Genetic and developmental defects of the mouse corpus callosum

D. Wahlsten

Department of Psychology, University of Alberta, Biological Sciences Building, Edmonton (Alberta, Canada T6G 2E9)

Summary. Among adult BALB mice fewer than 20% usually have a small or absent corpus callosum (CC) and inheritance is polygenic. In the fetus at the time when the CC normally forms, however, almost all BALB mice show a distinct bulge in the interhemispheric fissure and grossly retarded commissure formation, and inheritance appears to result from two autosomal loci, provided the overall maturity of fetuses is equated. Most fetuses recover from the early defect when the CC axons manage to cross over the hippocampal commissure, and thus there is developmental compensation for a genetic defect rather than arrested midline development. The pattern of interhemispheric connections when the adult CC is very small is topographically normal in most respects, despite the unusual paths of the axons. The proportion of mice which fail to recover completely can be doubled by certain features of the maternal environment, and the severity of defects in adults can also be exacerbated by new genetic mutations which create new BALB substrains. The behavioral consequences of absent CC in mice are not known, nor have electrophysiological patterns been examined. The mouse provides an important model for prenatal ontogeny and cortical organization in human CC agenesis, because these data are not readily available for the human condition.
Key words. Recombinant inbred strain; hippocampal commissure; fetus; axon guidance; brain development; incomplete penetrance; spontaneous mutation; animal model.

Introduction

Surgical section of the human corpus callosum interferes with transmission of information between the cerebral hemispheres, producing a sort of 'split brain' syndrome[68]. The operation is usually done to prevent epileptic seizures which cannot be controlled with drugs[20,97]. On the other hand, it happens that some people never form a corpus callosum, and in these cases the neurological results are profoundly different from surgical section. Agenesis or congenital absence of the corpus callosum does not prevent interhemispheric transmission[67], although the speed of transmission may be slightly reduced because there is no direct axonal pathway[50]. Agenesis disrupts certain aspects of language and motor coordination[9,28,29], but its effects are remarkably subtle and require sophisticated neuropsychological tests to detect. The dramatic difference between absence and section of the structure indicates that there must be substantial plasticity in the processes that form the synaptic connection in the forebrain[67].

Several problems confront those wanting to know more about the causes and consequences of the defect in humans, and certain of these can be addressed by studying an animal model. The corpus callosum (CC) arises prenatally in humans and is already quite large at birth[60]. The normal sequence of events in development of the human fetal brain is fairly well known[54], but almost nothing is known about sequelae of events that result in absence of the CC. This is mainly because there is no way to know whether a fetus lacking a CC at one stage would have formed a normal structure later, but also because agenesis is so rare that an immense collection of fetuses would be needed to locate enough cases. A mouse model of CC agenesis can provide invaluable data about the embryology.

Another problem is that many cases of CC agenesis in humans come to the attention of researchers because of some form of neurological impairment or external malformation[30,65]. For example, the Andermann syndrome[43], found in a region of Quebec in Canada, also results in neuromuscular degeneration, so it is not at all obvious which symptoms should be attributed to neuromuscular defects and which are specific to the absence of the CC. Compensatory mechanisms in forebrain development would be better studied in cases where CC agenesis is the only anomaly. A mouse model can provide this feature and thus is most useful for basic research on brain development, although no mouse model is yet available for clinically significant human syndromes.

Of course, a mouse model cannot be a complete substitute for detailed investigation of the human condition, because normal mice lack many human functions, such as language. It is expected that a mouse model will have greater validity at earlier stages of ontogeny, when the embryos of mammals are remarkably similar. A primate model of CC agenesis would be ideal, but none has yet been reported. Surgical damage to tissue important for CC formation can induce a condition in the adult that is virtually the same as hereditary agenesis[45,66], and this procedure might be possible with primates, although at great expense. Nevertheless, hereditary agenesis provides certain advantages over a surgical approach and can help to clarify results of early surgical intervention, which inevitably damages tissues other than ones of primary interest.

A complete understanding of the role of the CC in cortical development and function can be attained only by integrating diverse information from several mammalian species[12,24], while being careful to note species differ-

ences. Certain aspects of cortical function may be examined best using a cat with a highly developed visual system. For example, it is now apparent that the CC of the cat is very important for establishing normal visual acuity and binocular sensitivity of cortical neurons, but that the CC can be sectioned after the end of a critical period without a noticeable loss of these functions[13–15]. Electrophysiological study of the mouse visual cortex is certainly possible[48], but testing of visual acuity and depth perception is not at all easy in this species. Certain questions are better addressed with a cat or monkey than a mouse. Concerning developmental anomalies, however, the mouse is the only available non-human source of hereditary CC absence.

