DEMASCULINIZATION VIA PERINATAL ANTI-ANDROGEN

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Abstract—The male rat's corpus callosum is significantly larger than the female's. This dimorphism depends in part on the early presence of testosterone, since postnatal administration of testosterone to female pups enlarges their callosa in adulthood to the size of males. However, castrating males on day 1 is ineffective in reducing (demasculinizing) the size of their callosa as adults. We then addressed the question as to whether testosterone acts prior to day 1 to enlarge the callosa of males. To investigate this hypothesis pregnant rats were administered a non-steroidal androgen blocker, flutamide, during the last 5 days of pregnancy, while controls received vehicle only. Male pups from flutamide litters were castrated on day 3 to prevent postnatal recovery following clearance of flutamide, while others received sham surgery. Callosal sex differences were found between males and females of control litters, but not between males and females from flutamide litters. The absence of sex effects among flutamide litters was a consequence of small callosal size in flutamide-castrated males as compared to control males. We concluded that the prenatal production of testosterone in the male rat pup contributes to sexual dimorphism in the callosa of adult rats.

Key words: sex differences, flutamide, development, masculinization.

The rat corpus callosum is sexually dimorphic, with the cross-sectional area of the male being larger in 3-day-old pups¹³ and adults.¹ This dimorphism appears to be due in part to the fetal production of testosterone, since Zimmerberg and Scalzi¹³ found that prenatal alcohol exposure reduced the size of male rats' callosa in infancy and eliminated the callosal sex difference found in controls. They attribute this effect to a shift in the prenatal testosterone peak resulting from alcohol-induced stress.^{8,12} The sensitive period for the effects of testosterone on the callosum appears to extend postnatally, since Fitch *et al.*⁵ demonstrated that giving female rats 1 mg testosterone propionate (TP) on postnatal day (PD) 4 enlarged their adult callosa to the size of males'. It is of particular interest that TP did not affect the brain weight of treated females, substantiating our assertion that sexual dimorphism in the size of the rat callosum is not a reflection of differences in brain size.⁴

In the same series of experiments, castration on PD 1 failed to reduce the size of the males' callosa in adulthood.⁵ This raised the question of whether testosterone influences callosal development in the male. Since other researchers have shown effects of prenatal testosterone manipulations on male callosa,¹³ and male cortical thickness asymmetry,^{7,11} we decided to administer an androgen blocker during the prenatal period. Furthermore, since others have suggested that the post-parturition surge of testosterone may influence sexual differentiation,^{2,10} and since we have shown that TP on PD 4 masculinizes the callosa of females,⁵ we continued our treatment into the postnatal period.

EXPERIMENTAL PROCEDURES

Six pregnant female rats were administered 25 mg/kg of flutamide (FLT), a non-steroidal androgen receptor blocker, on days 17–21 of pregnancy (birth occurred on day 22).⁹ Four pregnant control females received the vehicle, polyethylene glycol.

To insure against postnatal recovery in treated males, the prenatally FLT-treated pups received injections of FLT on PDs 1 and 2 at the same dose per body weight, while controls received equivalent vehicle injections. On day 3, FLT pups were cryogenically anaesthetized and surgically examined for the presence of testes. If testes were found they were removed, and the pup was

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labelled a male castrate via a small ink-injection in the paw. If no testes were found, the pup was labelled a female. Controls pups were also cryogenically anaesthetized, and received sham surgery. At this time litters were culled to four males and four females, or as close as the birth ratio allowed. All pups were warmed and returned to the nest following surgery.

Pups were weaned on day 21, and housed in like-treated litter pairs. At 110 days animals were perfused, and the brains removed and immersed in sucrose-formalin. Brains were sagittally sectioned at a thickness of 45 μm , mounted on slides in a gelatin medium, and stained with cresyl violet. The closest intact section to midline in the right hemisphere was chosen for each subject, and a drawing of the callosum was made using a projection scope at a magnification of $\times 23$. This drawing was retraced five times onto a digitizing tablet connected to a Macintosh Plus computer, and the software package Stereology was used to obtain measures of area, length, perimeter, and 99 widths taken along equally spaced percentiles of the longitudinal axis. These measures were averaged across the five tracings. The 99 width measures were grouped into factors as defined by prior factor analyses.⁴ These factors, starting at the anterior (genu) of the callosum, are widths 1–5 (W1–5), W6–17, W24–38, W46–57, W62–72, W79–95 and W96–99.

Materials

Flutamide was obtained from Schering Corporation, Bloomfield, NJ.

RESULTS

Since multiple littermates were present for each condition, values were pooled for each sex within each litter. This procedure avoided the contaminating effects of littermate correlations,³ and yielded one male and one female mean value for each control and FLT litter. These data are summarized in Table 1, along with tests of significance (all probabilities < 0.10 are reported).

