Deficiency of Corpus Callosum Varies with Strain and Supplier of the Mice

By: Douglas Wahlsten

Wahlsten, D. Deficiency of corpus callosum varies with strain and supplier of the mice. Brain Research, 1982, 239, 329-347.

Summary:
Six inbred strains of adult mice either obtained from commercial suppliers or bred in the author's laboratory at Waterloo were assessed for size of body, brain and corpus callosum (CC) at a wide range of ages. Because almost all measures varied significantly with age, a simple regression procedure was used to minimize age effects statistically. The strains A/J, C57BL/6J and DBA/2J differed substantially in brain size and size of CC, but none of these animals ever showed severe deficits of CC fibres crossing the midsagittal plane. For these 3 strains the sizes of body, brain and CC were larger when bred at the Jackson Laboratory than at Waterloo. Two BALB/c strains obtained from different suppliers had over 30% of mice with defective CC, ranging continuously from total absence of transcortical fibres to slightly reduced CC size. For these two strains bred at Waterloo, brain sizes were larger and CC defects were much less frequent than when bred by commercial suppliers. The strain 129/J had a 71% frequency of CC defects when reared at the Jackson Laboratory but 38% defects when bred and reared at Waterloo. However, bodies of 129/J mice bred at Jackson were larger than those bred at Waterloo. These results show that relatively small differences in rearing conditions can have relatively large effects on certain aspects of brain development.
Key words: corpus callosum — brain development — inbred strain — mouse — nutrition — early experience

Article:

INTRODUCTION

The axons of the corpus callosum fail to cross from one cerebral hemisphere to the other in many mice of the inbred strain BALB/cJ, and in many of their siblings the corpus callosum is much smaller than normal[14]. Similar anomalies are known to occur in the 129/J strain as well (R.E. Wimer, personal communication).

The wide range of expression of the defect in BALB/cJ mice poses interesting questions about both the hereditary and developmental aspects of corpus callosum size. A previous study of mode of inheritance[14] was inconclusive because the extremely poor reproductive performance of BALB/cJ mice made a large breeding experiment impossible. Consequently, BALB/c mice from 7 different suppliers were studied[15], and mice from Carworth Farms as well as Charles River Breeding Laboratories were found to breed well in spite of frequent anomalies of corpus callosum.

Preliminary work with the BALB/cCF strain revealed three additional facts which complicated the analysis of hereditary influences but revealed much about brain development. Size of mouse corpus callosum continued to increase for many months and then declined at an age of over one year, which made it difficult to specify a single criterion for deficiency of the structure. Furthermore, frequency of the defect proved to be much lower in animals bred in the author's laboratory than in their parents purchased from commercial suppliers. Finally, in the course of an experiment with hybrid crosses between BALB/cCF and other inbred strains, a few mice of the strains A/J and DBA/2J were discovered which had a rather small amount of callosal fibres crossing midplane but which clearly were not afflicted by the same developmental anomaly as the BALB/c mice.

The present study was therefore done in order to : (a) document changes in sizes of body, brain and corpus callosum with age in several inbred strains and to apply a simple statistical correction for age effects; (b) assess strain differences in corpus callosum size and possible relationships to whole brain size; (c) determine the extent of changes in the brain from the commercial suppliers to the author's laboratory; and (d) devise a reliable criterion for abnormality of mouse corpus callosum by considering the shape of the structure as well as size.

MATERIALS AND METHODS

Inbred mice of the strains A/J, C57BL/6J, DBA/2J and 129/J were obtained at 6-8 weeks of age from the Jackson Laboratory; and BALB/cCF and BALB/cCRBL mice were procured at about 8 weeks of age from the Charles River Breeding Laboratories. The CF substrain was previously maintained by Carworth Farms, but this enterprise was taken over by Charles River. Although the CF substrain is no longer available from Charles River, a large breeding colony is maintained in the author's laboratory, and breeding stock will be provided to interested investigators. The CF substrain was established by Carworth Farms from pedigreed BALB/c mice obtained in 1968 from the Laboratory Animal Center in England, and the CRBL substrain was derived from pedigreed BALB/cAnN mice obtained from the National Institute of Health in 1975 (G.J. Pucak, personal communication).

Within any shipment of mice it was not known which ones were littermates. Animals were chosen for breeding haphazardly within a shipment, and some of the initial matings may have been between brother and sister, whereas others were probably between close cousins. Because all strains were highly inbred, one generation of non-sib mating should have had negligible effects. All subsequent coatings in the laboratory were between brother and sister. Data reported in this study were derived from S generations of BALB/cCF mice and at least two generations of the Jackson strains bred at Waterloo. Only one generation of the BALB/cCRBL strain was bred at Waterloo.

All animals in the laboratory were maintained on a 12 h light-12 h dark schedule at 22 ± °C with free access to tap water and non-autoclaved Master MLM rodent food from Maple Leaf Mills, Toronto. They were housed in 29 × 18 × 13 cm opaque plastic mouse cages with `Betta-Chip' hardwood lab bedding (Northeastern Products, Warrensburg, NY) and a few sheets of toilet tissue for nesting material.

One male was placed with one or more females, and each female was isolated as soon as she was visibly pregnant. A litter was left undisturbed until the pups showed hair growth, whereupon the cage bedding was changed. Large litters, which were very rare, were not culled. Mice remained with their mother until one month after birth, when they were weaned and placed in standard cages with same-sex littermates. The mother was then re-mated to the same male to obtain a second litter. A few females produced 3-5 litters.

Each mouse was subsequently anesthetized with pentobarbital sodium, body weight and tail length were measured, and then the animal was perfused intracardially with 10 ml saline followed by 10 ml buffered 10% formalin. After the brain was fixed at least 2 more days in formalin, the spinal cord, cranial nerves, olfactory bulbs and paraflocculi were carefully trimmed away with a scalpel, and then the brain was gently blotted on a paper towel to remove surface fluid and weighed to the nearest mg.

The corpus callosum was studied with one of two methods. In some cases serial sagittal sections were cut at 33 µm on a freezing microtome and stained with metachromatic thionin to reveal Nissl substance and myelin. The midsagittal section was then used to draw an outline of the corpus callosum and adjacent fibre tracts using a Leitz Tracing Device.