The BALB mouse

A glance at current catalogs of mutant genes in the mouse[51] will locate the *ac* gene, representing 'absent corpus callosum' reported by Keeler[35] in 1933. Linkage of the gene was never determined and King expressed doubts about its mode of inheritance[37]. Extinction of the stock[36] left nothing but a rather dubious '*ac*' for posterity.

Keeler's stock was derived from descendants of mice bred by Halsey Bagg. The Bagg albino strain was the ancestor of today's BALB strains, which are now found in numerous laboratories around the world, where they are favorite subjects for studies of the immune system, cancer and behavior. Among inbred strains, BALB is almost as popular as the C57BL/6 mouse. Many investigators think that strains such as BALB, C57BL/6 and DBA/2 are 'normal' or at least free from major defects, whereas those carrying mutations such as quaking (*qk*), reeler (*rl*) and staggerer (*sg*) are 'mutants'. It turns out that almost all of these common strains have distinct anatomical or neurological anomalies. The only 'normal' mouse most researchers ever encounter is in the pantry, a barn, or a house cat's jaws.

Richard Wimer[94] reported in 1965 that the BALB/cJ and 129/J strains have absent CC, and he later encouraged me to investigate this further with the large samples which would be required. We had already found some novel defects of the fornix and anterior commissure in the A/J strain[74], but these defects seemed minor compared to the chasm in the middle of the forebrain of some BALB mice. This phenomenon caused such excitement that we immediately shifted the emphasis of our work. However, our laboratory was not the first to publish a diagram of the defect in BALB. A 1973 report[16] included photographs showing lesions of the amygdala in a C57BL/6J and a BALB/cJ mouse brain. Perhaps because the nissl stain accentuated cell bodies and spared axons, the authors and many readers failed to notice the complete absence of the CC in the BALB. Just as a chemical selectively stains certain components of the brain, so our gaze is often directed to a small region of immediate interest and we fail to observe something that could provoke even greater interest.

Richard Wimer (personal communication) has systematically surveyed stained sections of mouse brains from over 60 strains maintained at the Jackson laboratory, and his list of those with CC defects now includes I/LnJ, with total absence of the CC. Ozaki and colleagues[56] in Japan have also observed CC agenesis in their ddN strain. Thus, the acallosal BALB is hardly a rarity among laboratory mice. It was chosen for further study mainly because it was in common use and readily available from many suppliers.

Initial studies[74] were done with the BALB/cJ strain from the Jackson Laboratory, but the extremely poor reproduction of this strain in our laboratory, most evident in poor maternal behavior and lactation, prompted a search for a substrain of BALB with defects of the CC and good breeding behavior. Seven substrains from different suppliers in the United States were tested[76], and the one from Carworth Farms was chosen for further work. The Bailey (By) substrain was later added to our colony. In every substrain where at least 20 mice were examined histologically, definite abnormalities of the CC were observed. This pattern suggests strongly that the hereditary factors responsible for CC agenesis in BALB arose before the various substrains became genetically differentiated from Bagg's original stock, and it leads to a suspicion that absence of the CC in Keeler's mice resulted from hereditary material derived from the BALB ancestor.

a) *Criteria for abnormality*

In many neurological mutations, it is obvious from the behavior or brain anatomy that a certain mouse has a particular genotype. That is, the quantitative difference between the mutant and its normal littermates is so large that it is virtually qualitative. This convenient property does not occur with the CC defect in BALB, however (see fig. 1)[82]. Some adult mice have a CC that looks histologically normal, and the cross-sectional area of the CC at the mid-sagittal plane can be anywhere from 1.5 to 0.0 mm^2 [74]. It is desirable that the line between normal and abnormal CC be drawn so that no mouse from a strain lacking CC defects is classified as abnormal. Because of continued myelination of the CC for several months after birth[47, 71], the criterion is age-dependent. It need not be judged relative to brain size because the defect of CC in BALB bears no relationship with brain size[78]. These considerations lead to the conclusion that at 250 days of age an abnormal CC must have an area less than 0.85 mm^2 and length less than 3.00 mm. At 100 days of age the values are 0.82 mm^2 and 2.9 mm, respectively[79]. Properly speaking, the defect is not total absence of the CC but rather is *deficiency* of the CC.

b) *Incomplete penetrance*

According to these criteria, 11% of a sample of 656 BALB/cCF mice were abnormal in the first generation in

