Retarded growth of the medial septum: a major gene effect in acallosal mice

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Abstract:

Absence of the corpus callosum is a hereditary brain defect that appears with varying severity in four inbred mouse strains and is the result of more than one major genetic locus. If relatively few, perhaps two or three, loci are involved in the prenatal ontogeny of the abnormal corpus callosum, it should be possible to identify a distinct morphological process which shows a major gene effect. Because available evidence suggests the source of callosal agenesis occurs in the substrates of axon guidance near the midsagittal plane rather than in the axons themselves, morphometric analysis was done for sagittal sections of the medial septal region in embryos of normal hybrids and four acallosal strains. The anterodorsal zone of the medial septum subadjacent to the cavum septi grew much slower in acallosal BALB/c and I/LnJ mice whereas the ventral septal region was apparently normal. In the Bailey recombinant inbred strains derived from an acallosal BALB/c progenitor, one recombinant (CXBG/By) closely resembled BALB/c whereas the others resembled the normal C57BL/6 parent strain. This pattern of results supports a major gene influence on fusion of the cerebral hemispheres near the region where the corpus callosum first crosses midplane over the dorsal septum.

Key words: Corpus callosum; Anterior commissure; Prenatal development; Inbred strain; Recombinant inbred strain; Morphometry

Article:

INTRODUCTION

The precision of axon growth over long distances to a remote target requires a suitable substrate or cellular environment[3,9,21]. The formation of the corpus callosum is especially interesting in this regard because axons from diverse regions of the cerebral cortex converge in a small area at the midsagittal plane just dorsal and anterior to the hippocampal commissure[11,17,21,25], which crosses midplane prior to the corpus callosum after following a very different path[13,23]. Cells of the subventricular layer emanating from the lateral ventricles are thought to play an important part in directing callosal axons toward midplane[21,32], which requires an abrupt change of direction to prevent them from entering the lateral septum. When the subventricular cells do not extend to the midsagittal region, the corpus callosum may fail to form[19,25].

Four inbred strains of mice lack a corpus callosum in the adult; instead, they have putative callosal axons assembled in a longitudinal bundle (the Probst bundle) and projecting ipsilaterally. Adult mice of the I/ LnJ strain never have a corpus callosum[12,14], whereas in the BALB/cWahl and 129 strains about half the mice have very small or absent corpus callosum[24,25]. In the ddN strain the callosal defect is relatively infrequent, and large samples are required to find enough acallosal mice for research[16]. All studies of inheritance find that callosal defects in adult animals involve more than one recessive gene[12,26]. Although the four inbred strains are quite dissimilar genetically[1,14,27], crosses between them yield many cases of deficient corpus callosum, with the exception of the BALB/c by 129 cross, which is almost always normal[14]. In BALB/c embryos the hippocampal commissure is also markedly deficient but it eventually recovers and grows to normal adult size[25]. In the embryo the combined size of the hippocampal commissure and the corpus callosum shows a pattern of inheritance typical of two autosomal, recessive loci[29]. In adults of the F_2 hybrid cross of BALB/ cWah 1 and

129, a. three-locus model is supported[28]. These data suggest that relatively few loci are responsible for callosal agenesis in mice.

Tracing axons in the embryo with lipophilic dyes demonstrates that callosal axons reach the vicinity of the midsagittal plane normally and have normal growth cone morphologies until then[17], but they fail to cross to the opposite hemisphere when they encounter the interhemispheric fissure, For some reason, the dorsal septal region has not grown far enough to provide an adequate bridge or scaffold to allow axonal traverse of midplane, If the major cause of the callosal agenesis occurs in the substrate cells, and if the problem arises from only two major loci, it should be possible to identify some feature of the axonal surroundings which is defective and has single locus inheritance. Consequently, the present study sought evidence of a specific morphological. defect in acallosal mice at the midsagittal plane and tested for single locus inheritance with recombinant inbred strains[2].

An essential question in understanding a hereditary brain defect is where and when the defect first becomes manifest, The sequelae of callosal agenesis are relatively well documented, but the crucial events causing the first axons to divert away from midplane are inadequately understood. In this study a wide range of morphological ages was examined to assess possible abnormalities prior to the time callosal axons usually arrive at midplane, relying on genetic and morphometric analysis to localize the source of' the problem. The degree of abnormality was quantified with reference to a standard F_2 hybrid mouse population which has substantial genetic differences among individuals but never shows outright deficiency of forebrain commissures[29].

MATERIALS AND METHODS

Mice

The strains and sample sizes are given in Table I, The standard for normal development was based on F_2 hybrid embryos produced by mating B6D2F_1 /7 mice purchased from the Jackson Laboratory; parents were the F_1 hybrid offspring of a C57B1.16j female mated with a DBA/2.1 male. The acallosal strains 129/5 and 129/ReJ were also purchased from the Jackson labs, and the I/LnJ mice were a generous donation from Dr. R.L. Collins of the Jackson labs. Acallosal strains BALB/cWahl and BALB/cWah2 are maintained by full-sib inbreeding at the University of Alberta. The ddN strain had been inbred for over 35 generations at the Kagawa Medical School in Japan and was obtained via the Japan Clea Co. of Osaka, courtesy of Dr. H.S. Ozaki who has studied them extensively. The Bailey recombinant inbred strains and their two progenitors were purchased from the Jackson labs. Most of the mice were maintained and bred at the University of Waterloo as described previously[24,25], but all the ddN and several of the 129/Rej and I/LnJ mice were studied at the University of Alberta under similar conditions, In order to fill in gaps in the body weight. distribution in three important groups and pro-vide equal sample sizes, some previously stained tissue collected with the same histological methods was traced anew and measured more extensively for the B6D2F_2 /J normal[25] and the BALB/cWahl and 2 acallosal groups[5].

TABLE I

Description of mice used in the study

Group	*Sample size*	*Weight range (g)*
B6D2F_2 /J	31	0.18 to 1.16
BALB/cWah1	32	0.31 to 0.80
BALB/cWah2	30	0.26 to 1.11
129/J & 129/ReJ	18	0.26 to 0.93
I/LnJ	12	0.16 to 0.83
ddN	6	0.42 to 0.68
BALB/cByJ	10	0.26 to 0.76
C57BL/6ByJ	10	0.30 to 0.96
CXBD/By	5	0.18 to 0.58
CXBE/By	6	0.36 to 0.83
CXBG/By	11	0.21 to 1.06
CXBH/By	7	0.23 to 0.70
CXBI/By	12	0.25 to 1.06
CXBJ/By	15	0.19 to 1.03
CXBK/By	7	0.30 to 0.79

Matings, litter extractions, and histology

One to three females were mated with one male at about 08.30 h and were then checked for vaginal plugs around noon and 16.30 h. when the male was removed until the next morning. The time of conception was defined as the midpoint between detection of a plug and the previous check without a plug, which made gestation age accurate to within 2 h. In several cases the mice were mated overnight in order to obtain litters with half-clay gestation ages, which were accurate to only 8 h. The plugged female was weighed and then housed alone until extraction of the litter. Litters of the normal B6D2F_2 /J group were obtained at gestation ages El4 to E18, whereas those from all other groups were observed at the four ages E1.5 to El8 because inbred mice are known to develop more slowly than hybricls[26,30]. In the CXBD/By group, no litter was obtained at El8 because of very poor breeding, and the body weight range for that group (Table I) was restricted.

