

# Sex Differences in Anterior Commissure Size in the Rat

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**ABSTRACT:** Earlier studies have shown that the corpus callosum of rats tends to be larger in males than in females. We report here that the anterior commissure of rats is also larger in males than in females. The sizes of the two commissures were positively correlated in both sexes, but significantly more so in females than in males. The anterior commissure size difference in rats reported here is opposite in direction from that reported elsewhere for humans, and we speculate that this may derive from differences in the relative proportions of the constituent fibers which make up the anterior commissure in the two species. © 1998 Elsevier Science Inc.

**KEY WORDS:** Anterior commissure, Corpus callosum, Sexual Dimorphism

## INTRODUCTION

In rats, the mean midsagittal area of the corpus callosum (CC) is greater in males than in females. This has been reported for Purdue-Wistar [4, 10] and Long-Evans [22, 26] rats, and the difference is seen at 3 days [27], 110 days [4, 10] and to a lesser extent at 215 days of age [4].

Whether there is a comparable size difference in the CC of humans has been a topic of some debate, with initial reports producing conflicting results [for reviews see 3, 5]. In some reports [e.g., 14], human females, not males, have shown greater callosal area; however, the difference between the sexes in CC area has most often proven to be statistically unreliable [e.g., 6, 7, 19]. It has also been argued that there may be a consistent difference in the shape of the callosum even if its overall size is comparable between the sexes. The posterior end of the callosum—the splenium—has been reported to be thicker and more bulbous in women than it is in men [3, 8]. However, in a recent meta-analysis of 49 studies which examined this issue, Bishop and Wahlsten [5] found (1) that the CC is larger in human males, not females, a finding attributable to the males' larger overall brain size; and (2) no reliable differences between the sexes in corpus callosum shape.

To date, less attention has been paid to the second largest inter-cerebral bundle, the anterior commissure (AC). The size of the AC in humans has been investigated in two studies by Allen and Gorski [1,2]. In both studies, the AC was found to be approximately 12 % larger in women than in men. We are aware of only one study which has examined sex differences in AC size in rats. Zimmerberg and Mickus [26] reported that the mean mid-sagittal area of the AC was very slightly greater in male rats, but not significantly so. However, this was based upon a relatively small number of subjects in their untreated control group.

We report here on two assessments of commissural size in rats based upon larger sample sizes. In both cases the investigations utilized brains which had been previously prepared in our laboratory for other purposes. We confirm the male-greater-than-female size difference for callosal area, and we find a similar male-greater-than-female difference for the anterior commissure.

## STUDY ONE (Pilot Investigation)

### *Materials and Methods*

The brains of 21 male and 20 female untreated Long-Evans (Blue-Spruce) rats which had been prepared previously as part of another project served as material in this investigation. At approximately 110 days of age (range 104-118), the rats had been perfused intracardially with a saline-formalin sequence. The whole brains had been post-fixed in 10% neutral buffered formalin for 7 days and then embedded in paraffin. They had been sliced coronally at 8 microns, every 20th section being preserved and stained with cresyl violet.

Because of the high inter-slice distance in these previously-prepared brains, the anterior commissure of each brain usually appeared in only 3-5 preserved sections; consequently, only an unrefined estimate of its size could be obtained. As a crude measure of anterior commissure size, its AP "length" (i.e., its extent along the anterior-posterior dimension) was computed as the total number of successive sections in which it was present, multiplied by 160 microns (the inter-slice distance). A comparable measure was also computed for the corpus callosum.

As a measure of gross brain volume, each stained section was projected onto a Houston Instruments digitizing tablet (working surface 30.6 cm sq, resolution 93.3 pixels/cm, magnification 14X). The perimeter of the entire brain was traced in each coronal slice in which any neocortical tissue was evident (i.e., for the entire anterior-posterior extent of the cerebral hemispheres). Total brain volume was computed as the sum across sections of the area inscribed corrected for magnification and multiplied by the distance between sections. All determinations of length and area were made in a process blind to the sex of the subjects. Twelve percent of our measures were repeated by a second blind observer and the measures obtained on the same tissue samples yielded a very high inter-rater reliability ( $r = .99$ ).

### Results

Our male and female subjects had nearly identical mean whole-brain volumes which did not differ significantly ( $F(1,41) = 0.02$ , ns). By contrast, anterior commissure length was significantly greater in males (male mean =  $0.63 \text{ mm} \pm 0.09 \text{ sd}$ ; female mean =  $0.55 \pm 0.10$ ;  $F(1, 41) = 7.58$ ,  $p = .009$ ). Corpus callosum AP length was slightly larger in males than females (male mean =  $5.39 \pm 0.52$ ; female mean =  $5.25 \pm 0.39$ ), but this difference was not statistically significant.