Other brains were cut in half along the midsagittal plane with a razor blade and then placed flush against a piece of glass and photographed in the unstained state with Ektachrome 64 film at 2 × magnification. The resulting 35 mm slide, which clearly revealed major fibre tracts, was then projected onto a screen, and the outlines of corpus callosum and adjacent structures were drawn. This method was derived from a technique suggested by R.E. Wimer (personal communication). Although this method was not quite as accurate as the histological procedure,

it was much faster and less expensive. Fig. 1 shows results of the two methods for three BALB/cCF brains varying in corpus callosum size. As a check for perceptual errors in viewing the film, length of corpus callosum in the bisected brain was also measured with an ocular scale in a dissecting stereoscope.

Size of corpus callosum was then derived from the drawing by tracing the outline in light pencil onto another sheet of paper of known density, cutting out and then weighing the paper representing corpus callosum. By correcting for density of the paper and magnification of the microscope or camera/projector, cross-sectional area of corpus callosum in sq. mm was obtained for each formalin-fixed brain. This area was roughly proportional to the number of axons passing between the two halves of the brain via corpus callosum. Length of corpus callosum was derived from the stained mid-sagittal section by a simple linear measure from the most posterior portion of the splenium to the most anterior portion of the genu. For bisected, unstained brains the comparable measure was found using the stereoscope, not the drawing made from the 3 mm color slide.

The outline drawn around corpus callosum included the dorsal commissure of the fornix (CFD, commissura fornicis dorsalis) as well as the genu, truncus and splenium of corpus callosum proper. This posed a problem in those brains with only a very small corpus callosum, especially when they were unstained, because CFD sometimes appeared to join with the hippocampal commissure (CFV, commissura fornicis ventralis) when the corpus callosum was absent. This ambiguity about the identities of CFD and CC in abnormal brains will have to be resolved using techniques to trace the actual pathways. In the present study it means that the distinction between total absence of CC and very small CC is not reliable. Fortunately, this distinction has little consequence for differences among strains and between suppliers, because the event is so rare and the numerical difference in area is so small.

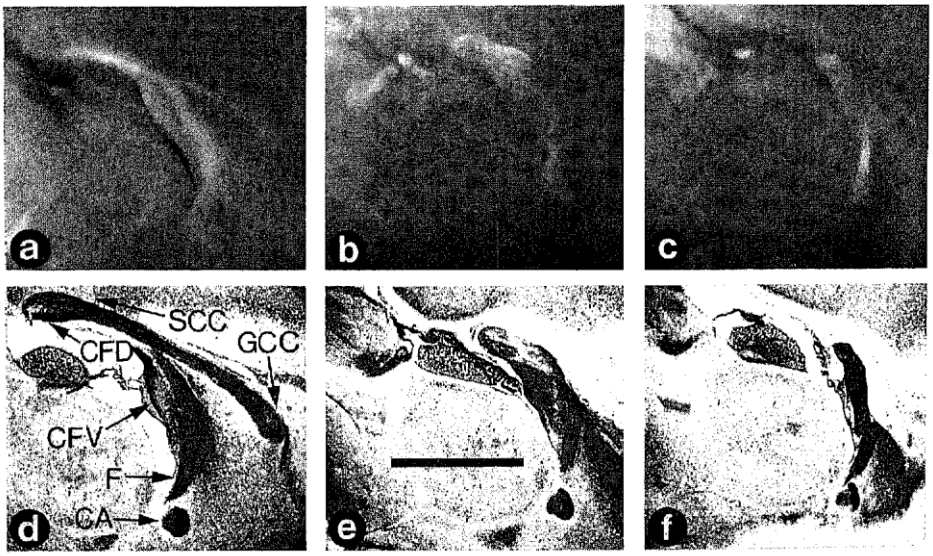

Fig. 1. Midsagittal sections of 3 BALB/cCF mouse brains, unstained and stained. Fig. 1a, b and c show the 3 brains after they were fixed in formalin, cut in half with a scalpel, placed flush against a thin piece of glass and photographed in tungsten light. These 3 photographs were made with Panatomic-X film, but brains were routinely photographed with Ektrachrome 64 colour slide film to enhance the discriminability of 'white' matter and 'gray' matter which are actually different shades of yellow and brown. After the photographs were taken, each brain was sectioned sagittally at 33 μm and then stained with metachromatic thionin for Nissl substance and myelin. The whole sections closest to midline are shown in Fig. 1d, e and f adjacent to their photographs unstained. Certain structures are evident in the unstained brains which are not seen in the stained sections because the bisected brains are slightly transparent and thus reveal fibre tracts as much as 0.5 mm lateral to midplane. All photographs are at the same magnification as shown by the bar in Fig. 1e which represents 2.0 mm. Fig. 1a and d are from a brain with normal corpus callosum. Fig. 1b and e are from a brain with a short corpus callosum. Fig. 1c and f are from a brain with no fibres of the corpus callosum crossing midplane. The photographs show that an accurate and rapid estimate of the size of corpus callosum can be made with the photographic method. Abbreviations: CA, commissura anterior; CFD, commissura fornicis dorsalis; CFV, commissura fornicis ventralis; F, columna fornicis; GCC, genu corporis callosi; SCC, splenium corporis callosi.

For data analysis the following measures for each mouse were processed with the SPSS program package : cross-sectional area and length of corpus callosum at the midplane, brain weight, body weight, tail length, age at perfusion and sex.

RESULTS

Changes with age

Analysis of age was done only for mice bred at Waterloo because those purchased from commercial suppliers usually had similar birth dates and ages at perfusion, resulting in a very narrow range of ages. Analyses were done separately for males and females. For the strains 129/J, BALB/cCRBL and BALB/cCF which were prone to defects of corpus callosum, only those animals that appeared to have a normal corpus callosum were used to assess changes with age. The preliminary criterion of normality was set at CC area of at least 0.85 mm^2 or CC length of at least 2.6 mm on the basis of a previous study[18]. This procedure rendered the test of age effects more sensitive and allowed a proper evaluation of differences between normal mice and those with defective corpus callosum in subsequent analyses.