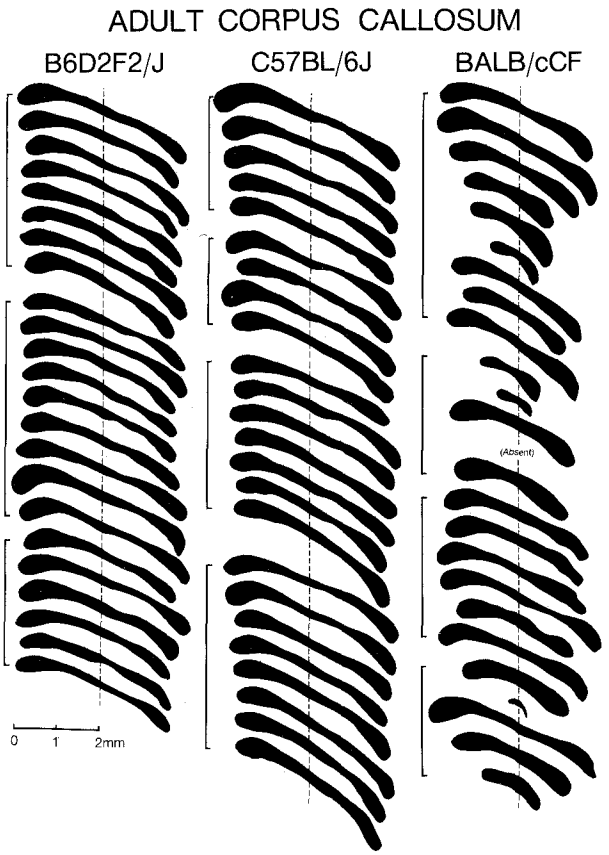

Figure 1. Outlines of the corpus callosum at the mid-sagittal plane for three strains of mice at 100 days after birth. Brackets indicate littermates. Dashed line passes through the middle of the hippocampal commissure for each brain. Anterior is to the right. Reprinted with permission from the Journal of Comparative Neurology[82]. Copyright by Alan R. Liss, Inc.

our laboratory[84]. Only 2% of a sample of 2878 brains had no CC axons crossing between the cerebral hemispheres. How could it be that inbred mice with the same genotype had radically different outcomes of forebrain development? It was conceivable that there was genetic variability within our BALB strain resulting from a spontaneous mutation or even genetic contamination from some other albino strain, which had occurred at one supplier of BALB mice[33] and is a constant danger in any breeding colony[18]. To evaluate this, breeding tests were conducted within our colony of BALB/cCF mice to contrast the hypothesis of genetic segregation against the alternative that all mice have the same probability (0.11) of showing the defect[84]. There was no significant association between the occurrence of abnormality in the parents and frequency of abnormality in the offspring, and separate families propagated by full-sib inbreeding did not differ in frequency of the defect over the first few generations. Evidently the incomplete penetrance arises from developmental rather than genetic variability.

c) *Laboratory and maternal environment*

The frequency of deficient CC in two BALB substrains as well as 129/J purchased from commercial suppliers was at least twice the frequency in their offspring bred in our laboratory at Waterloo[78], which demonstrates that the laboratory environment can alter the degree of penetrance. The Jackson laboratory husbandry procedures differ in many respects from those at Waterloo, but one aspect of the breeding regime is known to be of great importance. For reasons of productivity, most commercial suppliers of mice leave the male stud in the cage with the mother continuously , so that he can impregnate the female soon after birth of her litter because mice have a postpartum estrus. On the other hand, we routinely remove the male before the litter is born. A controlled study revealed that the frequency of CC defects in adult mice which had been in utero while their mother was nursing the first litter was 25%, much higher than the 10% seen in second litters which did not overlap the first[80]. The breeding regimen is not the only modulator of penetrance. There have been rather large fluctuations in our entire colony of BALB mice from one generation to the next, even though no females became pregnant during the postpartum estrus. For example, 12.7% of mice in generation 7 in 1980 showed deficient CC and then the frequency increased dramatically to 25.4% the next generation, which must have resulted from a change in the lab chow diet, water, bedding or some other feature of the environment common to all BALB mice in the lab[84]. Whatever the cause, it was not sufficiently strong to create overt signs of malnutrition.

d) *Substrain differentiation*

Spontaneous mutations occur in every breeding laboratory and, if a strain derived from inbred stock is perpetuated by a closed system of breeding, will accumulate gradually and give rise to a substrain genetically different from the ancestral strain. For a sample of 155 known loci in mice, the probability that a new allele is created and incorporated into an inbred strain is about 0.0004 per generation[91]. This may seem comfortably low, but one should keep in mind that the same risk occurs every generation and independently in every substrain, and that each mouse has vastly more than 155 loci in its chromosomes and mitochondria. The process proceeds inexorably, so that the more generations a substrain is removed from its ancestral stock, the more likely it now carries one or more new alleles somewhere in the genome. A mutation may not affect the particular characteristics of the brain and behavior that are under investigation at the time, but this is essentially a matter of luck.