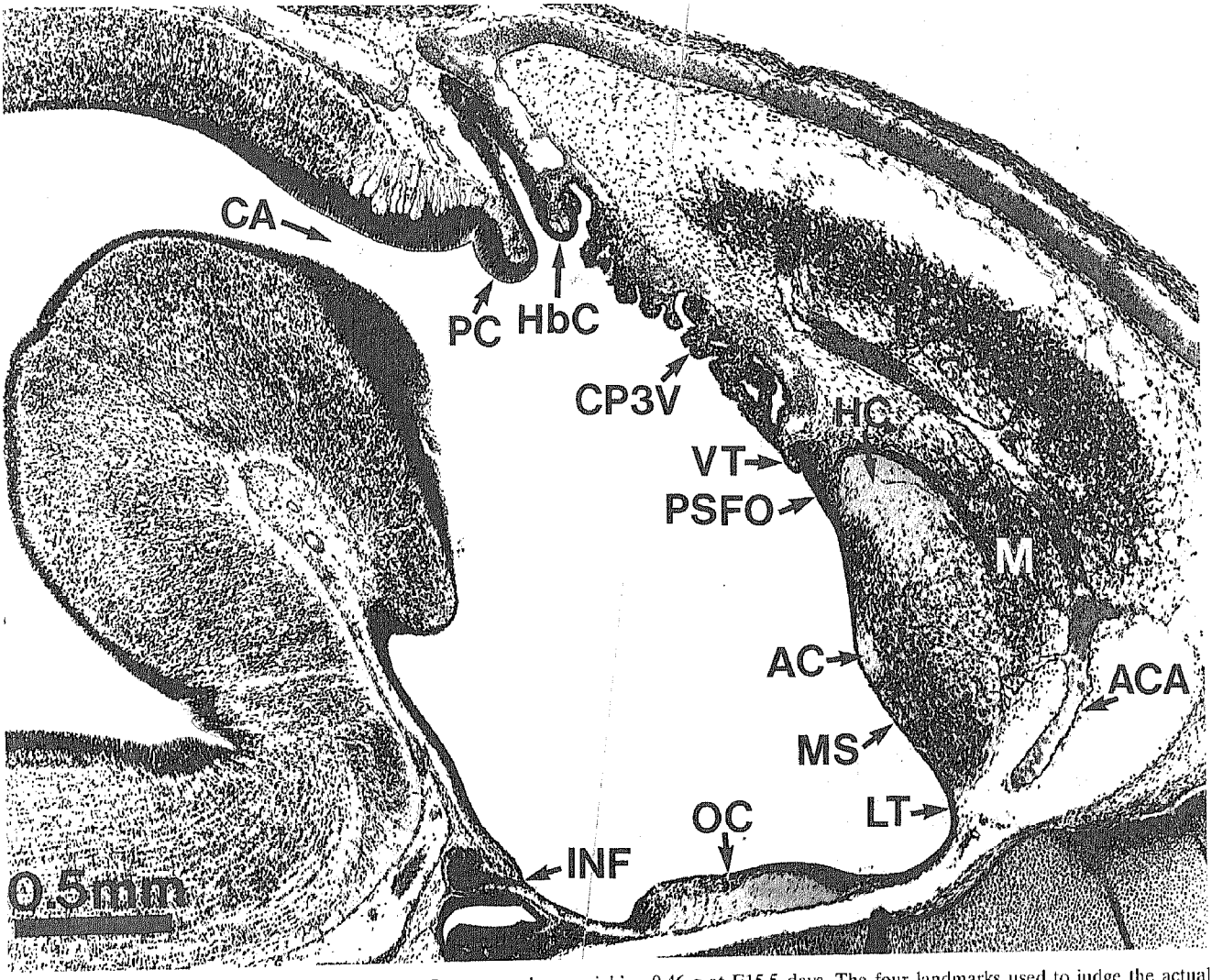

Fig. 1. Midsagittal section of a normal B6D2F_2 /J mouse embryo weighing 0.46 g at E15.5 days. The four landmarks used to judge the actual angle of sectioning are the anterior cerebral artery (ACA) dorsal to the hippocampal commissure (HC), the anterior commissure (AC), the infundibulum (INF) and the posterior commissure (PC). The appearances of these structures and nearby tissue changes rapidly while moving away from midplane, whereas the velum transversum (VT) and lamina terminalis (LT) remain in the same locaton in many serial sections. Scale bar = 0.5 mm.

Within one hour of the designated gestation age, the pregnant female was deeply anesthetized with sodium pentobarbital (120 mg/ kg), the uterus was removed surgically and plunged into 4°C saline, and then each embryo or fetus was removed from the amnion. blotted gently and weighed. The whole mouse was immersed in Bouin-Duboscq fixative for 48 h and then transferred to several changes of 70% ethanol. Shortly after being placed in the fixative, the animal had the scalp removed and small slits cut in the skull lateral to midline to aid penetration of fixative. The skull was left intact near midline to avoid damage to the meninges and blood vessels in the interhemispheric fissure. Mice were chosen for histology from among a larger group of littermates such that each group had a wide range of body weights, which yielded good statistical estimates of growth rates despite modest sample sizes in some groups. Whole heads were embedded in paraffin for sectioning in the

sagittal plane, then serial sections were cut at 10 μm and stained with hematoxylin and eosin. Tracings were made with a Leitz tracing device and measurements were made with a Numonics 2200 graphics tablet and the Sigma Scan program from Jandel Scientific.

Correction for artifacts

Dehydration shrinks neural tissue substantially and slicing the paraffin block dorsal to ventral compresses the section to some extent, which necessarily affects measures of area and thickness. The magnitudes of these artifacts were estimated for a subset of the brains by piercing the midbrain region of the unfixed fetus with four tungsten needles in a square array with tips 1.0 mm apart. The distances between holes in the stained tissue averaged 0.8 mm in the anterior-posterior dimension affected only by shrinkage, whereas in the dorsal-ventral dimension affected by both shrinkage and compression, the average was 0.7 mm at the University of Waterloo[25] and 0.6 mm at the University of Alberta. Distance measures were corrected by multiplying the raw measure by the appropriate correction factor (1.25 = 1/0.8, etc.), and areas of structures were corrected by multiplying by 1.25 times the appropriate dorsal-ventral factor.

The desired plane of' sectioning was sagittal, but a perfectly sagittal alignment was rare. When the actual plane of sectioning deviates from the true sagittal plane by an angle θ, the measured area of a right cylindrical structure is increased by $1/\cos\theta$, and the corrected area can be obtained by multiplying the measured area by $\cos\theta$. To find θ for each brain, four landmarks were. identified (Fig. I) for which reliable judgments could be made that the midsagittal plane passed through the structure in a particular section; the shape of the structure or nearby tissue changed abruptly as the sections moved away from midplane. These were (a) the anterior commissure, (10 the anterior cerebral artery dorsal to medial septum, (c) the posterior commissure dorsal to the cerebral aqueduct, and (d) the infundibulum. A standard coordinate system was established with the origin (0,0,0) at the center of the anterior commissure at mid-plane and Y-axis passing along the most caudal portion of the hippocampal commissure. Distances of structures were measured with respect to these axes and then corrected for shrinkage and compression. Finally, the value of θ was estimated using analytic geometry. Linear distances were corrected separately for angular deviations in the y (dorsal-ventral) and z (anterior-posterior) dimensions, respectively. In most cases the corrections for shrinkage and compression were much greater than for angle of sectioning.