In an effort to determine if the sex difference in anterior commissure size was robust, we examined sagittal sections from a larger sample of brains in a second investigation.

## STUDY TWO

### Materials and Methods:

Tissue from eighty-three (42 male, 41 female) Blue-Spruce Long-Evans rats were examined in this investigation. The brains had been previously prepared as part of an anatomical study [21] in which 2 microliters of horseradish peroxidase (HRP) solution had been injected into varying locations in the neocortex two or three days before euthanasia. Male and female rats received identical injections. Upon microscopic examination, most of the brains showed small bundles of HRP-labeled axons crossing the midline within the corpus callosum. None showed evidence of HRP-labeling in the anterior commissure.

In the HRP study, at ages ranging from 69 to 175 days (mean 111.8), the subjects had been euthanized by  $\text{CO}_2$  inhalation, and perfused intracardially with isotonic saline followed by a glutaraldehyde/paraformaldehyde solution. The brains had been extracted, bisected along the mid-sagittal plane and blocked around the corpus callosum. The tissue blocks were post-fixed by immersion in the fixative solution for 6 hours. For cryoprotection, they were then immersed in 30% sucrose for 12 hours. Frozen 40-micron sagittal sections were taken serially from the uninjected hemisphere beginning at the midline. The first section in which the callosum was intact (i.e., the section closest to the midline in which the callosal stump was wholly represented) had been preserved and treated with

diaminobenzidine (DAB) before gelatin mounting on glass slides and counter-staining with cresyl violet.

For purposes of the present study, the sagittal section for each subject was projected onto paper (magnification 23X) and the perimeters of the corpus callosum and the anterior commissure were traced. These drawings were subsequently video digitized at a resolution of 300 dpi and the area within each perimeter was assessed utilizing the Image-Pro software package. In this study, we were not able to obtain an index of gross brain size from these previously-blocked tissue samples.

### Results

CC area was entered into a regression equation with sex, age and the interaction of those two factors as variables. All of these influences proved to be significant. The corpus callosum was significantly larger in males than in females ( $F(1,79) = 14.65$ ,  $p = 0.0003$ ); its area increased with age in both sexes ( $F(1,79) = 38.74$ ,  $p < 0.0001$ ); and the increase in size with age was both more consistent and at a greater rate in females than in males ( $F(1,79) = 9.44$ ,  $p = 0.003$ ). This can be perceived in Table 1 which presents mean male and female values tabulated into upper and lower age ranges, and in the correlation coefficients relating CC size with age ( $0.314$ ,  $p < .05$  for males;  $0.788$ ,  $p < .001$  for females).

TABLE 1  
MEAN COMMISSURE AREA ( $\text{MM}^2$ )  
BY SEX AND AGE (DAYS),  $\pm$  STANDARD DEVIATION

	Age	Male	Female
CC area	69-100	$3.81 \pm .35$	$3.26 \pm .22$
	101-175	$4.03 \pm .44$	$3.85 \pm .37$
	All ages	$3.97 \pm .42$	$3.50 \pm .41$
AC area	69-100	$0.34 \pm .02$	$0.30 \pm .04$
	101-175	$0.36 \pm .04$	$0.34 \pm .04$
	All ages	$0.36 \pm .04$	$0.32 \pm .04$

When AC area was separately entered into a comparable regression equation, similar results were obtained. Anterior commissure area was also significantly larger in males than females ( $F(1,79) = 8.33$ ,  $p = 0.005$ ); its area also increased with age ( $F(1,79) = 9.77$ ,  $p = 0.003$ ); and, here too, both the consistency and the rate of increase with age was greater in females than in males ( $F(1,79) = 4.15$ ,  $p = 0.045$ ). These results are also summarized in Table 1. The correlation coefficient relating age with AC size was  $0.142$  (ns) for males and  $0.453$  for females ( $p < 0.01$ ).

The sizes of the two commissures also proved to be highly correlated ( $r = 0.668$ ;  $p < .001$ ). Accordingly, we entered AC area into another regression equation with CC-area, gender, age, and all of their possible interactions as variables (entering main effects, 2-way interactions, and the 3-way interaction in step-wise fashion). In this analysis, the 3-way interaction was not significant and its contribution was therefore dropped from the equation. Only sex and the

sex-by-CC area interaction terms were significant ( $F(1,76) = 4.50$ ,  $p = 0.04$ ; and  $F(1,76) = 4.89$ ,  $p = 0.03$ , respectively). These effects can be seen in the correlation coefficients relating AC size with CC size. They were significant in both sexes, but the relationship was weaker in males ( $r = 0.434$ ,  $p < 0.01$ ) than in females ( $r = 0.690$ ,  $p < 0.01$ ).

## DISCUSSION

The absence of sex differences in gross brain volume found in Study 1 is compatible with similar findings reported in some studies of this type [12, 13] but not in others [4, 9, 10, 26]. In the face of this absence of differences in overall size, we used actual values for commissure length rather than proportional ones.