Several substrains have been established from the original Bagg albino stock[1], and certain of these are known to differ at identified loci[63]. In many others there is a clear difference on a certain phenotype which has not yet been associated with a change in genotype. For example, the Bailey (By) substrain is much less likely to engage in fighting than the Jackson (J) substrain[63]. Regarding deficient CC in BALB, the frequency has ranged from 28% for BALB/cJ to 2% (1 of 43 mice) for BALB/cDub[76]. Of

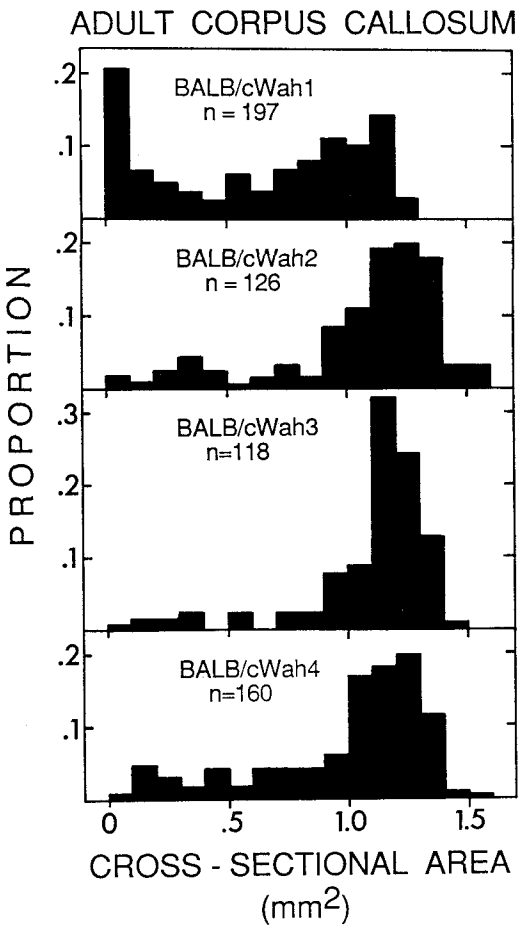

Figure 2. Frequency distributions of CC area at the mid-sagittal plane for 4 substrains of BALB/cWah mice. Reprinted with permission from the Journal of Neurogenetics[84]. Copyright by Gordon and Breach Science Publishers, Inc.

course, care must be exercised when attributing a substrain difference to genetic differentiation because environmental differences between suppliers could yield the same results.

Substrain differentiation can be seen most clearly when several strains are derived from the same ancestral stock and then propagated under identical conditions in the same laboratory. We established 16 lines from BALB mice obtained from Carworth Farms in 1976 and 1977. Over the first six generations there were no significant variations among them[84], but in 1980 one line was contaminated by a spontaneous mutation that produced a short tail, severe derangement of the foliation of the cerebellum as well as bizarre motor behavior, prompting us to call it the 'shaker short-tail' gene[86] (which turned out to be a new allele at the dreher (*dr*) locus on chromosome 1 and is now symbolized dr^{sst}). For the ongoing research program, something with impact on the CC would have been better. This fortuitous event did indeed happen in a different substrain, yielding over 50% of mice with defective CC, more than double the frequency in any other substrain[84]. After certain of the substrains had been maintained for over 20 generations by full-sib mating, they were given the international symbol 'Wah', comprising the four substrains indicated in figure 2 with stable differences in severity of CC defects. The relevant genetic difference between BALB/cWah1 and BALB/cWah2 is probably at a single locus. More recently, another spontaneous mutation producing a kinky tail and almost 100% severe deficiency of the CC has been discovered in BALB mice in our laboratory[6]. Thus, substrain differentiation can provide an invaluable source of genetic variants.

Heredity and CC in the adult

Just as the CC in the adult mammal is a very complex structure connecting diverse cortical regions homotopically as a commissure and heterotopically as a decussation via many thousands or even millions of myelinated and smaller unmyelinated axons[24], so must the genetic influences on the CC be numerous. The cross-sectional area of the adult CC must reflect the developmental rate of the animal, the number of cortical neurons sending axon collaterals, the proportion of axons with myelin, the number of lamellae in the myelin sheaths, the extent of axon loss following birth, the degree of branching of the dendrites and consequent availability of synaptic sites, etc. Several features of the CC are sensitive to environmental influences such as enriched experience[3,32] and malnutrition[40,93], and these phenomena must involve diverse physiological processes. It would not be at all surprising if the precise size of the CC is influenced by the actions of enzymes and other proteins derived from several hundred or even thousands of genetic loci on the chromosomes and mitochondria.

