Sex and species differences in mouse and rat forebrain commissures depend on the method of adjusting for brain size

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Abstract:
Sex differences in the forebrain commissures (corpus callosum, hippocampal commissure, and anterior commissure) were examined in B6D2F$_2$ hybrid mice and Sprague–Dawley rats. Twenty-four male–female littermate pairs of mice were perfused at each of 21, 42 and 63 days of age and the midsagittal area of the commissures was measured from en bloc stained tissue. Twenty-two male–female littermate pairs of rats were examined at 110 days of age using the same methods. Male mice had larger bodies than females but no sex differences were found for mouse brain weight or commissure areas. In contrast, a significant sex difference was found for rat body, brain, corpus callosum and hippocampal commissure sizes. Four methods were used to adjust for differences in brain size (ratio, geometric, linear regression, and allometric). When the two species were analysed separately, neither mice nor rats showed significant sex differences in commissure areas relative to brain size if regression or allometric adjustments were made. Even when data from mice and rats were combined into one large group with a wide range of values, no species or sex differences were apparent after adjustments were made for brain size with either the regression or allometric methods. The use of ratios to adjust for differences in overall size is not recommended, especially because this method does not effectively remove the influence of brain size from commissure size; a substantial correlation is often present between the ratio and brain size.
Keywords: Corpus callosum; Hippocampal commissure; Anterior commissure; Cerebral cortex; Allometry; Morphometry

Article:

1. Introduction

The cerebral hemispheres of the brains of placental mammals are connected by three main commissural pathways: the corpus callosum (CC), hippocampal commissure (HC) and anterior commissure (AC). The corpus callosum and anterior commissure connect primarily homotopic regions of the neocortex while the hippocampal commissure connects the radiate parts of the hippocampus in rodents [23]. Controversy regarding whether or not there are sex differences in the degree of connectivity between the hemispheres was sparked by a 1982 report by de Lacoste-Utamsing and Holloway [6] which stated that, in humans, the posterior portion of the corpus callosum, the splenium, was both considerably larger and more bulbous in females than in males and that this neuroanatomical sex difference may be related to gender differences in cognitive functioning.

Since 1982, at least 48 studies have been published on sex differences in the human corpus callosum; these have been reviewed in two meta-analyses of effect sizes [3,9]. Effect size (*d*) compares the difference between group means to the standard deviation (SD) within a group. Brain size and corpus callosum area are clearly larger in men than in women (*d* = 1.20 and 0.21, respectively; [3]), whereas splenial area shows almost no difference (*d* = 0.04 on the basis of 29 studies).

In rodents, corpus callosum area tends to be consider-ably larger in male than female Purdue–Wistar [2,7, 11, 12,21], Sprague–Dawley [22], and Long–Evans hooded rats [24,41], even at three days of age [42], although a

few studies w19,20x have not found a significant sex difference in CC size. Zimmerberg and Mickus [41] did not find a significant sex difference in anterior commissure (AC) size in Long–Evans hooded rats but Noonan et al. [24] reported that the anterior commissure was 12.5% larger in adult male than female rats. For mice, on the other hand, several strains have no sex difference in CC area [33,35].