TABLE I

Proportion of variance in each of 5 measures for 6 inbred strains bred at Waterloo which was attributable to linear and quadratic regression on age of mice with normal corpus callosum

Strain:	*A/J*		*C57BL/6J*		*DBA/2J*		*129/J*		*BALB/cCRBL*		*BALB/cCF*	
Sex:	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
Sample size:	41	24	40	34	11	14	17	8	35	35	935	970
Youngest:	80	80	83	84	140	200	150	180	96	96	59	52
Oldest:	470	470	414	414	305	340	410	392	329	433	513	561
Linear trend												
CC area	—	0.242	0.369**	0.391**	0.470	0.315	—	—	0.123**	0.095**	0.010**	0.030**
CC length	0.182*	0.132	0.146	0.296**	0.583*	0.322	—	—	—	0.044**	0.040**	0.031**
Brain wt.	0.106	—	—	0.111*	—	0.321	—	—	0.045**	0.402**	0.057**	0.082**
Body wt.	0.273*	0.259	0.642**	0.480**	0.493	0.376	0.354	0.825*	0.146*	0.501**	0.405**	0.427**
Tail-lth.	0.452**	0.388*	0.468**	0.452**	—	—	—	—	0.337*	0.567**	0.454**	0.484**
Quadratic trend												
CC area	—	—	—	0.155*	—	—	—	—	0.278**	0.289**	0.008*	0.004
CC length	—	0.170	—	—	—	—	—	—	—	0.316**	—	0.007*
Brain wt.	—	—	—	0.152	—	—	—	—	0.307**	—	0.012**	0.052**
Body wt.	0.101	—	0.125**	0.119*	—	—	—	—	0.211*	—	0.075**	0.070**
Tail-lth.	—	—	—	—	—	—	—	—	0.147*	0.193**	0.062**	0.057**
*Percent change from 100 to 365 days****												
CC area	—	19.8	20.6	42.1	50.1	70.6	—	—	0.0	20.8	2.4	6.4
CC length	3.8	5.4	3.2	6.4	15.1	22.1	—	—	—	0.0	6.6	5.7
Brain wt.	4.0	—	—	5.2	—	22.0	—	—	0.0	10.6	2.9	4.3
Body wt.	26.8	22.9	30.9	29.1	24.6	58.6	15.5	55.2	2.6	31.9	18.7	25.0
Tail lth.	14.9	11.6	9.1	9.0	—	—	—	—	4.8	17.2	9.9	10.9

* $P < 0.01$, one-tailed.
** $P < 0.001$, one-tailed. All other proportions not indicated as significant at the 0.01 or 0.001 levels are based on trends which are significant only at the 0.05 level.
*** These values were derived from the regression equations given in the Appendix. The expected values were determined for 100 and 365 days of age, and then the percent increase at 365 days over the expected value at 100 days was calculated. Change are shown only for groups where regression on age was significant at the 0.05 level or better.

For each of 12 strain-sex groups hierarchical regression on age at perfusion was done for the 5 variables shown in Table I. Linear regression on age was assessed first, and if a significant quadratic trend was evident above and beyond the linear change with age, then a quadratic regression equation was computed. Otherwise, only the significant linear trend was considered in further analyses.

Table I presents the proportion of variance in each measure which was attributable to changes with age in each group where the relationship was statistically significant ($\alpha = 0.05$). The proportion for linear trend expresses the goodness of fit of the linear regression equation alone (r^2), whereas the proportion for quadratic trend expresses the *improvement* in goodness of fit (multiple r^2) resulting from the inclusion of the quadratic term in the regression equation. Thus, the goodness of fit of the quadratic equation is the sum of the two proportions shown in Table I (e.g. 0.405 + 0.075 = 0.480 for body weight of BALB/cCF males).

Because so many regression equations were estimated, those trends significant only at the 0.05 level should not be taken too seriously. Furthermore, the 5 equations for each group were not independent, being based on the same animals. Nevertheless, many relationships were highly significant for each of the 5 measures, especially for groups with larger sample size and wide range of ages. The linear trends generally accounted for more variance and were more highly significant than quadratic trends. One reason for this apparent lack of curvature is that very few animals were more than 1 year old in several groups.

The magnitude of age-related changes in units of measure is apparent from examples of the lines of best-fit shown in Fig. 2 for C57BL/6J mice. Table I also presents the expected percentage changes in each measure from 100 to 365 days of age, derived from the regression equations for each group. It is apparent that changes in body weight and tail-length were very substantial, but that changes in brain weight were small and changes in corpus callosum size were relatively modest.

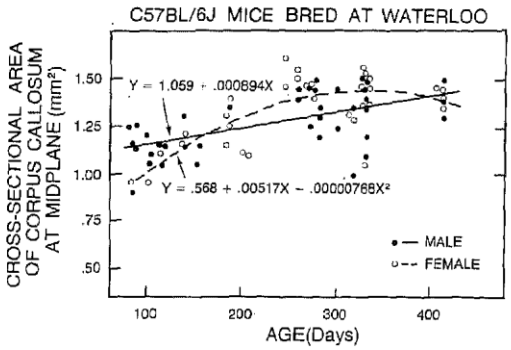

Fig. 2. Cross-sectional area of corpus callosum at the midsagittal plane for C57BL/6J mice of various ages bred in the author's laboratory at the University of Waterloo. Solid black dots represent males and open circles represent females. The continuous line is the straight line of best fit derived from hierarchical regression of CC area on age for males; the quadratic trend is not significant. The dashed line is the line of best-fit for females, which includes a significant quadratic trend. These regression equations were used to transform each mouse's score in order to remove variance attributable to change in the CC area with age, as discussed in the text. It is apparent that differences among relatively few older animals resulted in a quadratic trend for females but not males. Hence, the two equations were useful for correcting the data for age effects, but they do not mean that age changes are linear for males and curvilinear for females in the more general sense.

Correction for age

Because all measures change with age, the mean value of a measure for a single group is influenced by the distribution of ages in the group. Statistical correction for age can therefore serve the dual purpose of: (a) eliminating artifactual group differences in means caused by group age differences; and (b) increasing the power of a test of mean difference by reducing within-group variance.