Sagittal reconstruction

In embryos and fetuses the cerebral hemispheres are separated by a deep and narrow interhemispheric fissure (Fig. 2), the bottom of which demarcates the surface of the medial septum. In many fetuses the medial septum extends more than 0.5 mm in the dorsal-ventral dimension. With a section thickness of 0.01 mm, an angle of cutting of 0.02 radian (1.1°) or more will cause the dorsal and ventral portions of the septum to be at midplane in different sections. To obtain a valid representation of the midplane section of the medial septum, that section must be reconstructed front serial sections, each of which contains a portion of the true midplane; the greater the value of θ, the more sections must be included in the reconstruction. The interhemispheric fissure contains the meninges lining the fissure as well as a dense plexus of blood vessels emanating from the anterior cerebral artery and entering the medial septal region. There is also a zone of widely spaced glia-like cells and their processes just anterior to the HC where the cavum septi later forms in normal mice[6,19,20,25]. The medial septum proper was defined in this study as the contiguous, closely packed neural tissue composed of neurons, glia, their precursor cells and cells of the neuroepithelium lining the third ventricle but *not* including meninges or blood vessels in the fissure. When the angle of sectioning is imperfect, the region of the fissure clearly contains meninges and blood vessels, and a line can be drawn to demarcate the limit of the medial septum (MS). To assemble the fragments of the MS into a whole image, landmarks are needed which do *not* change appreciably as sections move away from midplane. Three of these were identified which maintained nearly constant distances from each other in serial sections near midplane: (a) the anterior commissure, (h) the lamina terminalis (LT) at the ventral limit of the MS, and (c) the velum transversum (VT) which separated the choroid plexus of the third ventricle from the primordium of the subfornical organ[6]. Each successive section was aligned with respect to these three landmarks and the portion at midplane was traced onto the composite. When the entire midplane region from the VT to the LT had been covered, a reasonably smooth line was drawn to define

the midstigittal septum (see Fig. 3). This method reconstructs the portion of the medial septal region ventral to the cavum septi and does not include the zone where the hemispheres fuse in advance of the growing CC. The outlines of the anterior commissure and the CC plus the FIC were not reconstructed but instead were traced from the section passing through each commissure at midplane. Tracings, reconstructions and measurements were done on coded slides without knowledge of the animal's age or strain, except for the tissue from previous studies where the strain was known.

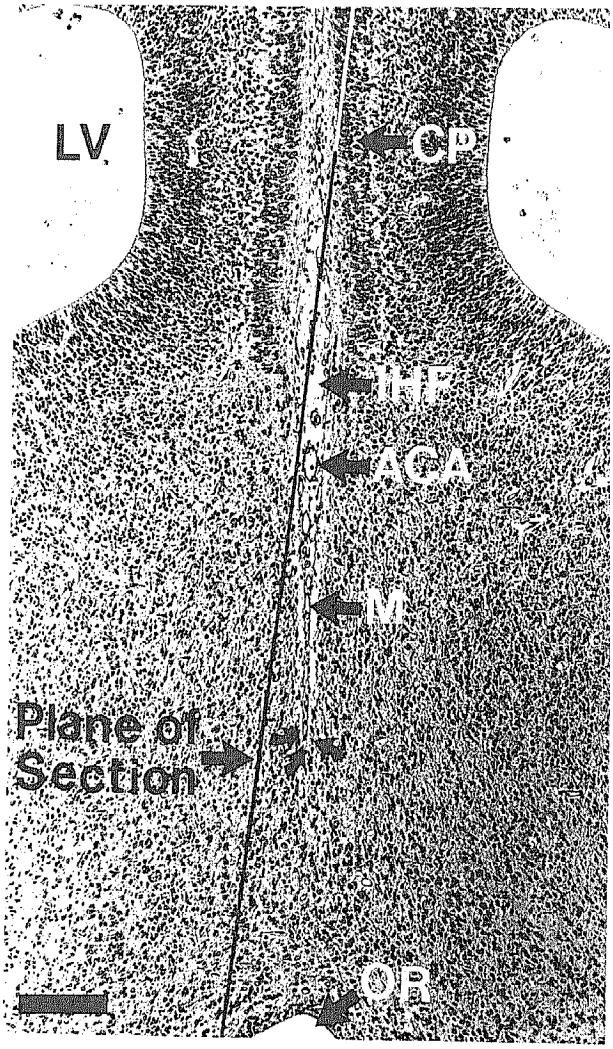

Fig. 2. Coronal section through the septal region anterior to the crossing of the anterior commissure in a BALB/c embryo weighing 0.38 g at E16 days; plastic section cut at 2 μm and stained with Methylene blue and basic fuchsin. The line represents a plane of sectioning which departs from the true sagittal plane by 10°. The dorsal portion of the section would show tightly packed cells of the cortical plate (CP) above a cell-sparse molecular layer of the right cerebral cortex and cells of the septal region of the left hemisphere. Only the portion of the section just dorsal to the anterior cerebral artery (ACA) would actually show the interhemispheric fissure (IHF) at the midsagittal plane. Small arrows indicate the ventral limit of the IHF and the meninges (M) lining the IHF. Scale bar = 200 μm.

Measurements

Cross-sectional areas were measured for the AC, the CC and 11C combined, the PSFO and the MS (see abbreviations in Table II). The dividing line between the PSFO and the MS \VW; defined in the younger embryos as the location of the flexion of the ventricular layer, which almost always coincided with the zone of first crossing of HC axons[13], and in older fetuses as the place where the I-IC was closest to the third ventricle (Fig. 3). The thickness of the MS was, recorded at 11 sites equally spaced along a line from the most dorsal LT to the PSFO boundary and perpendicular to that line (see Fig,. 4). The *(x,y)* locations of the dorsal limit of the PSF(:), the PSFO-NIS boundary and the LT were also recorded, as were the (*x*,*y*,*z*) coordinates of the AC, ACA, PC and INF. After correcting all linear measures for artifacts, as described above, distances among various pairs of points were computed.

Statistical analysis

Data were analyzed with multiple regression methods as de-scribed previously[17]. For the normal $B6D2F_2$ /J mice, the linear increase in a measure with respect to body weight was first found and then the quadratic trend was assessed by noting whether the addition of a quadratic term increased the estimated multiple R^2 significantly. Because so many tests were performed, only effects significant at $\alpha = 0.01$ were considered

worthy of attention. The significance criterion was not adjusted for the total number of tests because many effects were obvious or were known to be large from previous research.