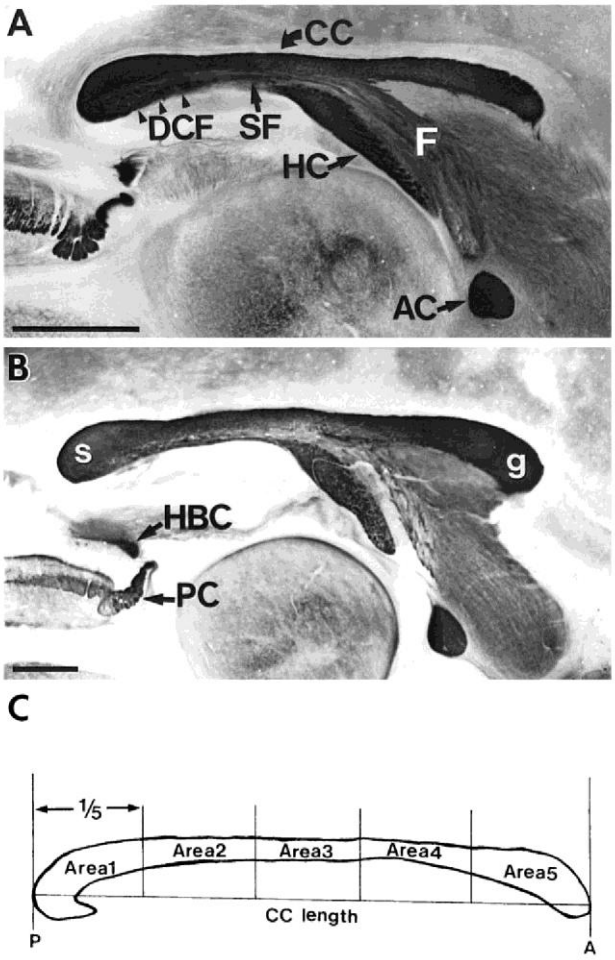

Fig. 1. Midsagittal sections showing myelinated fibre tracts. The corpus callosum (CC), splenium of the corpus callosum (s), genu of the corpus callosum (g), hippocampal commissure (HC), anterior commissure (AC), fornix (F), dorsal commissure of the fornix (DCF), superior fornix (SF), posterior commissure (PC), and habenular commissure (HBC) are labelled. (A) An adult female B6D2F_2 mouse, 63 days old, brain weight = 0.412 g. (B) An adult male Sprague–Dawley rat, 110 days old, brain weight = 2.215 g. (C) Diagram of the CC at the midsagittal plane of a rat brain, showing the definitions of maximum anterior–posterior length of the CC and subdivision of the CC into fifths. Area = splenium; Area 5 = genu; P = posterior; A = anterior. This tracing does not closely resemble the photographs because it does not include the adjacent DCF, F or SF. Scale bars = 1.0 mm.

Although studies of humans and rats frequently find a larger corpus callosum in males than in females, some researchers contend that it is the relative size of the CC in relation to brain size that is larger in females w16x. There is a substantial sex difference in brain size in both humans [15] and rats [39], with males having larger brains on average than females. The corpus callosum connects widely distributed areas of the cerebral cortex and therefore might be expected to be larger in subjects with larger cortices and larger brains. Growth factors that increase the number of neurons, axons and the degree of myelination might be expected to increase both overall brain size and the cross-sectional area of the corpus callosum. Thus, differences in commissure sizes between groups may be a manifestation of differences in overall size rather than a consequence of some fundamental difference in the commissures specifically due to sex. By statistically adjusting the samples for overall brain size, this question may be addressed. However, considerable disagreement exists regarding when and how to take account of overall size [13].

Four methods of making the adjustment are prevalent. Suppose X is brain size, Y is commissure size, and YX is commissure size adjusted for brain size. (1) A simple ratio of commissure cross-sectional area to brain weight or volume ($Y' = Y/X$) is commonly reported. A ratio may be appropriate when the relation between variables is isometric, being linear and having a Y-intercept of zero [25], but this does not hold for commissures vs. brain size. Inappropriate use of a ratio can artifactually create differences or mask real ones, and often it does not completely remove the correlation with variable X [8,25,26,29]. (2) A geometric adjustment notes that volume increases as the cube of the linear measure, whereas cross-sectional area, the usual metric for commissure size, increases as its square. Geometric adjustment is made when commissure area is divided by (brain weight)$^{2/3}$, or $Y' = Y/(X^{2/3})$. Unfortunately, this method inspired by geometry is nevertheless a ratio and suffers from the faults of a simple ratio (Y/X). (3) The regression method takes into account the actual degree of linear relation between two variables in the data set. Thus, if there is little or no relation, this method makes little or no adjustment in raw commissure size. Suppose the slope is b, such that area Y tends to increase b units for every unit increase in brain size X. If the mean brain size or a value near the middle of the distribution is M, the adjustment is made by deducting points from the commissure area of a relatively large brain and adding points for a small brain using the equation $Y' = Y - b(X - M)$. If all groups are assigned the same slope, this is equivalent to an analysis of covariance, but multiple regression analysis can take into account different slopes for different groups. (4) In most organisms the size of a part has an allometric relation with the whole [14,17], and the coefficients of the allometric equation Y s aX^b are best estimated from the data after a log transformation: $\log(Y) = \log(a) + b \log(X)$. Allometric adjustment for brain size is done using $\log(Y') = \log(Y) – b[\log(X) - M]$, where M is the mean of the $\log(X)$ values, and then the antilog is taken to obtain Y'. When the range of X and Y values is not large, a simple linear equation may fit the data as well as a power function [32], even though the relation over a wider range certainly is not linear [18].