Genetic analysis seeks to simplify this situation so that the contributions of one or two genes can be better understood. Comparing two inbred strains, they may have different alleles at only a few loci pertinent to CC structure, whereas they may have the same genotype at hundreds of other important loci. A genetic locus may be associated with an enzyme that is of critical importance for CC formation, it may be an integral part of heredity, yet it may not be a primary cause of a strain difference. Some strains, especially substrains, differ at relatively few loci, whereas others express many allelic differences[72]. The question thus is asked: If the BALB strain has hereditary agenesis of the CC whereas the C57BL/6 strain is genetically 'normal' in this respect, how many loci are responsible for the strain difference? The answer to this question will determine the direction of future work, because powerful molecular genetic techniques will likely be fruitful only if the genetic difference is relatively simple, involving only one or two loci.

The mode of inheritance of deficient CC is clearly recessive. On every occasion when BALB was crossed with a strain having CC size in the normal range, all the F_1

hybrid offspring were normal[6, 74, 79]. There is no sex difference in frequency of absent CC[78], so the recessive gene(s) must be autosomal.

As a test for single locus Mendelian inheritance, backcrosses of an F_1 hybrid to BALB should yield 50% of mice with the homozygous recessive genotype. The expected frequency of backcross mice with deficient CC should then be half the penetrance in BALB. As shown in the table, three separate studies found the frequency of CC defects in backcrosses to be far less than expected for single locus inheritance with incomplete penetrance[6, 74, 79]. At least two loci must be involved and probably many more than two.

To proceed further in pursuit of identifiable genes, one could conduct more elaborate breeding experiments with even larger samples of adult mice to scrutinize the *outcome* of CC development, or one could look more closely at the *processes* of CC development around the time when the malformation occurs. We chose the latter.

Frequency of deficient CC in BALB parent strain and mice from backcrosses to BALB, testing a single locus hypothesis

Study	% penetrance in BALB	Crossed with	Deficient CC in backcrosses Expected	Observed	Sample size
I[a]	39.2%	A/J	19.6%	2.0%	49
II[b]	10% to 19%	A/J	6.9%	3.0%	329
		C57BL/6J	8.5%	1.1%	265
		DBA/2J	8.3%	2.1%	235
III[c]	22.9%	C57BL/6	11.4%	2.3%	473

[a] Wahlsten (1974), BALB/cJ substrain[74]. [b] Wahlsten (1982), BALB/cCF substrain[79]. Penetrance varied with year of breeding. [c] Cassells (1988), BALB/cCRBL substrain[6].

CC development in the fetus

In most placental mammals the CC axons first cross between the cerebral hemispheres in the fetus, prior to birth. If the CC is absent in the adult, it seems likely that something went wrong in the fetus. The sequence of formation of the commissures in the hybrid mouse forebrain[77] is the anterior commissure (AC) at about 14.5 days of gestation, the hippocampal commissure (HC) at 15.0 days and finally the CC at about 16.0 days. Prior to crossing of the HC and CC, the region at the middle of the forebrain consists of the thin lamina terminalis at the bottom, the primordial septum in the middle, and the primordial subfornical organ and the choroid plexus of the third ventricle at the top[19] (see fig. 3). Unlike the AC which grows *through* the septal tissue, the HC and CC cross *over* the top part of the septum[19, 66]. The CC axons usually approach the midline region via a transitory layer of cells, an extension of the subventricular zone of cells extending from the lateral ventricles towards midline, termed the 'sling' or 'scaffold' by Jerry Silver and coworkers[22, 66]. It is seen in rats[34] as well as in mice[99]. Some of the early CC axons evidently contact the previ-