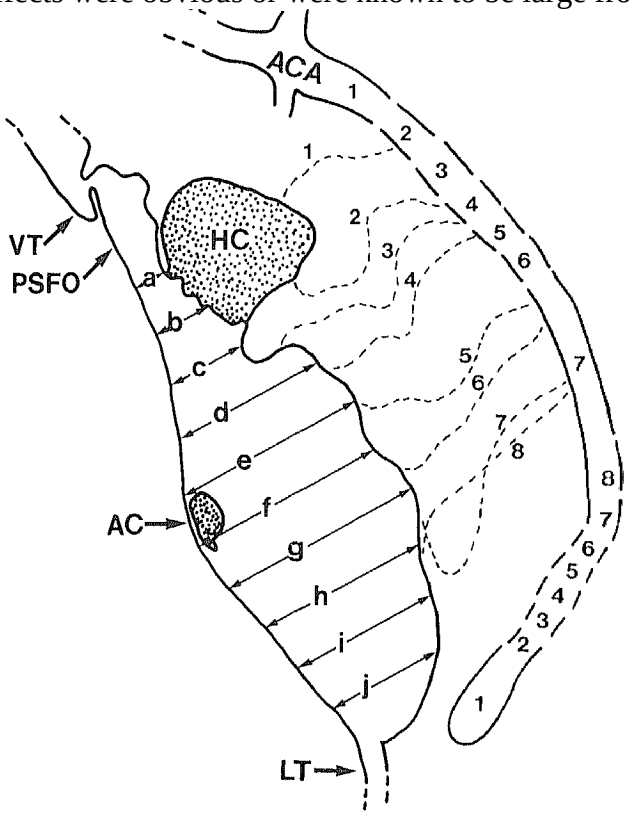

Fig. 3. Midsagittal reconstruction for B6D2F$_2$/J embryo brain. Numerals 1 to 8 represent the section number on the slide in question where the anterior cerebral artery (ACA) is exactly at midplane. The dashed lines represent the limit of contiguous septal tissue in each section. Letters a to j indicate the zones of the medial septum where thickness was measured in the reconstructed midsagittal section. Each line is perpendicular to a line joining the anterior aspects of the velum transversum (VT) and lamina terminalis (LT).

TABLE II

Abbreviations

AC	Anterior commissure
ACA	Anterior cerebral artery
CA	Cerebral aqueduct
CC	Corpus callosum
CP	Cortical plate
CP3V	Choroid plexus of the third ventricle
F	Fornix
HC	Hippocampal commissure
HbC	Habenular commissure
IHF	Interhemispheric fissure
INF	Infundibulum
LT	Lamina terminalis
LV	Lateral ventricle
M	Meninges
MS	Medial septum
OC	Optic chiasm
OR	Optic recess
PC	Posterior commissure
PSFO	Primordium of the subfornical organ
VT	Velum transversum

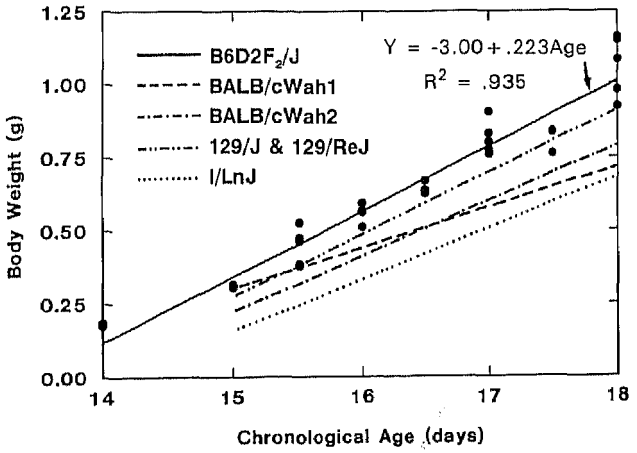

Fig. 4. Body weight versus chronological age timed from conception for the B6D2F$_2$/J normal reference group and four acallosal strains. The linear equation for the hybrids accounts for 93.5% of variance in body weight.

RESULTS

Chronological age and body growth

It is conventional to describe the developmental stage of a mouse embryo by its chronological age (E16, for example). As is widely recognized in research with amphibians[7], however, morphology may provide a superior indicator of developmental status when overall rate of growth varies substantially between individuals. This is certainly true for mice, too, because their rate of prenatal growth depends strongly on embryonic genotype and maternal environment[30]. As shown in Fig. 4, all inbred acallosal strains were smaller at a given chronological age than the normal hybrids. To discover which structures in the brain are specifically related to absent corpus callosum, it is essential that normal hybrids be compared with mice having about the same overall degree of maturity; otherwise, every feature of the brain which grows will appear to be smaller in the acallosal strains.

Previous studies have found that prior to birth, the body weight of a mouse is a reasonably good indicator of morphological maturity and is amenable to statistical analysis[5,29]. Brain size would be a superior index because prenatal brain growth is not perfectly correlated with body growth[31], but crucial tissues in the interhemispheric fissure are often damaged during brain extraction. For these reasons, growth of neural structures was assessed in relation to whole body weight.

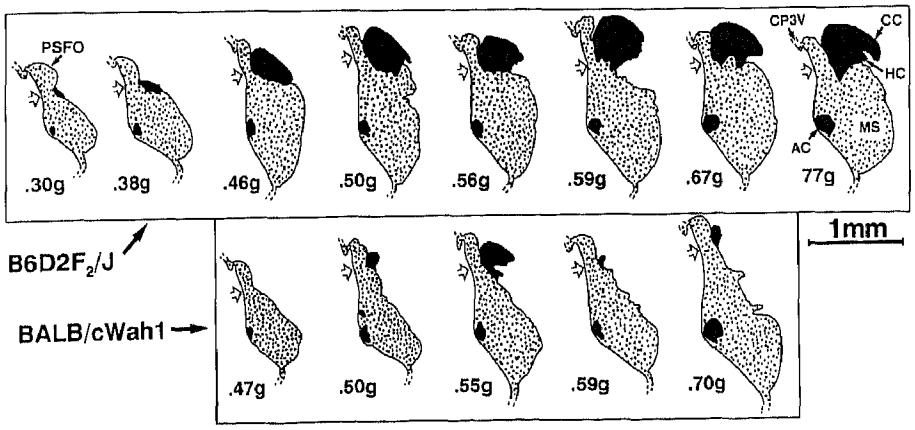

Fig. 5. Diagrams of the midsagittal plane (reconstructed) for a series of normal B6D2F$_2$/J embryos and BALB/cWah1 embryos matched for body weight. Note the location of the earliest hippocampal commissure (HC) at the ventral limit of the primordium of the subfornical organ (PSFO). The open arrows indicate the point where the HC is closest to the third ventricle.

Normal growth in hybrid mice

The major features of normal growth at the midsagittal plane in the septal region are shown in Fig. 5, where it is apparent that certain portions grow very rapidly while others change very little. Regression of each measure on body weight revealed that in almost every instance the relationship with body weight in this period of prenatal development was linear and strong $r^2 > 0.80$).