In this study, we compare forebrain commissure sizes of males and females as well as rats and mice examined with the same histological procedures, and we apply all four methods of statistical adjustment to the data. We ask whether sex and species differences in commissure sizes persist after making appropriate adjustments for brain size and whether the method of adjustment itself makes an appreciable difference in the results. By including two rodent species in the same analysis, the range of values is dramatically extended, which should provide a more stringent evaluation of alternative methods.

Table 1
Mean (SD) body, brain and commissural sizes for mice and rats

Species	Age (days)	Sex	N	Body (g)	Brain (g)	CC (mm^2)	HC (mm^2)	AC (mm^2)
Mouse	21	M	24	10.87 (1.64)	0.402 (0.025)	0.605 (0.074)	0.242 (0.032)	0.102 (0.012)
Mouse	21	F	24	10.98 (1.38)	0.398 (0.021)	0.613 (0.056)	0.230 (0.030)	0.103 (0.011)
Mouse	42	M	24	22.22 (2.26)	0.418 (0.018)	0.777 (0.047)	0.282 (019)	0.121 (0.009)
Mouse	42	F	24	17.97 (1.60)	0.418 (0.020)	0.769 (0.056)	0.286 (0.025)	0.122 (0.013)
Mouse	63	M	24	25.77 (2.59)	0.432 (0.021)	0.815 (0.058)	0.308 (0.026)	0.127 (0.012)
Mouse	63	F	24	20.28 (1.58)	0.431 (0.016)	0.797 (0.059)	0.304 (0.035)	0.125 (0.013)
Rat	110	M	22	462.0 (47.2)	2.209 (.074)	3.403 (0.263)	0.790 (0.091)	0.325 (0.028)
Rat	110	F	22	217.4 (35.9)	2.076 (0.079)	3.116 (0.179)	0.735 (0.046)	0.319 (0.027)

2. Materials and methods

2. 1. Subjects

B6D2F$_1$/CrlBR mice (C57BL/6NCrlBR female × DBA/2NCrlBR male) were purchased from Charles River Laboratories (Quebec, Canada), raised until 8 weeks of age in our laboratory and then mated to produce the B6D2F$_2$ hybrid mice, which have substantial genetic variation w1x. One male and three female mice were mated in a standard plastic mouse cage (29 × 18 × 13 cm), and each female was isolated when visibly pregnant. At mating and throughout pregnancy and lactation the parent mice were fed freely with high (1 1%) fat PMI Breeder Blox 5015 and given free access to Edmonton tap water. The cage contained Aspen Chip bedding and one compacted paper ‘nestlet’ for nest construction. The pups were weaned and housed in groups of same-sex littermates at 20–22 days after birth. All weaned mice received a normal (4.5%) fat diet (PMI Rodent Blox 5001), and they were housed in a temperature-controlled room with a 12 h light–12 h dark cycle (lights on at 6 h). Six mice from each F$_2$ litter, consisting of one male–female littermate pair of mice at 21, 42, and 63 days of age, were randomly chosen for perfusion. Thus, F$_2$ litters that did not have at least three females and three males were not used.

Outbred Sprague–Dawley rats were obtained from the Biological Sciences Animal Services at the University of Alberta. One male was mated with five females and then the females were isolated before birth of the litter. Offspring were weaned at 21 days after birth, ear marked to identify littermates, and then housed in groups of eight to 10 rats of the same sex. Rats were fed PMI Rodent Blox 5001 at all times. Twenty-two male–female littermate pairs of rats were perfused at 1 10 days of age (comparable with previous studies).

2.2. Histological procedures

At the appropriate age, mice and rats were weighed, anaesthetized with an overdose of pentobarbital (mice) or chloral hydrate (rats) and perfused intracardially with saline vascular rinse followed by neutral 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were extracted from the skull and placed in fresh fixative for one week. At this time, the brains were coded to conceal sex and age. Brains were then blocked to a standard configuration [34[, blotted, weighed and bisected sagittally. The left half of each brain was stained en bloc using gold chloride [31].