TABLE II

Means and standard errors (S.E.M.) for 3 measures of size for 6 inbred strains bred at commercial suppliers (C) and at Waterloo (W) given separately for males and females

	Males				*Females*			
	n	*Brain weight*	*Body weight*	*Tail length*	*n*	*Brain weight*	*Body weight*	*Tail-length*
A/J								
C	15	0.427 ± 0.007	26.7 ± 0.6	9.16 ± 0.12	19	0.432 ± 0.004	24.2 ± 0.4	9.10 ± 0.08
W	41	0.414 ± 0.003	24.3 ± 0.5	8.28 ± 0.09	24	0.417 ± 0.005	21.8 ± 0.6	8.21 ± 0.10
C57BL/6J								
C	25	0.483 ± 0.005	32.0 ± 0.6	9.20 ± 0.06	50	0.497 ± 0.003	24.7 ± 0.3	9.02 ± 0.04
W	41	0.477 ± 0.003	30.3 ± 0.3	8.09 ± 0.05	34	0.488 ± 0.002	23.4 ± 0.2	7.92 ± 0.04
DBA/2J								
C	9	0.431 ± 0.006	29.2 ± 0.9	8.66 ± 0.08	24	0.445 ± 0.004	25.4 ± 0.4	8.48 ± 0.05
W	12	0.428 ± 0.003	27.2 ± 0.4	8.00 ± 0.09	16	0.418 ± 0.004	21.5 ± 0.6	8.03 ± 0.08
129/J								
C	16	0.444 ± 0.005	27.1 ± 0.7	8.97 ± 0.11	18	0.449 ± 0.007	21.6 ± 0.6	8.79 ± 0.14
W	23	0.442 ± 0.003	25.4 ± 0.3	8.58 ± 0.08	16	0.437 ± 0.004	22.2 ± 0.6	8.52 ± 0.09
BALB/cCRBL								
C	10	0.470 ± 0.010	31.5 ± 1.5	9.43 ± 0.14	18	0.471 ± 0.005	28.3 ± 0.4	9.03 ± 0.10
W	36	0.493 ± 0.003	32.6 ± 0.5	9.28 ± 0.05	38	0.485 ± 0.004	25.7 ± 0.5	9.31 ± 0.04
BALB/cCF								
C	11	0.476 ± 0.004	29.6 ± 0.8	9.73 ± 0.09	18	0.443 ± 0.005	25.8 ± 0.4	9.11 ± 0.08
W	1103	0.489 ± 0.001	28.7 ± 0.1	9.12 ± 0.01	1181	0.498 ± 0.001	25.3 ± 0.1	8.95 ± 0.01

Correction for age was done for each measure in a group only if the regression on age was significant at the 0.05 level (one-tailed) or better, as indicated in Table I. The score of each mouse was transformed using the regression equation to an equivalent score it would have had at an age of 250 days, an age which was close to the average age of 226 days for all mice from commercial sources and 237 days for all mice bred at Waterloo (except strain BALB/cCF). Use of the rounded figure of 250 days as the common age made the changes in group means resulting from the transformations much smaller than if a younger age such as 100 days were used. The significant regression equations derived from each strain-sex group bred at Waterloo were also applied to the comparable groups bred commercially, even though the effect of this procedure was to increase within-group variance for certain commercial groups. Details of the transformation procedure are given in the Appendix.

Analyses of body and brain size

Means and standard errors of variables corrected for age are shown in Table II, and results of analyses of variance performed on these data are shown in Table III for all strains except BALB/cCF, whose sample size was so much larger than the others that its data were analyzed separately. All effects significant at the 0.05 level

or better are presented in Table III, but, because of unequal sample sizes and multiple tests, only those significant at the 0.01 level or better should be taken seriously. Linear correlations among certain variables for mice with normal CC are shown in Table IV.

TABLE III

Analysis of variance and covariance for 5 measures, showing the F ratio and degrees of freedom (df) for each effect

Effect	*All five strains*				*A/J, C57BL/6J and DBA/2J only*				
	df	*Brain weight*	*Body weight*	*Tail-length*	*df*	*CC area*	*CC area****	*CC length*	*CC length****
Strain	4	238.1**	99.7**	117.6**	2	290.5**	36.8**	488.9**	163.4**
Supplier	1	9.5*	54.6**	338.9**	1	42.5**	20.3**	76.9**	47.3**
Sex	1	4.4	553.5**	9.3*	1	15.4**	5.8	9.8*	—
Strain × supplier	4	9.6**	2.6	40.1**	2	5.1*	3.7	5.5*	8.3**
Strain × sex	4	2.8	12.3**	—	2	13.0**	10.4**	—	—
Supplier × sex	1	—	—	—	1	6.4	—	—	—
3-way	4	—	4.1	—	2	—	—	8.9**	8.3**
Residual	460				292				

* $P < 0.01$.
** $P < 0.001$. Effects not significant at even the 0.05 level are not shown in the Table. Effects significant only at the 0.05 level but not the 0.01 level or better are shown by the F ratio alone.
*** Analysis of covariance using brain weight as the covariate.

Sex differences in body weight were very large, as expected, but brain weights of males and females were generally quite similar. For the whole body, males weighed significantly more than females in every group, although the magnitude of the difference varied with group.

TABLE IV

Linear correlations among measures of mice with normal corpus callosum for males (M) and females (F) bred commercially (C) or at Waterloo (W)

		n	*CC area × CC length*	*CC area × brain wt.*	*Brain wt × body wt.*	*Brain wt × tail lth.*	*Body wt. × tail lth.*
A/J							
C	M	15	0.509	0.490	0.687*	0.491	0.506
	F	19	0.460	0.611	0.676*	0.659*	0.704*
W	M	41	—	0.374*	0.684*	0.466*	0.442*
	F	24	—	—	0.601*	0.566*	0.634*
C57BL/6J							
C	M	24	0.381	0.653*	0.519*	0.602*	0.750*
	F	50	0.402*	0.355*	0.395*	0.263	0.335*
W	M	40	0.312	0.316	—	0.302	—
	F	34	—	0.303	0.682*	0.644*	0.548*
DBA/2J							
C	M	8	0.852*	—	0.628	0.781	—
	F	24	0.409	0.632*	0.723*	0.501*	0.544*
W	M	11	—	—	—	—	—
	F	14	0.468	0.465	—	0.576	—
129/J							
C	M	7	0.903*	—	—	—	—
	F	6	0.788	—	0.950*	—	—
W	M	17	0.710*	—	—	—	—
	F	8	0.713	—	—	—	—
BALB/cCRBL							
C	M	8	0.622	—	0.779	0.664	0.818*
	F	9	0.739		0.730	0.885*	0.817*
W	M	35	0.532*	—	0.598*	0.552*	0.599*
	F	35	—	—	0.734*	0.337	0.431*
BALB/cCF							
C	M	8	0.784	—	0.678	0.874*	0.842*
	F	12	0.604	—	—	—	0.548
W	M	935	0.540*	0.153*	0.551*	0.301*	0.454*
	F	970	0.530*	0.173*	0.490*	0.297*	0.379*

* $P < 0.01$. All other correlation coefficients shown in the table are significant only at the 0.05 level. Those not given in the table are not significant even at the 0.05 level. All tests are one-tailed.