Stable zones. Two zones did not increase while nearby regions grew larger. The lamina terminalis at the base of the medial septum had a thickness of about 60 μm for all body sizes. The primordium of the subfornical organ[6], the region posterior to the hippocampal commissure, did not increase significantly in area, dorsal-ventral extent or thickness at the junction with the medial septum, although the average thickness of the PSFO did grow slightly (62 μm/g body weight, $r^2 = 0.24$, $P = 0.006$). This growth was about one-tenth the rate for the more active zones of the medial septum. This zone is evidently of some importance because the first axons to cross midplane and form the hippocampal commissure invariably do so across the dorsal surface of the medial septal region at the base of the PSFO[6]. For some reason, the HC axons do not initially grow across the PSFO. The population of surface molecules on cells of the PSFO versus those of the MS may be of great importance for guidance of HC axons.

Commissure growth. As observed by Glas[6], the earliest axons of the HC grow across the dorsal surface of the septum at midplane. Later additions to the HC grow across the existing bundle of axons and probably through it as well. The HC can usually be seen[13] at midplane when body weight is 0.4 g, and HC growth occurs rapidly in the dorsal direction, such that by 0.6 g body weight the HC is at or above the dorsal limit of the PSFO. The axons of the corpus callosum join the HC to form one large commissure, and general purpose cell or fiber stains cannot distinguish the two commissures at midplane until they are more mature[4,6,21]. Tracing callosal axons with postmortem lipophilic dyes reveals that axons from frontal cortex first reach midplane at about 0.69 g body weight in B6D2F$_2$ fetuses[17], and other evidence suggests fibres from cingulate cortex may arrive somewhat earlier[10]. In any event, the combined area of the HC plus the CC normally shows positively accelerated growth prior to birth (Fig. 6). The anterior commissure appears at midplane shortly before the HC[21,25], and it, too, shows nonlinear growth prior to birth. The fact that over 90% of variance in AC size is associated with body weight suggests that body size is a rather good indicator of brain maturity in this strain.

Medial septum. The area of the entire medial septum at midplane exhibited linear growth over a wide range (Fig. 6). Among the nine zones (b—j) in the MS where there was statistically significant growth in anterior-posterior thickness, only one central zone (MS(e), Fig. 3) showed El significant quadratic trend, which meant that linear regression analysis was particularly well suited to those measures. MS zones c—f grew at over 600 μm/g body weight, which in terms of chronological age for normal fetuses (Fig. 4) was about 150 μm/day. Growth in the dorsal-ventral dimension was also very rapid, exceeding 230 μm/ day for the distance between the LT and the ventral limit of the PSFO.

Structures beyond the septum. Distances between the AC and the ACA as well as the PC also increased rapidly, whereas the distance between the ACA and the PC actually decreased at about 130 μm/ day owing partly to a shortening of the choroid plexus of the third ventricle. Distances of the INF from the ACA and AC did not increase significantly, whereas the INF-PC distance grew but showed large variability ($r^2 = 0.32$ with respect to body weight). The INF was quite far from the other landmarks and was more prone to distortions from tissue damage during histology; hence, distances from this structure were not analyzed further. Correlations between deviations from expected values for different measures were usually positive, but they were much higher for structures closer together (Pearson $r \geq 0.7$) than for those further apart (most r's 0.1-0.3). This pattern of results implies that individuals differed primarily in the *shape* of the medial septal region and that mismatches between whole brain size and body size were generally not large (because brain—body mismatch would tend to make all correlations large).

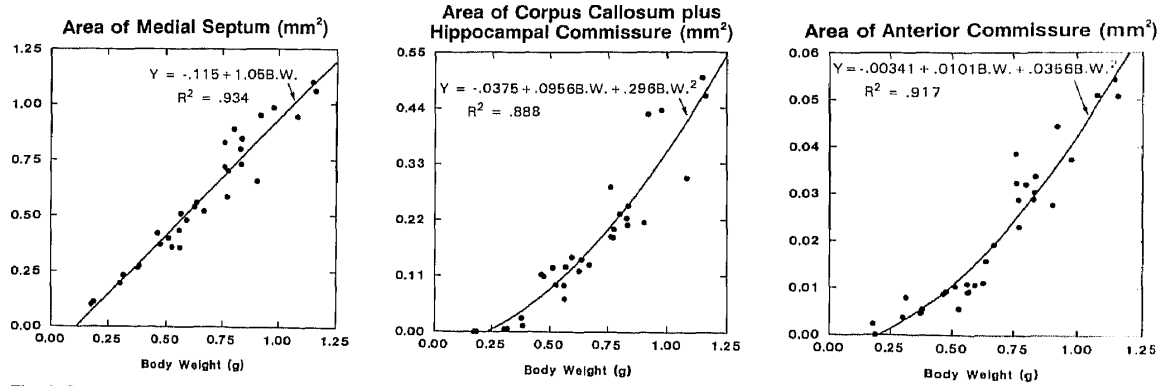

Fig. 6. Cross-sectional areas of three structures at the midsagittal plane versus body weight for normal B6D2F_2/J hybrid mice. Equations of best fit and goodness of fit (R^2) are shown.

Acallosal strains

Cross-sectional areas of the CC plus HC, AC and MS are shown in Fig. 7 for the five acallosal strains with reference to the lines of best fit for the normal hybrids from Fig. 6. Not surprisingly, the CC + HC size lagged far behind normal for all strains except ddN and a few BALB/ cWah2 mice above 0.7 g which showed signs of recovering from the severe early deficit, as observed previously[5]. The AC was remarkably normal in these strains, but the MS clearly showed slower growth on average (1.05 mm^2/g body weight for B6D2F_2 hybrids versus 0.68 mm^2/g for acallosal strains). Area of the medial septum was close to nor-mal in mice from acallosal strains weighing less than 0.5 g. It is noteworthy that even in the severely defective I/ LnJ mice which never form a CC, the size of the MS was normal until they reached 0.5 g body weight, whereupon a profound retardation of growth occurred. The ddN strain was similar to the other four acallosal strains, but its sample size and range were too small to allow adequate statistical comparisons with the other strains.

BALB / c versus normal hybrids. The strains BALB/ cWahl and BALB/ cWah2 were first compared with B6D2F_2 in a multiple regression analysis having nearly equal sample sizes per group (Table I). The F_2 hybrids were used as the reference group and dummy variables (DI and D2) indicated the difference between the mean value for each BALB/c strain and the mean of the hybrids. Body weight *(x)* was included in the equation and two other terms expressed the departure of the slope of the relation with body weight for each BALB/c group from the slope for the F_2 reference group. To achieve the best assessment of differences in slope, body weight was 'centered' for the BALB/c groups by using the difference (DX) of each animal's body weight from the strain mean (0.607 g for BALB/ cWahl and 0.657 g for BALB/cWah2), which effectively eliminated any correlation

between the interaction terms and the other independent variables. The general form of the regression equation was thus $\hat{y}\ B_0 + B_1D^1 + B_2D2 + B_3x + B_4(D1 \times DX) + B_5(D2 \times DX)$. The statistical significance of each regression coefficient (B_1 to B_5) is indicated in Table III for several measures. Results revealed that the rate of growth of the medial septum was significantly lower for the two BALB/c strains in zones MS(b) to MS(0, whereas in zones MS(h) to MS(j) and for several other measures, BALB/c and the normal controls did not differ significantly in growth rate. Because so many statistical tests were done, effects with a *t* ratio of less than 4.0 and more than — 4.0 were not considered to be major deficits. Growth in the dorsal-ventral dimension (AC to VT, AC to LT) showed no major deficit for BALB/c. The distance from AC to PC was similar in all three groups, which also suggests there was no major variation between groups in overall brain size in relation to body size. The anterior cerebral artery (ACA) tended to be closer to the anterior commissure in the BALB/c mice.