2.3. Measurements

All measurements were made at 40 × from tracings of the en bloc midsagittal section of each brain (Fig. 1) using the Sigma-Scan digitizer system from Jandel Scientific (v. 3.10). Areas of the corpus callosum, hippocampal commissure, and anterior commissure were measured from each tracing. Care was taken not to include the dorsal commissure of the fornix, the superior fornix, or the longitudinal striae in the corpus callosum area in each tracing. The maximum anterior–posterior length of the corpus callosum was measured and then the corpus callosum was divided into fifths (Area 1 to Area 5) by drawing lines perpendicular to the maximum length at equal distances along its length (Fig. 1). The area of each fifth was measured. Measurement reliability for the corpus callosum was 0.98, calculated from repeated measurement of ten randomly chosen tracings by two different investigators.

Table 2
Significant effects in repeated measures analysis of variance[a]

Species	Variable	Effect	F	p	Estimated ω^2
Mouse	Body	Age	624.1	< 0.000001	0.777
		Sex	127.8	< 0.000001	0.069
		Age * Sex	34.8	< 0.000001	0.003
Mouse	Brain	Age	39.2	< 0.000001	0.287
Mouse	CCA	Age	155.5	< 0.000001	0.681
Mouse	HCA	Age	94.6	< 0.000001	0.512
Mouse	ACA	Age	63.9	< 0.000001	0.440
Rat	Body	Sex	381.9	< 0.000001	0.895
Rat	Brain	Sex	46.1	0.000001	0.407
Rat	CCA	Sex	33.7	0.000009	0.277
Rat	HCA	Sex	10.8	0.004	0.110

[a]The criteria for significance used were $\alpha = 0.003$ for mice and $\alpha = 0.01$ for rats. For tests of age, sex and age * sex effects for mice, $df = 46$, 23, and 46, respectively. For tests of sex effects for rats, $df = 21$. Sex and interaction effects deemed not significant are not shown in the table. Estimated ω^2 values are partial effect sizes calculated without removing litter effects from the total variance.

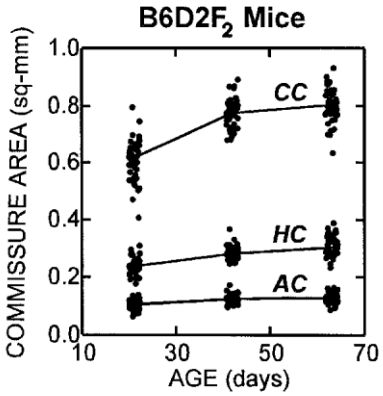

Fig. 2. Commissure area (mm^2) vs. age in days for the B6D2F$_2$ hybrid mouse corpus callosum (CC), hippocampal commissure (HC), and anterior commissure (AC) showing the line of average change for each growth period. Data have been 'jittered' with SYSTAT to reveal overlapping points.

3. Results

3.1. Body, brain and commissure sizes

The means and standard deviations of body, brain and commissure sizes are presented in Table 1. Age and sex effects were assessed using repeated measures analysis of variance, and statistically significant effects are shown in Table 2. Using the Bonferonni procedure to control the Type I error rate at $\alpha = 0.05$ for one analysis, only those effects with $p < 0.003$ for mice (15 tests) and $p < 0.01$ for rats (5 tests) were judged to be significant. Significant sex differences were found in rat body, brain, CC and HC sizes but not in AC size ($F = 0.81$, $p = 0.38$). The sizes of the sex differences in rats were very large ($d = 5.89$, 1.74, 1.30, 0.80 for body, brain, CC and HC size, respectively, when between-litter variance is included in the within-sex variance). In contrast, only body size in mice showed a significant sex effect ($d = 1.74$). However, there was a significant age trend for all measurements in mice. Examination of the age group means for body weight suggests that there is a sex

difference in body size in older mice (42 and 63 days) that is not yet evident in weanlings (21 days). No other measurements showed a sex by age interaction, indicating that the lack of a sex difference persisted throughout the three ages studied. Fig. 2 illustrates that the commissures increased in size most from 21 to 42 days and less so from 42 to 63 days of age.