Strain differences in body size and brain weight were obviously large. A substantial portion of the variation in brain size was attributable to size of the whole body. Comparing strains, those with heavier bodies tended to have heavier brains (Spearman $r = 0.89$, $P < 0.05$). Within a group (Table IV) the linear correlation between brain weight and body weight was greater than 0.5 and significant at the 0.01 level in most cases.

The effects of supplier were surprisingly large on all measures. Inspection of the data in Table II revealed that mice bred at the Jackson Laboratory were consistently larger than their progeny bred at Waterloo; bodies averaged 1.8 g heavier, tails averaged 0.7 cm longer and brains averaged 11 mg heavier at 250-days-equivalent

age. For the BALB strains bred commercially, brains averaged 25 mg lighter than their progeny bred at Waterloo, but bodies averaged 0.7 g heavier and tails 0.2 cm longer than at Waterloo.

Analysis of corpus callosum size

Frequency distributions of corpus callosum cross-sectional area and length at midplane are shown in Fig. 3. Sex differences were generally very small, so data of males and females were pooled for presentation.

Among the 3 strains which always had normal corpus callosum, CC area was greater for the C57BL/6J mice with heavier brains. This relationship was also evident from the moderate correlations (Table IV) between CC area and brain weight within a group. The relationship was far from perfect, however; DBA/2J mice had slightly smaller CC area than A/J mice but slightly larger brains. Furthermore, the ratio of CC area to brain weight differed very significantly among groups.

Analysis of covariance which first removed the linear relationship between brain weight and CC size revealed significant differences in CC size above and beyond differences in whole brain size (Table III). The large strain-by-sex interaction was attributable to the larger CC area of females compared to males in C57BL/6J.

The supplier effects closely paralleled those on brain weight. CC area and length were generally greater for mice bred at the Jackson laboratories than at Waterloo (Fig. 3). The supplier effect was highly significant even when variance attributable to brain weight was first removed using analysis of covariance (Table III).

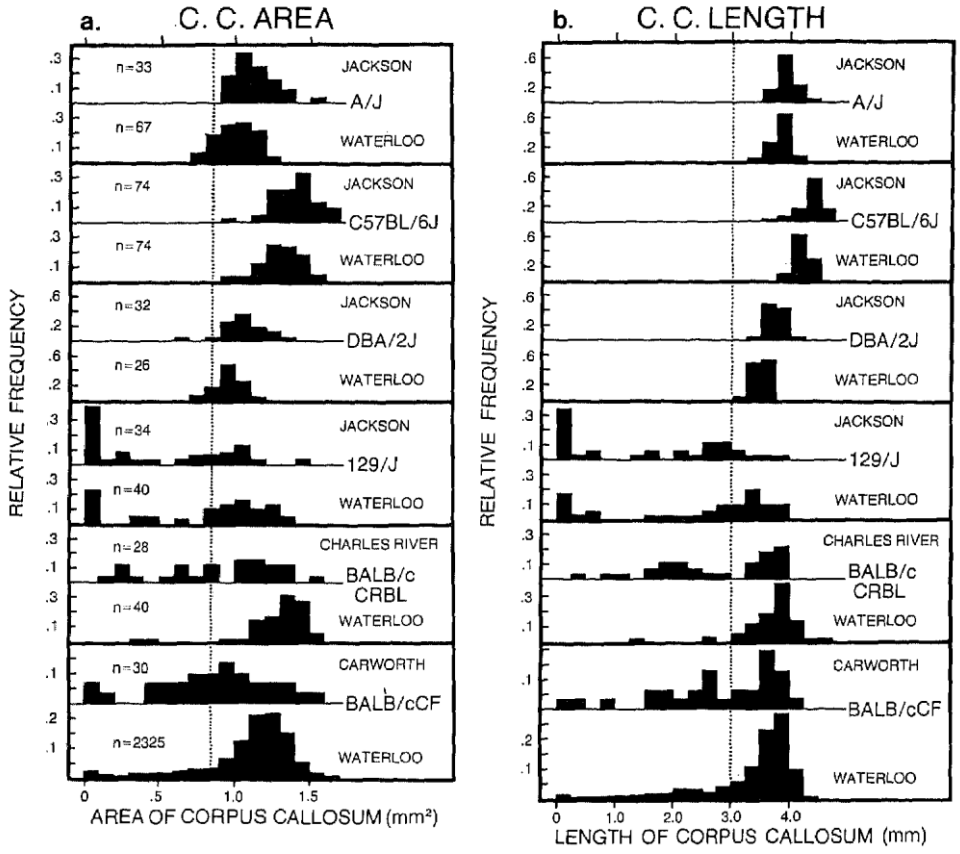

Fig. 3. Frequency distributions of area (a) and length (b) of corpus callosum at the midsagittal plane for mice of 6 inbred strains bred commercially and their descendants bred at the author's laboratory at the University of Waterloo. Commercial breeders were the Jackson Laboratory, Charles River Breeding Laboratories and Carworth Farms. Data for males and females in each group were pooled after any significant changes with age were eliminated by transforming all scores to an equivalent age of 250 days, as described in the text. Dotted lines show the criteria for abnormal corpus callosum which were derived from the two-way scatterplot shown in Fig. 4.

For the 3 strains which sometimes had deficient or absent corpus callosum at midplane the usual analysis of variance was inappropriate because data were very skewed. Distribution-free tests were therefore used.