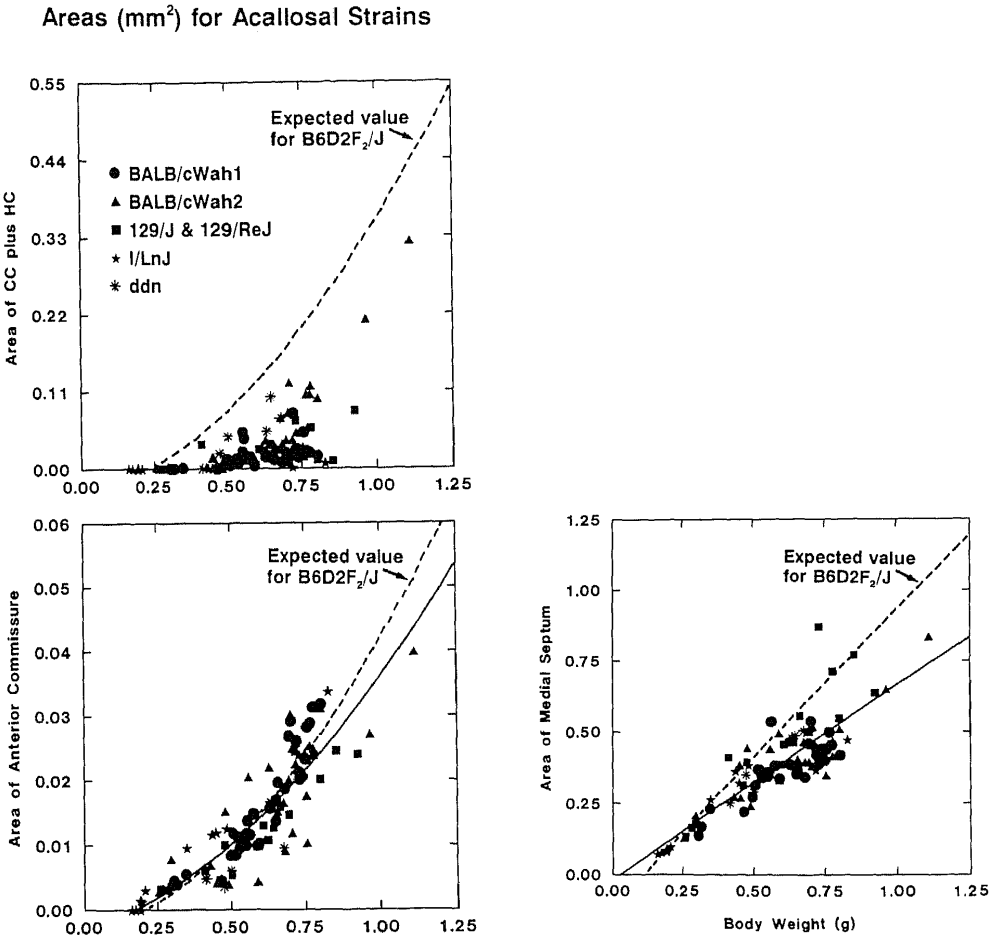

Fig. 7. Cross-sectional areas of three structures at the midsagittal plane versus body weight for mice of five acallosal strains. Dashed lines are equations of best fit for normal B6D2F$_2$/J mice shown in Fig. 6, whereas solid lines are equations of best fit for the entire sample of acallosal strains. Note that growth of the anterior commissure (AC) is reasonably normal in acallosal strains but medial septal growth is markedly retarded for all but strain 129.

The regression analysis indicates that a major deficit in BALB/c mice occurs in the anterodorsal portion of the medial septum but not the more ventral septum. The magnitudes of these effects are shown with vectors for growth rates in Fig. 8. Inspection of mice from acallosal strains revealed many instances of a distinctive gap in the medial septum where the walls of the interhemispheric fissure had not fused (Fig. 9). This was most apparent in fetuses greater than about 0.7 g body weight. The precise dorsal-ventral boundary between the abnormal and normal zones of the medial septum varied between individuals, which accounts for the reduced size of the statistical effect for zone MS(g) in Table III.

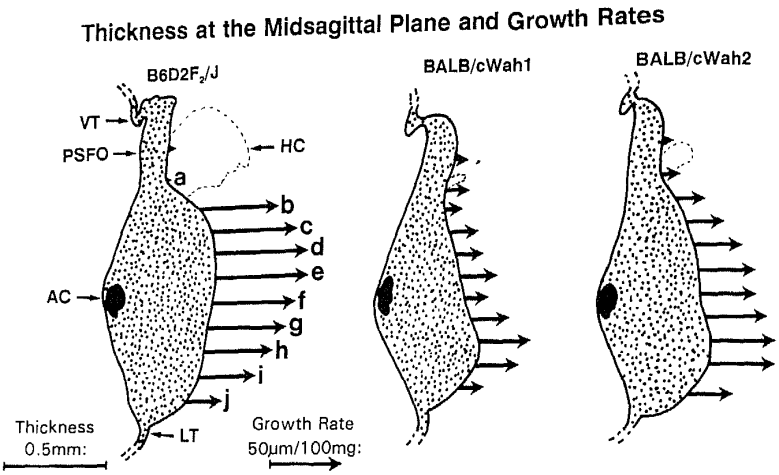

Fig. 8. Midsagittal sections for mice at 0.6 g body weight. The thickness of each zone of the medial septum is derived from a multiple regression equation and the growth rate (length of vector) at each zone is the regression coefficient or slope of change in thickness (μm) with body weight (mg). Location of the hippocampal commissure (HC) is shown by a dashed line.

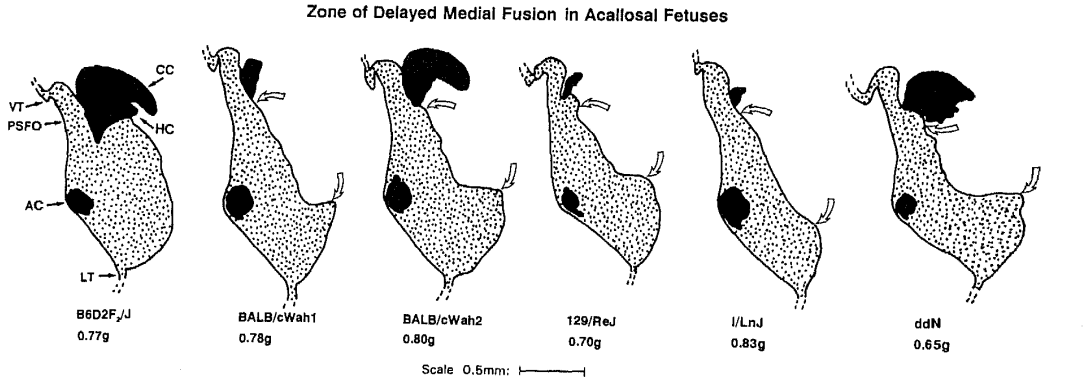

Fig. 9. Midsagittal sections through the medial septum for a normal B6D2F$_2$/J fetus and fetuses from five acallosal strains. Open arrows demarcate the limits of the unusual septal notch where the hemispheres have not yet fused in these mice.