The work presented here in Study Two confirms a size difference between the sexes for corpus callosum area which has been presented in a number of earlier reports [4, 10, 22, 26]. There is also precedent for our findings regarding changes in callosal area with age, and for the interaction between age and sex. Our data show that callosal size increases between 69 and 175 days of age, and that the rate of increase is greater for females than for males. Juraska and Kopik [18] reported a similar pattern of increase in CC area with age, and Berrebi et al [4] report a similar interaction between sex and age.

The principal new contributions we make in this report is the documentation of a significant sex difference in the anterior commissure, and that AC size shows the influence of a similar sex by age interaction. This finding closely parallels the differences which exist for the corpus callosum, and suggests that greater interhemispheric connectivity in males compared to females may be a general theme in the rat and not limited to callosal connections [however see 26]. It has been shown that perinatal testosterone exposure results in thicker corpora callosa of greater area compared to untreated control subjects [10]. The parallel sex difference found here for the anterior commissure suggests that it may be similarly sensitive to androgens during development.

We cannot yet say whether the size differences we report here are associated with increases in the number of crossing fibers [cf. 20]. However, it is tempting to speculate that sex differences in the anterior commissure may derive from differences in one or more of the hemispheric structures which project across the midline via this pathway. It is noteworthy that size differences between the sexes have already been documented for the bed nucleus of the stria terminalis and the medial amygdaloid nucleus [13], both of which appear to send contralateral projections through the anterior commissure [25].

The fact that anterior commissure size is correlated with corpus callosum size in our sample is not surprising. Allometrically, larger brains would be expected to have larger components generally. Of more interest is the fact that the relationships of AC size with both age and CC size was much stronger in females than in males. Female rats with large CCs had a high likelihood of also having a large AC. Males tended to be larger on average than females for both commissures, but the degree to which the size of one

commissure could be predicted from the other was considerably lower for males. If the correlation between the sizes of the two commissures reflects a generalized allometric factor, it appears that this influence is more clearly expressed in females. In males, it appears that androgens (or other developmental influences) promote greater size in both commissures, but they appear to influence the two commissures quite variably. This suggests that other, as yet unknown, factors play much more important roles in determining commissure size in males than in females.

It is difficult to reconcile our findings with those reported for humans [1, 2]. In the human, the AC area has been reported to be approximately 12% larger in *females*. In our Study Two, we found the AC to be about 12% larger in *males*. It has been argued elsewhere that there has been considerable evolutionary divergence between humans and rodents in the organization and constituent fibers of the anterior commissure [17]. However, it is not yet clear to what degree differences in the make-up of the AC between the species should be best viewed as qualitative or quantitative in nature. In both primates and rodents, the AC interconnects the olfactory complex, the bed nucleus of the stria terminalis, and the amygdaloid complex [11, 16, 17, 23, 24]. Additionally, in the primate there are extensive interconnections of neocortical regions in the inferior temporal lobes [11, 16, 23], as well as projections that extend to, and may involve, perirhinal cortex [23]. In the rat, it has been established that the AC interconnects piriform cortex along the rhinal sulcus [17], and Horel and Stelzner [15] have demonstrated that the AC of the rat also includes extensive projections from lateral neocortical areas which lie above the rhinal fissure—areas which may be homologues of the primate temporal regions. Thus, at a qualitative level, it might be argued that the AC is comprised of fibers originating in the same basic structures in both taxonomic groups. Quantitatively, however, the picture is quite different. The largest contribution to the primate AC is made by its posterior limb, which carries the neocortical projections, whereas the largest contribution in the rodent AC comes through the anterior limb, which is comprised for the most part of olfactory connections. It is possible that these differences will ultimately account in some way for the divergence between the rat and human in the direction of AC size differences. For both species, it will be interesting to examine the size of the anterior and posterior limbs of the AC separately in order to determine the degree to which the sex differences can be attributed to one or both of these branches.