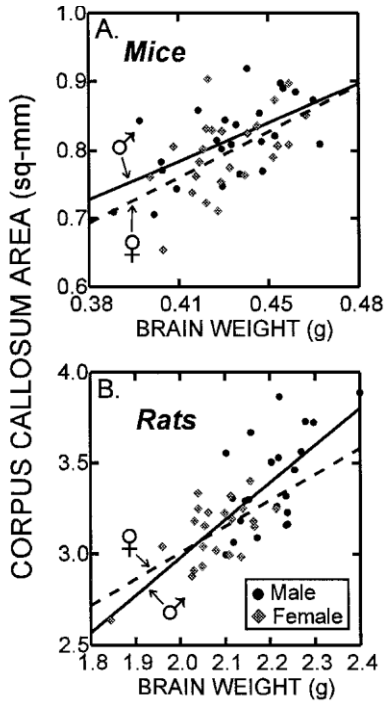

Fig. 3. Brain weight (g) vs. cross-sectional area of the corpus callosum (mm^2) at the midsagittal plane for males (circles) and females (diamonds). (A) Data from 63-day-old mice showing the line of best fit for males ($Y = 0.086 + 1.69X$; $r^2 = 0.29$) and females ($Y = -0.070 + 2.01X$; $r^2 = 0.36$). (B) Data from rats showing the line of best fit for males ($Y = -1.13 + 2.06X$; $r^2 = 0.40$) and females ($Y = 0.138 + 1.43X$; $r^2 = 0.34$).

Table 3
Mean (SD) corpus callosum subdivision sizes (mm^2) for mice and rats[a]

Species	Age (days)	Sex	*N*	Area 1	Area 2	Area 3	Area 4	Area 5
Mouse	21	M	24	0.174 (0.018)	0.091 (0.018)	0.080 (0.013)	0.106 (0.017)	0.156 (0.021)
Mouse	21	F	24	0.178 (0.017)	0.091 (0.011)	0.082 (0.007)	0.103 (0.013)	0.154 (0.020)
Mouse	42	M	24	0.219 (0.021)	0.113 (0.010)	0.100 (0.010)	0.137 (0.013)	0.200 (0.013)
Mouse	42	F	24	0.213 (0.018)	0.114 (0.013)	0.100 (0.012)	0.141 (0.014)	0.197 (0.018)
Mouse	63	M	24	0.223 (0.023)	0.123 (0.015)	0.103 (0.011)	0.140 (0.016)	0.220 (0.017)
Mouse	63	F	24	0.222 (0.021)	0.120 (0.012)	0.101 (0.009)	0.138 (0.015)	0.214 (0.020)
Rat	110	M	22	0.779 (0.073)	0.423 (0.035)	0.499 (0.055)	0.642 (0.068)	1.060 (0.090)
Rat	110	F	22	0.724 (0.050)	0.409 (0.062)	0.459 (0.040)	0.583 (0.029)	0.950 (0.063)

[a]Splenium is Area 1; genu is Area 5.

Table 4
Correlation (Pearson r, sexes pooled) between brain weight and adjusted commissure sizes after four methods of adjustment for brain size

Variable	Ratio	Geometric	Regression	Allometric	Unadjusted
A. 63-day-old mice					
CC Area	−0.020	0.208	0.000	0.000	0.562**
HC Area	0.054	0.212	0.000	0.004	0.473**
AC Area	0.219	0.374**	−0.001	−0.002	0.604**
B. 110-day-old rats					
CC Area	0.221	0.454**	0.000	0.002	0.729**
HC Area	−0.149	0.025	0.001	0.011	0.349*
AC Area	−0.308*	−0.128	−0.001	0.002	0.250

** $p < 0.01$.
* $p < 0.05$.

3.2. Subregions of the corpus callosum

The mean and standard deviations for the fifths of the corpus callosum (Area 1 to Area 5) are presented in Table 3. A repeated measures analysis of sex and age effects for the mouse callosal subdivisions replicated the results found for CC area as a whole: no significant effects of sex were evident, all subdivisions increased significantly with in-creasing age (p values < 0.001), and no sex by age interactions were indicated. Significant sex differences were found for all rat callosal subdivisions (p values < 0.001; male > female), except the fifth adjacent to the splenium (p = 0.324). Therefore, analysis of the size of subdivisions (fifths) of the corpus callosum for the most part paralleled the effects found for callosal size as a whole. An analysis of the bulbosity of the splenium (Area1/CC area) found no sex difference in mice or rats (p values > 0.05).

In both species, brain size was significantly correlated with commissure areas (63-day mice: Pearson r values= 0.562, 0.473, 0.604; rats: Pearson r values = 0.729, 0.349, 0.250 for CC, HC and AC size, respectively). A plot of CC size against brain size for mice and rats (Fig. 3) reveals that this relationship was substantial for both species.