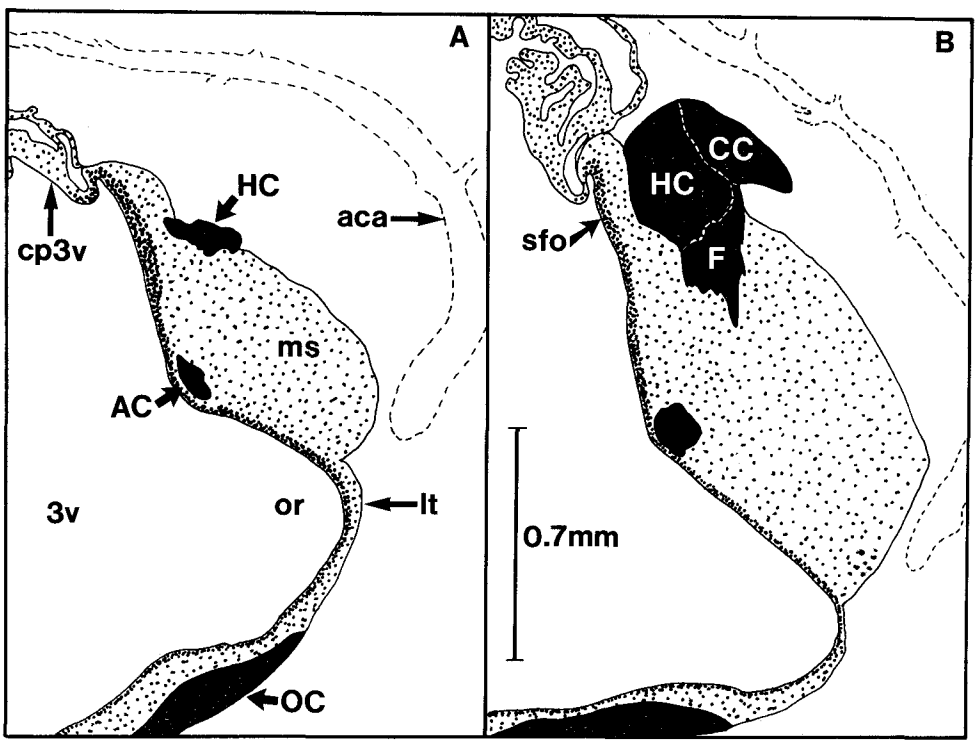

Figure 3. Diagrams of the septal region at the mid-sagittal plane in normal hybrid mouse fetuses. *A* B6D2F$_2$/J fetus at 15.5 days gestation age, weighing 0.37 g, before the first crossing of the CC. *B* B6D2F$_2$/J fetus at 16.0 days, 0.51 g, showing location of early CC. Stippling indicates neural tissue. Meninges and small blood vessels, which are abundant in the mid-sagittal region, are not shown. Abbreviations: AC, anterior commissure; CC, corpus callosum; F, fornix; HC, hippocampal commissure; aca, anterior cerebral artery; cp3v, choroid plexus of the third ventricle; lt, lamina terminalis; ms, medial septum; or, optic recess; sfo, subfornical organ; 3v, third ventricle.

ously formed HC, but most of them cross via the sling a little above and in front of the HC. Once the first wave of axons has traversed the hemispheres, later arriving ones fasciculate along previous ones, and the whole CC grows very rapidly, first to the front to form the genu (GCC) and later towards the rear to form the splenium (SCC)[81]. The CC axons extend a considerable distance into the opposite hemisphere by three days after birth in rats[26,95] and reach all layers of the cerebral cortex about one week after birth. In the ensuing weeks, large numbers of CC axons perish[38,41,70] but the size of the CC continues to increase because of myelination[47,71], which commences at about 10 days after birth in the mouse[75].

a) *Retarded development in BALB*

To identify the anatomical problem in BALB, comparisons must be made with mouse fetuses from strains that never show CC defects, such as C57BL/6 and B6D2F$_2$ hybrids. When this is done at 17.0 days gestation age, it appears that virtually *every* measure of BALB mice is significantly less than in controls because the *entire* organism is developmentally retarded prenatally. Judging from external morphology and body size, BALB fetuses are more than 1.0 days behind hybrids of the same chronological age[88]. Apparently a BALB fetus can show an unusually small or even absent CC for either of two reasons: The whole fetus may be retarded, or the CC may be small relative to whole brain or body size[81]. It is extremely important to distinguish between these alternatives, because mere retardation of overall development is not likely to produce permanent deficits specific to the CC. Indeed, experimental treatments such as prenatal ethanol can increase the frequency of absent CC in fetuses by retarding overall growth[7], yet not result in deficient CC in the adult[89]. The adult BALB brain is quite large[62] and all commissures except the CC are normal size[82]. Therefore, we want to find fetal defects which are specific to the CC and its locale. This requires that we compare BALB fetuses to control fetuses *matched* for external morphology or body size, which in turn requires that BALB and control fetuses have *different* chronological ages.