CC area was significantly larger for 129/J mice bred at Waterloo than at Jackson (Mann-Whitney IT-test, P = 0.026, two-tailed), which runs counter to the smaller body and brain weights for 129/J mice bred at Waterloo. However, the improvement in CC size paralleled an even more dramatic improvement for the two BALB strains. CC area for BALB mice bred at Waterloo was substantially and significantly greater than for their ancestors bred at Charles River or Carworth Farms (Kolmogorov-Smimov two-sample test; two-tailed tests, $P <$ 0.001 in both cases). Similar effects were present for CC length.

The relationship between CC size and brain size also proved to be unusual for the 129/J and BALB strains. Animals having small or absent corpus callosum were well within the range of brain weights typical of the strains A/J, C57BL/6J and DBA/2J, and hence deficiency of corpus callosum was not merely a consequence of gross nutritional inadequacy early in life. However, among the 129/J and BALB mice with CC size in the normal range, the correlations between CC area and brain weight were very low (Table IV), being significant only for the two BALB/cCF groups with very large sample sizes.

This important finding suggests that the relation between CC development and development of the rest of the brain was somehow disrupted in these strains and that even those 129/J and BALB mice with corpus callosum of normal size were in some respects unlike those of strains A/J, C57BL/6J and DBA/2J.

Abnormality of corpus callosum

The frequency distributions in Fig. 3 reveal that size of corpus callosum is indeed a continuous variable and that no clear dichotomy exists between normal and abnormal brains. For either area or length of CC, there is a lower limit below which no animals of the strains A/J, C57BL/6J or DBA/2J are ever found. However, neither area nor length of CC is by itself a satisfactory criterion of abnormality.

The problem is apparent from a scatterplot of length versus area of CC, shown in Fig. 4. (Of course, area depends on length, but in the present study the two variables were measured separately.) It is clear that mice of strains A/J and DBA/2J sometimes had a corpus callosum of rather small area but of seemingly normal length; that is, a long but thin corpus callosum. For BALB/cCRBL mice, on the other hand, most animals had quite a large corpus callosum, comparable to the C57BL/6J mice with similarly large brains. Those BALB mice which had CC areas near the lower end of the A/J and DBA/2J distributions, however, had unusually short CC lengths.

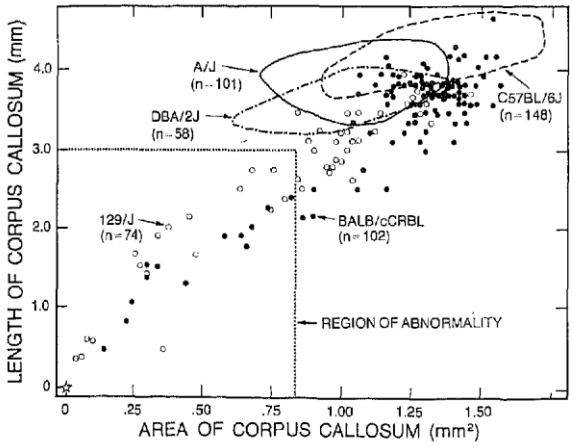

Fig. 4. Scatterplots of length versus area of corpus callosum at the midsagittal plane for 5 inbred strains. Data for males and females as well as mice bred commercially and at the author's laboratory were corrected for significant changes with age and then pooled for each strain, yielding the indicated sample sizes. The curved lines for each of the strains A/J, C57BL/6J and DBA/2J enclose all of the mice in those groups but do not show central tendencies, which are evident in Fig. 3. The location of each BALB/cCRBL mouse is shown by a solid black dot, and the location of each 129/J mouse is shown by an open circle. The star at the origin of the graph represents 20 mice of the 129/J strain with no fibres of CC crossing midplane. The two intersecting dotted lines represent the criteria for area and length of corpus callosum, both of which must be met in order to designate a brain as abnormal.

Fig. 5 compares two mice with CC areas of about 0.61 mm^2. The DBA/2J corpus callosum was structurally normal but thin, whereas the BALB/c corpus callosum was short with a most unusual shape that is never seen in A/J, C57BL/6J or DBA/2J mice.

From a purely statistical standpoint, a criterion of CC length at 3.0 mm would distinguish every A, C57 and DBA mouse from 129 and BALB mice which are clearly abnormal. Unfortunately, this would also include BALB mice with relatively large CC areas of 1 mm^2 or more.

The distribution of CC area for the large sample of BALB/cCF mice (Fig. 3a) is helpful in that the areas at the upper end appear to be approximately normally distributed, much as they are for A; C57 and DBA mice. The mode of this distribution is close to 1.2 mm^2, and for those animals above this mode 94% have an area less than 1.5 mm^2 or 0.3 mm^2 above the mode. Supposing that 'adequate' corpus callosum is normally distributed, then only about 3.5 % of these 'normal' animals would have CC areas below 0.85 mm^2.