3.3. Mouse commissure sizes adjusted for brain size

Commissure areas for 63-day-old mice were adjusted for differences in brain size using the four methods described in the introduction. Because the slopes for the regression equation predicting commissure area from brain size did not differ significantly between the sexes, average slopes of b = 1.813, 0.799, and 0.412 were used for CC, HC and AC area, respectively. The allometric method adjusted the mouse commissure areas to a brain weight of X = 0.45 g using the equation: $\log(Y') = \log(Y) - b * [\log(X) - \log(0.45)]$. The use of averaged allometric coefficients was also justified (b = 0.985, 1.087, and 1.429 for CC, HC and AC area, respectively).

No significant sex differences were found in relative mouse commissure sizes using any of these methods (paired t-tests, all p values > 0.05). If an adjustment method adequately removes the variability due to influences of overall brain size, there should be little or no correlation between the adjusted commissure size and brain weight. As can be seen in Table 4A, all of the adjustment methods reduced the correlation, but the correlation coefficients were generally close to zero only for the linear regression and allometric methods. The correlation for AC area adjusted using the geometric method even achieved statistical significance (p < 0.01).

3.4. Rat commissure sizes adjusted for brain size

Rat commissure sizes were adjusted using the same four methods. They were adjusted to a brain weight of X = 2.0 g using the regression equation: $Y' = Y - b * (X - 2.0)$, where b = 1.937, 0.266, 0.068 for CC, HC and AC areas, respectively. The equation: $\log(Y') = \log(Y) - b * [\log(X) - \log(2.0)]$ (where b = 1.259, 0.677, 0.433 for CC, HC and AC area, respectively) was used for the allometric adjustment.

A significant correlation was found between brain size and CC size adjusted using the geometric method (Table 4B; p < 0.01). The linear regression and allometric methods were more successful in removing the variance due to overall brain size effects (all p values > 0.05). No significant sex differences were found in rat relative commissure sizes (t-tests, p values > 0.05) using the ratio, linear regression and allometric methods. However, when the geometric method was used to adjust CC size for brain weight, a significant difference between males and females was found (p = 0.009).

Table 5
Mean (S.D.) adult commissure sizes after four adjustments for brain size when species and sexes are pooled

Species	Commissure	Ratio	Geometric	Regression	Allometric
Rat (N = 44)	CC	1.522 (0.086)	1.961 (0.122)	1.619 (0.188)	1.676 (0.098)
	HC	0.357 (0.034)	0.459 (0.043)	0.458 (0.072)	0.494 (0.046)
	AC	0.151 (0.013)	0.194 (0.016)	0.192 (0.027)	0.206 (0.017)
Mouse (N = 48)	CC	1.867 (0.113)	1.411 (0.087)	1.624 (0.049)	1.678 (0.102)
	HC	0.709 (0.062)	0.536 (0.048)	0.458 (0.029)	0.495 (0.044)
	AC	0.292 (0.023)	0.221 (0.019)	0.191 (0.011)	0.207 (0.018)

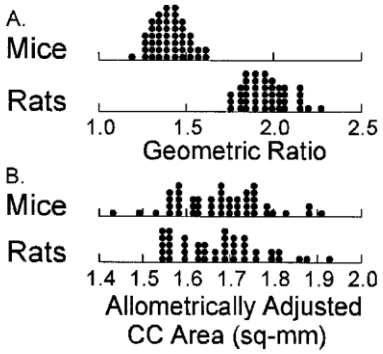

Fig. 4. Distribution of adjusted midsagittal area of the CC (mm^2) for 63-day old mice and 110-day old rats. (A) Geometric method for adjustment of CC size. (B) Allometric method for adjustment of CC size. See also Table 5.

3.5. Comparison of rat and mouse adjusted commissure sizes

In order to determine whether the forebrain commissures of rats and mice differ relative to brain size, data for both species were combined, which greatly extended the range of values. Rat and mouse commissures were adjusted for differences in brain size and then sex, species and sex by species influences were tested using multiple regression. Rat and mouse commissures were adjusted to a common brain size of X= 1.0 g using the linear equation: $Y' = Y - b * (X - 1.0)$, (where b = 1.439, 0.267, 0.114 for CC, HC and AC area, respectively) and the allometric equation: $\log(Y') = \log(Y) - b * [\log(X) - \log(1.0)]$, (where b = 0.873, 0.571, 0.587 for CC, HC and AC area, respectively). Means and standard deviations of adjusted commissure sizes are given in Table 5.