b) *Retarded commissure formation*

When BALB fetuses weighing 0.5–0.75 g at 17.0–17.5 days are compared with equivalent size control fetuses at 16.0–16.5 days, several interesting results appear[82]. a) Unlike adults, almost all BALB fetuses show obvious defects at this stage, and this complete penetrance of the BALB heredity greatly aids genetic analysis; b) the HC forms relatively late in BALB but grows at a normal rate once formed; c) there is a large bulge in the longitudinal cerebral fissure and the sling does not extend close to midline. The problem is not specific to CC axons but rather resides in the *substrate* for axon growth near midline. In most BALB fetuses the CC axons do eventually find a path via the HC, but this happens rather late on day 18 or even 19, just before birth. The later the crossing, the smaller the CC will be in the adult, because many CC axons turn longitudinally and grow into a Probst bundle[57,58,66] when they fail to locate a bridge across the gap between the hemispheres. Partial absence or reduced size of the adult CC is not the result of arrested midline development, as was previously believed[46,57,77], but is the result of compensatory or plastic processes of development.

If the CC axons have not found a path to the other hemisphere by day 19, they never will. There is a critical period for CC formation, and fetuses with the most severe defects of the sling and the largest bulge at midline are never able to cross the threshold. Precisely why there is a midline bulge and failure of sling formation remains to be learned.

Heredity and CC in the fetus

These discoveries about the fetal BALB brain were crucial for achieving a better understanding of the genetics of CC defects.

a) *Index of abnormality*

Any genetic crossing experiment which examines fetuses at the same chronological age is going to confound variation in overall rate of development with effects specific to the structure of interest. Generally speaking, hybrid fetuses develop faster than inbred fetuses[88], and development in a hybrid maternal environment is faster than in an inbred mother[4]. Furthermore, within a litter con-

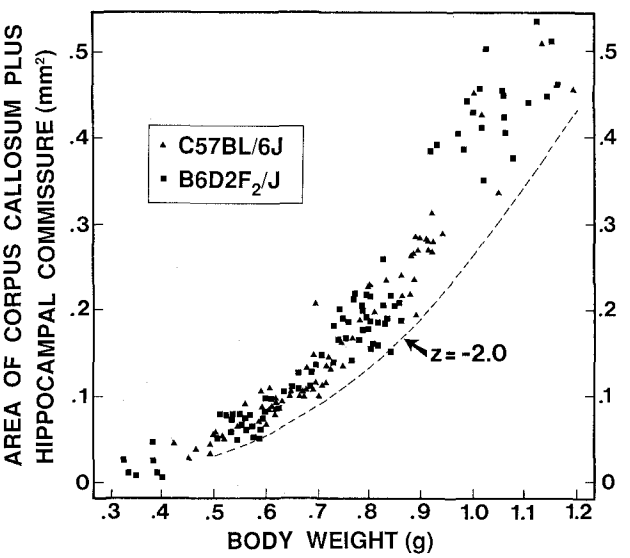

Figure 4. Area of CC plus HC at the mid-sagittal plane versus body weight for a sample of 163 normal mouse fetuses. Based upon these data, an index of abnormality was devised to express the number of standard deviations that the CC + HC value of a given fetus departs from the value expected on the basis of its body weight. A value of z below -2.0 is considered abnormal. Reprinted with permission from the Journal of Heredity[87]. Copyright by Oxford University Press.

ceived at the same time there are often large differences in rate of progress amounting to more than one day's growth at 17 or 18 days of gestation. To know whether a particular fetus has a CC or HC that is abnormally small in relation to the maturity of the whole animal, fetuses can be obtained in a fairly narrow *range* of body sizes, such as 0.5–0.8 g, and then we can construct an index of the degree of commissure retardation which will allow meaningful comparison of fetuses at different body sizes. This has been done for the combined areas of the HC and CC of a standard series of mouse fetuses (see fig. 4)[87]. The result is two quadratic equations, one providing the value of CC + HC areas expected for a fetus given its body size, and the other providing the expected standard deviation of the measure at a given body size. For any fetus in a genetic experiment, a standard score (z) is derived by dividing a) the difference between its actual CC + HC and the CC + HC value expected from its body size, by b) the expected standard deviation. A fetus with a z score less than -2.0 is considered to have abnormally retarded commissure growth.

b) *Simplified genetics*

In a series of classical crosses between BALB/cWah and C57BL/6J, the z index was very successful in correcting for maternal effects on and within-litter variations in overall rate of development[87]. As would be expected, inbred and F_1 hybrid groups with no genetic variation had variances of the z index close to 1.0. Single locus inheritance was ruled out because the distribution of scores in the backcrosses was not bimodal, but the rather high frequency of extreme negative z scores in backcross fetuses contrasted sharply with the rarity of CC defects in adults and suggested a two locus difference. Fetuses were also obtained from the Bailey strains BALB/cByJ and C57BL/6ByJ, their F_1 hybrid and their seven recombinant inbred strains (CXBD, E, G, H, I, J and K). The 10 genetic groups showed mean z scores in four clusters: (C57, F_1, CXBI), (CXBD, CXBE, CXBK), (CXBH, CXBJ) and (BALB). The only overlap between these clusters was for CXBG. Several but not all CXBG fetuses were as severely affected as extreme BALB fetuses. These data indicate two loci are involved[2].