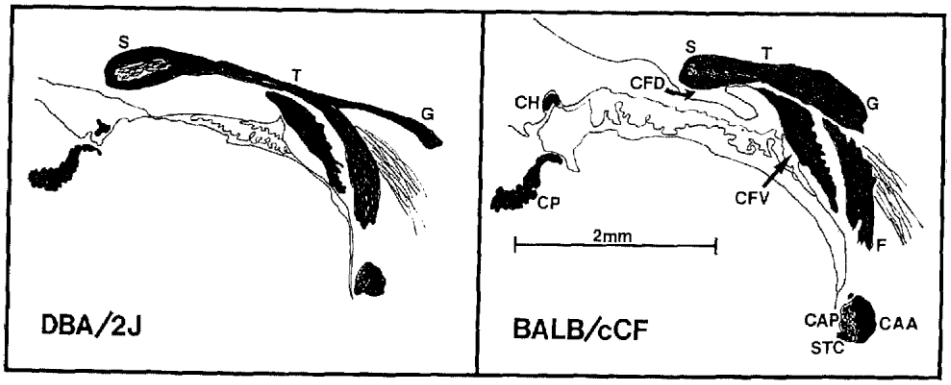

Fig. 5. Drawings of major myelinated fibre tracts at the midsagittal plane of two mice with equivalent cross-sectional areas of corpus callosum. Drawings were made from 33 μm sections stained with metachromatic thionin. The DBA/2J mouse was a 253-day-old female with a brain weight of 0.422 g, and the BALB/cCF mouse was a 177-day-old male with a brain weight of 0.531 g. The CC areas were 0.618 and 0.607 mm^2 for the DBA and BALB mice, respectively, which were rather small compared to many mice shown in Fig. 3a. However, the CC length was 3.32 mm for the DBA mouse, well within the normal range. The splenium (S) of this animal was clearly normal but the genu (G) was unusually thin, as was the truncus (T) of CC. The BALB mouse with CC length of only 1.99 mm presented quite a different picture, however. Its splenium was quite unusual, and its genu was abnormally short, not even extending beyond the anterior commissure (CAA) in the anterior direction. Abbreviations: CAA, commissura anterior, pars anterior; CAP, commissura anterior, pars posterior; CFD, CFV, F as in Fig. 1; CH, commissura habenularum; CP, commissura posteriorum; STC, stria terminalis, pars commissuralis.

TABLE V

Mean values of 3 measures for BALB/cCF mice bred at Waterloo which showed either normal or deficient corpus callosum (CC)

	n	*Brain weight (g)*	*Body weight (g)*	*Tail-length (cm)*
Males				
Normal CC	957	0.4887	28.06	9.12
Deficient CC	142	0.4886	28.58	9.19
Females				
Normal CC	1040	0.4984	25.32	8.95
Deficient CC	182	0.4969	25.32	8.96

Thus, a good first approximation of deficient corpus callosum is one which is shorter than 3.00 mm *and* smaller than 0.85 mm^2 in area. This is an artificial dichotomy imposed on continuous phenomena, but it should be useful for certain kinds of investigations such as mode of inheritance. Fortunately, it is very close to the criterion used to derive the regression equations on age and linear correlations (Tables I and IV).

Using the above criteria, mice of strains 129/J, BALB/cCRBL and BALB/cCF were divided into groups with deficient CC and non-deficient CC, and a t-test was done to compare brain weights, body weights and tail-lengths within each strain-sex-supplier group where there were at least 6 mice with deficient CC. In none of 8 groups were either the body weights or tail-lengths of mice with deficient CC significantly less than those with normal CC. Regarding brain weight, only female BALB/c mice bred at Charles River showed heavier brains for

those with normal CC (0.478 g) than deficient CC (0.463 g), but the difference was only marginally significant ($t = 1.77$, $P = 0.05$, one-tailed) and could easily have resulted from sampling error.

The striking similarity of mice dichotomized by CC size is apparent in Table V which gives data for the large sample of BALB/cCF mice bred at Waterloo. It is obvious that deficiency of corpus callosum in these animals has nothing to do with some kind of nutritional or growth deficiency that is expressed in size of the brain or body.

TABLE VI

Percent of mice of 3 inbred strains bred commercially or at Waterloo which had deficient corpus callosum (n = sample size)

Supplier	*129/J*	*BALB/cCRBL*	*BALB/cCF*
Commercial	70.6% (34)	39.3% (28)	33.3% (30)
Waterloo	37.5% (40)	2.7% (74)	14.0% (2321)
z	2.57	2.78	2.73
p*	0.005	0.003	0.003

* One-tailed tests.

The criterion of deficient CC was also used to assess sex differences in frequency of the defect within the various groups. No significant differences between males and females were found using chi-squared tests ($\alpha = 0.05$).

Finally, the criterion was used to assess the relative frequency of the deficiency of corpus callosum in the various groups. Results are shown in Table VI, pooling males and females. For each strain the proportion of deficient CC was greater for mice from commercial suppliers than those bred at Waterloo (chi-squared tests, $P < 0.01$).

DISCUSSION

Having eliminated age effects statistically, several highly significant strain differences in sizes of body, brain and corpus callosum were detected. Almost identical strain-specific brain weights have been reported previously[12,22,23], and closely comparable strain differences have consistently been found when adjustments are made for minor differences in methods of weighing brains[18]. The present data also replicate two previous studies of corpus callosum size in strains which never show the BALl3- type deficits[14,22]. The impressive stability of these measures of brain structure from year to year and across different laboratories contrasts sharply with frequent reports of dramatic reversals of strain rank-orders on tests of learning in different labs[18]. Brain size and structure are clearly subject to mild environmental influences at different laboratories, however. The mice bred at Jackson Laboratory were generally larger in body, brain and corpus callosum than their descendants bred at Waterloo. The only exception was strain 129/J which at Waterloo had smaller body size, equivalent brain size and larger corpus callosum size than parents obtained from Jackson. The two BALB/c strains showed slightly smaller body sizes when bred at Waterloo than at commercial suppliers, but their brain sizes and corpus callosum sizes were substantially larger at Waterloo. These results indicate that some aspects of rearing conditions at the author's laboratory are especially beneficial for brain development relative to whole body growth.

The differences in rearing conditions among the Jackson, Carworth, Charles River and Waterloo labs are very numerous and remain to be investigated systematically. Diets certainly vary, for example. At Jackson each inbred strain routinely receives a special strain-specific diet designed to yield optimum growth and reproduction (E.P. Les, personal communication; see Wahlsten[19], Table IV); at Charles River and Carworth mice receive(d) an autoclaved Purina diet (G.J. Pucak, personal communication); whereas at Waterloo all strains receive the same non-autoclaved diet. Each of these diets presumably is adequate for growth and reproduction, having no major deficiencies of nutrients, but seemingly small differences in composition can have important effects on growths and different mouse strains may thrive on one commercial diet but not another[2]. Furthermore, it is quite

possible that dietary factors are especially important for inbred strains which are generally less viable than hybrids[4].

Another important factor may be the breeding regimen. Most commercial suppliers of mice leave the father in the cage with the mother so that she gets pregnant during postpartum estrus and delivers one litter after another without pause, whereas at Waterloo fathers are removed before birth and returned to their mates after the first litters are weaned. Litter size of embryos in gestation while the first litter is still suckling may be substantially reduced[10] and risk of malformations may be increased for the second litter[9]. A study recently conducted in the author's laboratory indicated that defects of corpus callosum in BALB/cCF mice are more frequent in second litters conceived while the mother is nursing[21].