Control male vs control female

Male and female sham values were compared within control litters as an independent test of our prior reports of callosal sex differences.^{1,4,5} Given the replicability of prior findings, a one-tailed test was used to measure significance of sex effects within controls. These results are shown in the sixth column of Table 1. Significant sex effects were found for callosal area, length, perimeter, brain weight, and W1–5. The sex difference in region W79–95 approached significance. The male values were larger for all measures. These findings are consistent with our earlier reports of strong sex differences in anterior and posterior callosal regions.^{1,4,5} The stability of these effects is particularly robust given that the current data were obtained with only four control litters.

Control male vs flutamide castrated male

Our hypothesis was that prenatal FLT would reduce the size of males' callosa when measured in adulthood. The statistical evaluation of this hypothesis is shown in the seventh column of Table 1 (two-tailed tests). Prenatal FLT treatment plus postnatal castration decreased all male callosal values as compared to control male values, but did not affect brain weight. This decrease was significant for area, length, perimeter, W1–5, and was marginal for W24–38 and W46–57 ($P < 0.10$).

Flutamide castrated male vs flutamide female

In contrast to the sex differences found in our control litters, female callosal values in FLT litters did not differ from castrated male values for most measures. A marginal sex effect was found only in the most posterior region, W96–99 ($P < 0.10$; two-tailed test), with FLT male castrates being larger than FLT females.

Brain weight

It is important to note that a significant sex difference was found for brain weight in FLT litters, with mean brain weight values of FLT male castrates and females being approximately the same as those of male and female controls, respectively. Thus FLT treatment eliminated sexual dimorphism in callosal size, but sexual dimorphism in brain weight was not affected.

Table 1. Callosal (mm) and brain (g) measured by group, with S.E.'s

Variable	Control sham male	Control sham female	FLT castrated male	FLT sham female (P)	Control male vs control female (P)	Control male vs FLT castrate male (P)	FLT castrate male vs FLT sham female
Area	3.603 (0.108)	3.120 (0.121)	3.272 (0.084)	3.225 (0.085)	<0.014	<0.041	-
Length	6.809 (0.100)	6.532 (0.127)	6.599 (0.029)	6.547 (0.072)	<0.023	<0.042	-
Perimeter	15.38 (0.188)	14.71 (0.234)	15.00 (0.040)	14.78 (0.138)	<0.004	<0.039	-
Brain wt	1.45 (0.017)	1.35 (0.029)	1.44 (0.012)	1.36 (0.030)	<0.015	-	<0.016
W1-5	0.818 (0.014)	0.727 (0.016)	0.765 (0.013)	0.805 (0.023)	<0.036	<0.029	-
W6-17	0.833 (0.013)	0.774 (0.033)	0.794 (0.031)	0.787 (0.024)	-	-	-
W24-38	0.507 (0.013)	0.457 (0.019)	0.459 (0.019)	0.466 (0.032)	-	<0.098	-
W46-57	0.388 (0.023)	0.340 (0.011)	0.343 (0.012)	0.365 (0.018)	-	<0.094	-
W62-72	0.319 (0.016)	0.302 (0.012)	0.311 (0.012)	0.301 (0.007)	-	-	-
W79-95	0.554 (0.026)	0.508 (0.010)	0.542 (0.016)	0.523 (0.010)	<0.075	-	-
W96-99	0.609 (0.011)	0.576 (0.020)	0.599 (0.013)	0.560 (0.010)	-	-	<0.096
N	4	4	6	6	4 pairs	10	6 pairs

CONCLUSION

Callosal values for FLT male castrates were significantly smaller than those of control males on a number of measures. This confirmed our hypothesis that development of the male callosum is influenced by testosterone. In a related study we found that ovarian hormones mediate callosal development in the female.⁶ Thus sex-typical gonadal steroids appear to influence callosal development in both sexes.

Although our procedure blocked the effects of both the pre- and postnatal testosterone surges, evidence from the literature suggests that the sensitive period for masculinization of the callosum probably starts prenatally. First, Zimmerberg and Scalzi showed that prenatal exposure to alcohol reduced the size of male rat callosa at 3 days of age.¹³ Second, several reports show that prenatal stress demasculinizes the typical cortical thickness pattern normally found in male rats.^{7,11} Third, it has been demonstrated that both prenatal stress¹² and prenatal alcohol exposure⁸ depress the testosterone surge that normally occurs on gestational day 18 in the male rat fetus.

When taken in combination with the fact that TP administered on PD 4 is sufficient to masculinize the female callosum,⁵ we conclude that the sensitive period for masculinization of the callosum via testosterone in the rat begins prenatally and extends at least through PD 4.

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