Table 6
Comparison of commissure sizes in combined data for rats and mice adjusted for brain size with four different methods

(A) Statistical significance of sex and species differences (p values)

Variable	Effect	Ratio	Geometric	Regression	Allometric	Unadjusted
CC Area	Sex	0.049	0.004	0.033	0.021	0.000
	Species	0.000	0.000	0.857	0.955	0.000
	Sex * Species	0.996	0.147	0.134	0.647	0.000
HC Area	Sex	0.504	0.247	0.267	0.188	0.010
	Species	0.000	0.000	0.960	0.971	0.000
	Sex * Species	0.763	0.730	0.471	0.680	0.029
AC Area	Sex	0.728	0.839	0.402	0.901	0.378
	Species	0.000	0.000	0.767	0.952	0.000
	Sex * Species	0.228	0.355	0.231	0.401	0.604

(B) Correlation with brain weight (Pearson r, sexes and species pooled)

Variable	Ratio	Geometric	Regression	Allometric	Unadjusted
CC Area	−0.856*	0.943*	0.002	0.012	0.994*
HC Area	−0.960*	−0.640*	0.007	0.002	0.974*
AC Area	−0.965*	−0.613*	0.021	−0.006	0.980*

* $p < 0.01$.

With the ratio and geometric methods, the adjusted commissure areas remained strongly correlated with brain size (Table 6B), but the regression and allometric methods were both effective in removing the variance in commissure sizes due to overall brain size (p values > 0.05). With the regression and allometric methods, no effects on commissure areas due to species or sex were found (Table 6A; all $p > 0.015$). Adjustment using the ratio and geometric methods resulted in significant effects of species for all three commissure areas and a significant sex difference in CC area adjusted using the geometric method. Fig. 4 compares relative corpus callosum size for mice and rats adjusted using the (A) geometric and (B) allometric methods. When the proper adjustment is made for overall size effects, no sex or species differences are found in commissure areas of mice and rats. This indicates that, in general, differences in commissure sizes across these two species and the sexes can be attributed to factors that influence the size of the brain and its constituent parts rather than factors that influence the commissures specifically. Improper adjustment for overall size using ratios does not fully remove the variance due to overall size and, in some cases, indicates effects that do not exist when the proper

adjustment has been made. Only the allometric method eliminated the large difference between rats and mice in variance in commissure sizes (Table 5).

3.6. Subregions adjusted for brain size

No method of adjustment for brain size created a significant sex difference in any subregion in mice. In rats, the sex differences (Table 3) for area 1 (splenium), area 3 (truncus) and area 4 were eliminated by all methods of transformation, and no method created a significant sex difference in area 2 adjacent to the splenium. Linear regression eliminated the sex difference in the genu (area 5) of rats but the ratio and geometric methods did not. When data for rats and mice were combined, ratio and geometric adjustments but not linear regression retained the very large species differences but not sex differences for all but the genu. However, the sex difference in the genu (area 5) was significant with the ratio ($p = 0.01$), geometric ($p = 0.0005$), linear regression ($p = 0.004$), and allometric ($p = 0.008$) methods. This finding suggests that there may be a sex difference in the genu independent of variation arising purely from brain size effects when a wide range of values are considered.

4. Discussion

This study examined sex differences in midsagittal cross-sectional area of the forebrain commissures in mice and rats. No significant difference between male and female mice was found for brain weight or the size of the corpus callosum, hippocampal commissure or anterior commissure. As expected, when neither variable differed by sex, no method of adjusting one for the influence of the other generated an apparent sex difference. In contrast, brain weight and the midsagittal area of the corpus callosum and hippocampal commissure were significantly greater in male than in female rats. The anterior commissure was also larger in male rats, but the difference was not large enough to achieve statistical significance. However, when commissure sizes were adjusted relative to brain size using regression or allometric methods, no significant differences between the sexes or even between mice and rats were evident. Sex differences in the size of the rodent commissures beyond differences attributable to variation in overall brain size were not evident. Ratio and geometric methods, on the other hand, failed to remove variation associated with brain size. Both also retained an apparent species difference in all commissures.

The anterior commissure is considerably smaller than the corpus callosum, which might make it more difficult to measure accurately. The standard deviations of AC size (Table 1) were about 9% of their mean, whereas for the CC they were close to 7%. Supposing that there is a genuine but small sex difference in the rat AC, which seems likely from the available evidence, detecting it would require larger sample sizes and somewhat greater accuracy afforded by thinner histological sections.