Other possible factors include maternal parity and age at breeding[3], cage space[5] and season of breeding[7]. This latter factor may have contributed slightly to the supplier effects in the present study because the immediate offspring of mice bred commercially were of course born in different seasons than their parents; however, any effect would have been diluted by pooling data from several generations of mice bred subsequently at different seasons in Waterloo.

Whatever the case may be, the influence of relatively small differences in environment upon development of BALB corpus callosum in particular is noteworthy. These effects may complicate studies of inheritance, but they also suggest that a brain defect which occurs in only some mice of an inbred strain may be especially sensitive to environmental influences.

It is clear that overall amount, caloric value or protein composition of the diets cannot account for all observed changes in corpus callosum. These dietary factors have well-known effects on whole body and brain sizes[24], but body and brain sizes are no different for mice with normal and deficient corpus callosum within the 129/J and BALB/c strains. The supplier effect for the two BALB/c strains shows that the factor(s) which influence expression of corpus callosum also influence whole brain growth, but the effect on corpus callosum is not mediated by the change in brain growth.

The defect of corpus callosum in BALB/c mice apparently is not confined to those animals with no or few fibres crossing midplane. The very small correlations between corpus callosum size and brain weight among animals in the normal size range indicate that even these animals are different from other inbred strains in some respects. There is evidence that the temporal schedule of neuron generation in the hippocampus of BALB/cJ mice is highly unusual from 12 to 17 days after conception and that this pattern is related to an unusual topography of mossy fibre terminations on pyramidal cells of regio inferior[1,13]. The latter part of this prenatal period overlaps with the period when callosal axons are approaching midplane[11,20]. Thus, it is conceivable that there is something unusual about neural ontogeny in all BALB/c mice and that this aberrant process renders them very sensitive to minor perturbations in uterine environment which may result in defective corpus callosum.

It is pertinent to note in this context that BALB/cCF mice with deficient corpus callosum occur randomly across families in a pattern that is inconsistent with the hypothesis of Mendelian segregation within the BALB/cCF strain in the author's laboratory[11]. Animals with no transhemispheric callosal fibres often occur in the same litter with sibs which appear quite normal, even though they have the same mother at the same time. Whether the difference in corpus callosum morphology between BALB/cCF and inbred strains with normal brains reflects a difference at a single genetic locus is not entirely clear[18].

The criterion for abnormality derived from the present study will facilitate further research on hereditary processes, provided its somewhat arbitrary nature is kept in mind. The best approach would be to vary the criterion within limits evident in Fig. 4 to see if the choice of criterion makes a difference for the conclusions about mode of inheritance. There is reason to believe that a few mice above this criterion may be truly abnormal and that a few below criterion may not be so unusual. Jones[6] placed small lesions in neocortex of BALB/c mice

and found an unusual pattern of terminal degeneration for one animal which had corpus callosum size that was slightly larger than criterion and a seemingly normal pattern for another animal slightly below criterion.

The deficiency of corpus callosum in 129/J mice is similar in gross morphology to that in BALB/c mice, but the consistently normal appearance of the structure in hybrids between 129/J and BALB/cCF shows that the developmental mechanisms must be different to some extent[18]. The 129/J strain is also distinguished by a rather high frequency of the defect, even in the author's laboratory at Waterloo, as well as the surprisingly low levels of intercorrelations among body, brain and corpus callosum size. This evident disruption of developmental regulation may in turn be somehow related to the extremely poor breeding and maternal performance of this strain.

Research is underway to elucidate the hereditary, developmental, anatomical and behavioural aspects of this striking anomaly of the mouse forebrain. The hereditary defect of corpus callosum should help to analyze the processes of callosal organization in normal mice, and it should also serve to alert a wide spectrum of researchers that the brains of the BALB/c and 129/J inbred strains, which are commonly used in laboratory work, frequently suffer from gross defects of organization.

APPENDIX

The correction for differences in age was done differently for measures of corpus callosum which were sometimes at zero and measures which were always well above zero (brain weight, body weight, tail-length). For the latter 3 measures the variances around the line of best-fit generally did not change appreciably with age, so a simple additive transformation was used instead of standard scores (the z transformation). For a particular dependent measure (Y) of one group, its expected value at an age of 250 days was determined ($\hat{Y}_{250}$). Then for a particular mouse of age A, the expected value at that age was determined ($\hat{Y}_A$) and this value was subtracted from its actual score (Y). This difference was then added to $\hat{Y}_{250}$ to yield the transformed score (Y′) as follows:

$$Y' = \hat{Y}_{250} + (Y - \hat{Y}_A)$$

Thus, if a 375-day-old mouse had a brain weighing 0.008 mg less than the expected weight at 375 days, its transformed weight would be 0.008 mg less than the expected weight at 250 days.

For area and length of corpus callosum the above procedure would yield negative values for some mice older than 250 days, which, although acceptable for statistical analysis, would not help in explanation of the data to the readers. Hence the scores were transformed by multiplying the actual score (Y) by the ratio of $\hat{Y}_{250}$ to $\hat{Y}_A$ as follows :

$$Y' = Y(\hat{Y}_{250}/\hat{Y}_A)$$

This made the ratio between Y′ and $\hat{Y}_{250}$ the same as between Y and $\hat{Y}_A$. A check was done on the efficacy of this unorthodox procedure by regressing the transformed scores on age. In no instance was the relation with age significant for mice bred at Waterloo, showing that effects of age were minimized. A listing of the actual regression equations used to correct the scores of each group is available from the author upon request.

The correction for age changes in corpus callosum size using regression equations estimated from the large sample of BALB/cCF mice should be interpreted cautiously, keeping in mind that the equations were derived from a sub-sample chosen to exclude mice with unusually small corpus callosum. The compound criterion for exclusion was applied to all mice irrespective of age, which may have introduced a small bias by wrongly excluding a few of the youngest animals but including a few of the older animals which really had deficient CC. This would tend to reduce slightly the slope of the line of best-fit and the goodness of fit. The only way to overcome this minor problem would be to measure enough mice in each one-month block of ages to assess the

change in modal size of corpus callosum; the mode in BALB/c mice would be unaffected by the choice of criterion for abnormality.

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