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## REFERENCES

1. Allen, L.S.; Gorski, R.A. Sexual dimorphism of the anterior commissure and massa intermedia of the human brain. *J. Comp. Neurol.* 312: 97-104; 1991.
2. Allen, L.S.; Gorski, R.A. Sexual orientation and the size of the anterior commissure in the human brain. *Proc. Nat. Acad. Sci.* 89: 7199-7202; 1992.
3. Allen, L.S.; Richey, M.F.; Chai, Y.M.; Gorski, R.A. Sex differences in the corpus callosum of the living human being. *J. Neuroscience* 11: 933-942; 1991.
4. Berrebi, A.S.; Fitch, R.H.; Ralphe, D.L.; Denenberg, J.O.; Friedrich, V.L.; Denenberg, V.H. Corpus callosum: Region-specific effects of sex, early experience and age. *Brain Res.* 438: 216-224; 1988.
5. Bishop, K.M.; Wahlsten, D. Sex differences in the human corpus callosum: Myth or reality? *Neurosci. Biobehav. Rev.*, in press.
6. Byne, W.; Bleier, R.; Houston, L. Variations in human corpus callosum do not predict gender: A study using magnetic resonance imaging. *Behav. Neuroscience* 102: 222-227; 1988.
7. Constant, D.; Ruther, H. Sexual dimorphism in the human corpus callosum? A comparison of methodologies. *Brain Res.* 727: 99-106; 1996.
8. de Lacoste-Utamsing; Holloway, R.L. Sexual dimorphism in the human corpus callosum. *Science* 216: 1431-1432; 1982.
9. Denenberg, V.H.; Fitch, R.H.; Schrott, L.M.; Cowell, P.E.; Waters, N.S. Corpus callosum: Interactive effects of infantile handling and testosterone in the rat. *Behavioral Neuroscience* 105: 562-566; 1991.
10. Fitch, R.H.; Berrebi, A.S.; Cowell, P.E.; Schrott, L.M.; Denenberg, V.H. Corpus callosum: Effects of neocortical hormones on sexual dimorphism in the rat. *Brain Res.* 515: 111-116; 1990.
11. Fox, C.A.; Fisher, R.R.; Desalva, S.J. The distribution of the anterior commissure in the monkey (*Macaca mulatta*). *J. Comp. Neurol.* 89: 245-269; 1948.
12. Gorski, R.A.; Harlan, R.E.; Jacobson, C.D.; Shryne, J.E.; Southam, A.M. Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. *J. Comp. Neurol.* 193: 529-539; 1980.
13. Hines, M.; Allen, L.S.; Gorski, R.A. Sex differences in subregions of the medial nucleus of the amygdala and the bed nucleus of the stria terminalis of the rat. *Brain Res.* 579: 321-326; 1992.
14. Holloway, R.L.; deLacoste, M.C. Sexual dimorphism in the human corpus callosum: An extension and replication study. *Human Neurobiol.* 5: 87-91; 1986.
15. Horel, J.A.; Stelzner, D.J. Neocortical projections of the rat anterior commissure. *Brain Res.* 220: 1-12; 1981.
16. Jouandet, M.L.; Gazzaniga, M.S. Cortical field of origin of the anterior commissure of the rhesus monkey. *Exp. Neurol.* 66: 381-397; 1979.
17. Jouandet, M.L.; Hartensein, V. Basal telencephalic origins of the anterior commissure of the rat. *Exp. Brain Res.* 50: 183-192; 1983.
18. Juraska, J.M.; Kopicik, J.R. Sex and environmental influenced on the size and ultrastructure of the rat corpus callosum. *Brain Res.* 450: 1-8; 1988.
19. Kertesz, A.; Polk, M.; Howell, J.; Black, S.E. Cerebral dominance, sex, and callosal size in MRI. *Neurol.* 37: 1385-1388; 1987.
20. LaMantia, A.S.; Rakic, P. Cytological and quantitative characteristics of four cerebral commissures in the rhesus monkey. *J. Comp. Neurol.* 291: 520-537; 1990.
21. Noonan, M.; Sanfilippo, M.A.; Chmiel, D.J.; Smith, M.A. Mapping the midsagittal corpus callosum in the rat: Topographical correspondence of callosal regions with cortical subdivisions. *Soc. Neurosci. Abs.* 22: 675; 1996.
22. Nunez, J.L.; Kim, J.H.Y.; Juraska, J.M. The gross size of the rat corpus callosum: Sex differences, hormones and possible correlates with axon number. *Soc. Neurosci. Abs.* 21: 1065; 1995.
23. Pandya, D.M.; Edwardo, A.K.; Lele, P.P. The distribution of the anterior commissure in the squirrel monkey. *Brain Res.* 49: 177-180; 1973.
24. Turner, B.H.; Mishkin, M.; Knapp, M.E. Distribution of the anterior commissure to the amygdaloid complex in the monkey. *Brain Res.* 162: 331-337; 1979.
25. Yajeya, J.; Criado-Gutierrez, J.; Fernandez-de-Molina, A. Unitary activity in the bed nucleus of the stria terminalis after homo- and contralateral stria terminalis stimulation. *Acta Physiol. Scand.* 135: 241-248; 1989.
26. Zimmerberg, B.; Mickus, L.A. Sex differences in corpus callosum: Influence of prenatal alcohol exposure and maternal undernutrition. *Brain Res.* 537: 115-122; 1990.
27. Zimmerberg, B.; Scalzi, L.V. Commissural size in neonatal rats: Effects of sex and prenatal alcohol exposure. *Intl J. Dev. Neurosci.* 7: 81-86; 1989.