These results suggest that a large portion of the variability in the midsagittal area of the cerebral commissures may be attributed to influences affecting overall brain size as a whole. For species in which males and females differ in average brain size (humans and rats, but not mice), a sex difference in commissure sizes is expected. Covariation in brain and commissure sizes may be attributable to variation in common resources or growth processes. Finlay and Darlington [10] found that the sizes of brain components are highly predictable from absolute brain size across species and concluded that the overwhelming proportion of total variance in individual structure sizes is explained by the single factor of overall size, more usefully construed as brain size than body size. Ultrastructural analyses indicate that the adult commissures are composed of neuronal axons, myelin and associated glial cell bodies, and neuronal precursors. Differences in cell number and size arising, in part, from differences in the rate and duration of cell division, cell death, and cell growth may influence both the size of the commissures and the brain as a whole. Across species, very large differences in brain size are found [18], and the only factor that can produce differences of this magnitude is duration of neurogenesis [10]. Witelson et al. [38] reported higher neuron densities in female human brains, whereas Pakkenberg and Gundersen [27] found a major sex difference in total number of neocortical neurons, such that males have 16% more neurons than females and there is no difference in neuronal density. Brain weight and neuron number were significantly correlated in their data ($r = 0.56$). Common physiological mechanisms may tightly link the proliferation and survival of neurons in different parts of the brain.

However, not all of the variability in the midsagittal area of the cerebral commissures can be attributed to influences affecting overall brain size. Correlations between brain weight and commissure sizes in rats and mice are modest ($0.25 < \text{Pearson } r < 0.73$). The CC and AC connect neurons only in certain portions of the forebrain, and the HC neurons primarily in the hippocampus, so correlation with whole brain size is not expected to be large. Differences in cellular composition between the commissures, which consist primarily of myelinated and unmyelinated axons, and the rest of the brain may also contribute to the modest correlations. In an examination of larger and smaller rat and chick brains, Zamenhof et al. [40] suggested that differences in supportive tissue mass rather than differences in neuronal and glial cell counts may be important for brain size differences. Regional and cell-specific differences in gene expression are thought to control the size of specific neuronal populations [30]. Williams et al. [36] found no correlation between retinal ganglion cell number and brain weight ($r = 0.017$) for several isogenic strains of mice. Individual variability in brain component sizes may result from cellular and molecular mechanisms specific to a brain region or cell type as well as mechanisms affecting overall development [37]. Unlike large sex differences in the vertebrate nervous system such as the songbird vocal control regions or the sexually dimorphic nucleus of the preoptic area in rats w4x, moderate sex differences in the forebrain commissures of mice and rats do not exist beyond influences that produce larger and smaller brains overall.

The present study compared four methods to adjust for overall size. In general, the use of ratios, whether it be a ratio to brain size or brain size$^{2/3}$, is not recommended. Use of a ratio in this study detected species and sex effects in commissure sizes that disappeared when regression adjustment was used. Ratio methods also were not effective in removing the variability associated with overall brain size in several instances; the ratio often was positively correlated with brain size. The use of regression adjustments is instead recommended [5]. Because these methods are based on the relationship between variables in the actual data, applying the regression method to adjust for differences in brain size will not change the results for commissure size if no relationship exists. Thus, it is both wise and safe to adjust commissure size for brain size, even when their correlation is not highly significant.

Although both linear regression and analysis of log transformed data effectively removed the variability due to brain size within and between species, the use of regression without a log transform is simpler and easier to communicate. The allometric method has two clear advantages, however. First, as seen in Table 1, rats with much larger brains than mice also have much larger variances in commissure sizes. A log transform nearly equates these within-group variances (Table 5), whereas the linear regression method retains a marked heteroscedasticity and thereby violates one of the assumptions of regression analysis. Second, allometry aids meaningful comparisons within different species and across a wider range of species.

In summary, we recommend that researchers report the results of analyses first for unadjusted commissure sizes and then for sizes adjusted for brain size, even if the correlation of commissure size with brain weight is not statistically significant. Linear regression is usually an acceptable method if only one species is being studied, whereas allometry is clearly superior for species comparisons. Examination of relative commissure sizes using regression or allometric methods should lead to more consistent conclusions regarding sex differences in the cerebral commissures, especially when there is doubt about the extent of allometric growth for the human corpus callosum [28]. It should also indicate more clearly whether experimental treatments have sex specific effects beyond influences on brain size as a whole [7,19,21,22].

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