Other strains versus BALB / c. Variation among the acallosal strains was assessed with multiple regression equations using BALB/ cWah1 as the reference group and including centered interaction terms for each strain to assess differences in rate of change with body weight. The ddN strain was not included because of a small sample size (n = 6) and narrow range of body weights. Sample sizes of the other four strains were quite discrepant, which compromised the independence of tests of slope variations. In Table IV the t ratio for an interaction effect is represented as significant only if the strain average also differed from BALB/ cWahl. Neither the BALB/ cWah2 nor I/ LnJ strain differed substantially in the medial septum from BALB/ cWahl, whereas the 129/J and ReJ strains had greater growth rates of zones c to f, precisely those septal zones which were most retarded in BALB/ cWahl compared with normal hybrids. In fact, the growth rates of zones c—f for strain 129 averaged 545 μm/g, only slightly less than the average of 626 μm/g for the normal hybrids. Although a few 129 mice showed the notch in the MS characteristic of BALB/cWahl (Fig. 9), most did not. The 129 strain apparently does not suffer the same defect of the medial septum that is seen in BALB/c and I/ LnJ mice, even though the frequency of absent or deficient corpus callosum is somewhat higher in 129 than BALB/c.

Regression analysis of commissure growth, including a quadratic term for body weight, confirmed ($P < 0.001$) the previous finding that BALB/ cWah2 recovers more quickly from the initial retardation of the CC and HC[4]. Although the deficit in HC growth and total lack of a CC for I/ LnJ is well established, its difference from BALB/ cWahl in these data was not significant, owing to small sample sizes in the more mature fetuses and large individual variations. The anterior commissure was smaller ($P < 0.01$) and grew more slowly ($P < 0.01$) in 119 than the other three strains, but the difference (see Fig. 7) was not very large and in none of the mice was it remarkably aberrant.

TABLE III

Size [a] of effects in a multiple regression equation comparing acallosal strains to the normal B6D2F$_2$ / J hybrids

Measure	R^2	*Body weight*	*BALB / cWah1*		*BALB / cWah2*		*Major deficit?*
			Mean	*Slope*	*Mean*	*Slope*	
Thickness of:							
PSFO	0.39	4.1	−2.7	−3.4	NS	NS	No
MS(a) [b]	0.37	NS	NS	3.6	NS	NS	No
MS(b)	0.75	11.1	−9.2	−4.0	−8.7	−4.2	Yes
MS(c)	0.78	11.7	−9.9	−3.8	−9.4	−4.0	Yes
MS(d)	0.80	12.6	−10.5	−4.2	−9.1	−3.7	Yes
MS(e)	0.81	13.6	−10.1	−3.8	−8.7	−3.4	Yes
MS(f)	0.76	12.6	−7.4	−4.2	−6.3	−2.9	Yes
MS(g)	0.73	12.3	−4.3	−3.2	−2.9	NS	??
MS(h)	0.80	14.6	NS	NS	NS	NS	No
MS(i)	0.80	13.8	NS	NS	NS	NS	No
MS(j)	0.58	8.3	NS	NS	NS	NS	No
LT	0.22	NS	3.5	3.5	NS	NS	No
Distance from:							
AC to VT	0.38	3.2	NS	NS	3.1	NS	No
AC to LT	0.80	15.1	NS	NS	−4.2	NS	No
AC to ACA	0.75	10.3	−6.4	NS	−7.2	NS	Yes
AC to PC	0.54	8.0	NS	NS	NS	NS	No
ACA to PC	0.57	−9.2	2.8	3.3	NS	NS	No

[a] Sizes of effects of body weight, strain mean and deviation from the slope of B6D2F$_2$ /J reference group are indicated by t ratios, each having 87 degrees of freedom. Only effects signficant at $P \leq 0.01$ are shown. NS denotes a non-significant effect with $P > 0.01$.

[b] Locations of zones of the medial septum (MS) are indicated in Figs. 3 and 8.

TABLE IV

Significance (t ratio) of difference from BALB / cWah1 reference group in a multiple regression equation [a]

Measure	R^2	*Body weight*	*BALB / cWah2*	*129 / J & ReJ*	*I / LnJ*
Thickness of:					
PSFO	0.50	4.05	NS	4.56	NS
MS(a)	0.52	5.58	NS	−3.55 [b]	NS
MS(b)	0.56	2.46	3.06	NS	NS
MS(c)	0.57	2.06	NS	3.86 [b]	NS
MS(d)	0.61	NS	NS	4.83 [b]	NS
MS(e)	0.64	2.66	NS	3.99	NS
MS(f)	0.64	NS	NS	3.55 [b]	NS
MS(g)	0.68	2.88	NS	NS	NS
MS(h)	0.78	5.34	NS	NS	−2.74
MS(i)	0.78	5.29	NS	NS	NS
MS(j)	0.65	2.77	NS	3.14	NS
LT	0.22	NS	NS	−3.43	NS
Distance from:					
AC to VT	0.57	5.05	NS	NS	NS
AC to LT	0.82	7.30	NS	NS	NS
AC to ACA	0.62	4.80	NS	NS	NS
AC to PC	0.55	3.03	NS	5.25	NS
ACA to PC	0.72	NS	NS	5.97	4.63

[a] Only strain effects significant at $P \leq 0.01$ with 84 degrees of freedom are shown, whereas nonsignificant effects are indicated as NS. Details of equations are given in the text. Measures are the same as in Table III.

[b] Slope of relation with body weight also differs from BALB/cWah1 slope at $P \leq 0.01$.

Recombinant inbred strains

The same measures of the medial septa] region were also collected for the seven Bailey recombinant inbred (RI) strains and their progenitor strains BALB/cByJ, which suffers CC defects with moderate frequency[29], and C57BL/6ByJ, which has normal CC. If the difference between the BALB/c and C57BL/6 parents involves a major gene effect, the recombinant strains formed by inbreeding separate families from the F_2 hybrid cross should resemble one parent or the other and there should be none intermediate or more extreme than either parent[2]. Assessment of a large number of measures could easily find one or two for which the RI strains form two clusters purely by chance. Consequently, this third phase of the study focused on only a few measures. Of greatest interest was the thickness of medial septal zones b—f which differed to the largest extent in BALB/ cWah mice (Table III). Two new variables were created: the average thickness of the five zones b—f and the average thickness of the more ventral zones h, i and j, which were not abnormal in acallosal strains. Multiple regression analysis with dummy variables for strain was used to compare BALB/ cByJ and the RI strains to the C57BL/6ByJ reference group.

For the average of the more dorsal zones b—f, the equation with body weight and eight dummy variables yielded $R^2 = 0.76$, whereas adding eight interaction terms expressing strain differences in growth rate significantly *($F = 30.7$,* df = 8,65, $P < 0.0001$) increased the fit of the equation to $R^2 = 0.84$. *Two* of the strains had lower growth rate of zones b—f than the C57 reference group: the BALB/ cByJ parent *(t* = —2.83, P = 0.006) and CXBG/ By *(t* = -3.21, P 0.002). As shown in Fig. 10, BALB/ cByJ and CXBG/By were very similar to the BALB/cWahl and 2 strains, but these four had much lower slopes than B6D2F$_2$/J, C57BL/6ByJ and the other RI strains. The presence of two clusters of strains having different rates of growth of the anterodorsal septum at midplane provides strong evidence for the presence of a major gene effect.

Results were very different for the average thickness of zones h, i and j. The regression equation complete with interaction terms revealed that only the CXBD/ By strain differed significantly from C57BL/6ByJ, but this could have easily occurred by chance because of the restricted range of observations for strain D. None of the other strains differed markedly from the normal F_2 hybrids, which by contrast emphasizes the importance of the

defect in the anterodorsal septum in callosal agenesis. The thickness of the PSFO did not increase with body weight or differ significantly between the Bailey strains.

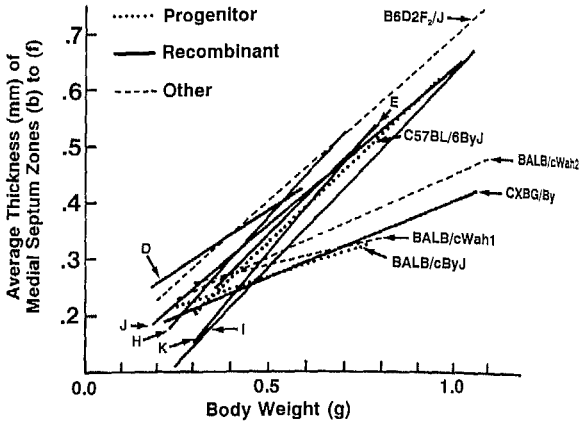

Fig. 10. Average thickness of zones (b) to (f) of the medial septum (see Fig. 3) versus body weight for the Bailey recombinant inbred strains (D, E, G, H, I, J, K; see Table I), their progenitor strains BALB/cByJ and C57BL/6ByJ, the two BALB/cWah strains, and the normal B6D2F_2/J hybrids. Each line is taken from a multiple regression equation and is drawn to span the actual range of data (Table I).

DISCUSSION

These results provide evidence of a major gene effect on growth of the dorsal septa! region at the midsagittal plane. Recombinant inbred mice are ideally suited to this kind of analysis because growth rates can be computed for each genotype, an impossible feat in segregating genetic crosses. The strength of evidence for a major gene derives not only from the multiple regression analysis of recombinant strains but also from the prediction of the specific outcome from independent facts. The probable zone of defect was first established by comparing BALB/cWah substrains with normal F_2 hybrids and then the defect was detected in precisely the one recombinant (CXBG/By) which sometimes shows deficient corpus callosum in adults[29]. Unfortunately, the Bailey strains provide only seven recombinants; hence, evidence of linkage cannot be entirely convincing[15] and cosegregation of the putative locus with a marker locus will need to be documented in backcrosses. For this purpose, fetuses greater than 0.8 g will be most appropriate because the two genotypes ought to show a bimodal distribution of septal sizes in the larger animals (Fig. 10).

The time of the initial defect in septal growth is identified within reasonably narrow limits by these data. Thickness of the medial septum is close to nor-mal in fetuses from acallosal strains weighing less than 0.5 g (Figs. 7, 10). Of course, the putative gene may act prior to this but the phenotypic consequences when it is aberrant are not apparent until the dorsal septum becomes much thicker than the primordium of the subfornical organ. In normal mice the hippocampal commissure first crosses midplane prior to 0.40 g body weight13 whereas this commissure makes a delayed appearance in acallosal strains[25]. This suggests a mid-plane defect is present prior to 0.45 g but is not yet evident in septal size. In more mature fetuses, the lack of subventricular 'sling' cells several hundred microns lateral to midplane is quite obvious in many acallosal fetuses[21,25]; however, at 0.5 g the cleft between the hemispheres is unusually deep but not remarkably wide. It is conceivable that the difficulty could originate in faulty migration of subventicular cells towards mid-plane, but it is also possible that abnormal closure of the interhemispheric fissure further impairs migration of cells into the medial septum.

There seems to be general agreement that cells separating the cerebral cortex from the septal region where callosal axons normally cross midplane consist of some kind of immature glial cells[8,21,22,32]. There is some uncertainty concerning the transcortical route of the first corpus callosum axons, which have been said to cross (a) *through* a commissural plate[18], (b) *over* the dorsal septum[6] or (c) *over* the hippocampal commissure[22]. The present observations clearly contradict (a) but cannot discriminate between (b) and (c) because the first callosal axons to arrive at midplane cannot be detected with general purpose stains. There is disagreement about the formation of the medial septum itself. Rakic and Yakovlev[18] observed fusion of 'a narrow slit-like cleft' (the

sulcus medianus telencephali medii) but not of the medial hemispheres themselves in human tissue, and Zaki[32] argued against fusion of murine tissue separated by mesenchymc. Others studying rodents attributed the medial septum to fusion of the hemispheres[19,21,25]. The present data clearly indicate a steady growth of the medial septal region, but they cannot reveal whether this occurs via a genuine fusion of the medial hemispheres, enlargement by migration of cells into the region which thereby pushes the meninges further anterior, or both processes. It can be stated with confidence, however, that the corpus callosum does not cross in the zone of rapidly growing septal tissue identified in this study; rather, it crosses near the dorsal limit of the hippocampal commissure, which itself crosses over the dorsal surface of the medial septum and rapidly fills the region anterior to the primordium of the subfornical organ. Discovering a distinct septal notch in acallosal strains does not imply that this is the only defective midplane zone. On the contrary, previous data clearly implicate the glial sling, and the zipper-like formation that precedes the anterior extension of the CC[20] may also be malformed in acallosal mice.

The presence of the septal notch in acallosal strains is one of several processes leading to absence of the adult corpus callosum. The adult defect clearly involves problems at several loci. If one of these loci yields a 'septa' notch,' there could be just one other major locus or several minor loci combining to yield a permanent structural abnormality. To distinguish these possibilities will require a much larger sample of recombinant strains. It is evident that a mouse can have the homozygous recessive genotype and yet have a normal adult corpus callosum. Almost all fetuses of the strains BALB/ cWah2 and CXBG/ By show the septal notch, yet most adults of these strains have a corpus callosum of normal size[29]. BALB/ cWah1 and BALB/ cWah2 suffer similar degrees of retarded septal growth, yet the former has a much higher frequency of severe callosal defects in the adult[26]. Presence of the septa] notch apparently acts as a risk factor which can be overcome through recovery processes which allow callosal axons to cross over the hippocampal commissure, except in the I/ LnJ strain where growth of the hippocampal commissure itself is so retarded that callosal axons cannot utilize it as a bridge. There can also be failure of corpus callosum formation when there is no septal notch, as often occurs in the 129 strain. Two strains can express a similar anatomical error for different genetic and developmental reasons. The hybrid cross of BALB/c and 129 has a normal corpus callosum[14]. This evidence of the involvement of different genetic loci in these strains is consistent with the present observations indicating that severe septal notch is prevalent only in BALB/c.

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