The results for fetuses allow a prediction about CC defects in adults of the recombinant inbred strains. Data for BALB/cWah fetuses suggest that only those about -5.0 standard deviations or more below the expected value would fail to recover from the prenatal defect. Among the Bailey recombinant inbred strains, many (all but CXBI) show z scores below -2.0 but only CXBG is ever below -5.0. Therefore, only CXBG should ever show deficient CC in the adult. This was confirmed, except for one surprising CXBD strain mouse[87].

c) *In search of single gene differences*

Recombinant inbred strains provide valuable information about the number of loci producing a difference between two parent inbred strains[2], but they can also simplify the genetics even further. If BALB and C57 differ at only two loci pertinent to the CC defect, then certain pairs of recombinant inbred strains derived from them must differ at only one locus, which should greatly facilitate the search for a specific developmental process related to a specific gene. Suppose two loci are involved, called A and B. The defect in BALB is recessive, so BALB would have genotype aa bb and C57 would be AA BB. Being homozygous at one locus might produce a more severe defect than the other so that aa BB would have lower z score than AA bb. If this scheme were correct, then C57 and CXBI should be AA BB; CXBE should be AA bb; CXBH should be aa BB; and BALB should be aa bb.

Taking further advantage of the two spontaneous mutations in our lab which affected severity of CC defects[6,84], there are now available several pairs of strains which may differ at a single locus relevant to the CC defect.

BALB/cWah1	vs	BALB/cWah2
BALB/c 'tail mutant'	vs	Siblings
CXBI/By	vs	CXBE/By
C57BL/6ByJ	vs	CXBH/By
BALB/cByJ	vs	CXBE/By
BALB/cByJ	vs	CXBH/By

The difference between a particular pair will very likely involve a locus unique for that pair. There is no reason to suspect that the kinky tail mutation was at the same locus as the enhancer of CC defects in BALB/cWah1, although this is possible and warrants a direct test of allelism.

Incomplete penetrance as an analytical device

If genetically identical organisms have radically different outcomes of ontogeny, investigation of the genetics will require larger samples and more elaborate breeding tests than when penetrance is complete, and molecular genetic analysis will be especially difficult. To some researchers, deficiency of the CC may seem just too messy for effective experimentation. A closer look reveals invaluable uses of this phenomenon for the study of developmental processes.

a) *An elegant experiment of nature*

Suppose one wants to find out why a mutation causes a constellation of effects on brain structure and alters certain behaviors. For example, neurological mutations such as staggerer (*sg*) or weaver (*wv*) result in loss or deficits of several types of cells in several brain regions. Is the change in one cell type secondary to loss of another cell type and its trophic influence, or does the mutation act separately in the two cell types? The pathways of causation are extremely difficult to analyze because all

cells possess the aberrant genotype and many of them may express it simultaneously. There is irony in the fact that the very consistency of gene effects which makes genetic analysis easy makes developmental analysis difficult. Researchers have turned to chimeras formed by fusing genetically different embryos in order to determine sites of primary gene effects and secondary consequences[23,25].

In this regard, incomplete penetrance provides an elegant experiment of nature for analyzing development. In the case of the BALB mouse, genetically identical littermates conceived at the same time and nurtured in the same uterus have slightly different degrees of a defect limited to the midline of the forebrain, and these slight differences in degree are magnified by a threshold to produce radically different axonal wiring patterns in the cerebral cortex. This is achieved without surgery and its inevitable damage to the meninges and blood vessels. The phenomenon provides an excellent opportunity to investigate processes of axonal guidance and the behavioral consequences of altered cortical organization.

b) *Cortical organization in CC agenesis*

A great deal of evidence supports the existence of competition between axonal inputs from different brain regions for available synaptic sites on a neuron[10,11,59]. Experimental removal or reduction of inputs from one source often results in expanded innervation from another region[24,49,52,61,96]. These plastic processes appear to be part of the normal course of development, because projections from one region to another early in ontogeny are often diffuse and later become topographically patchy or discrete[42]. For example, a tracer molecule such as horseradish peroxidase (HRP) injected into the occipital cortex of one hemisphere a few days after birth of a rat or mouse is transported to cells of origin in most zones of the occipital cortex on the other side[24,26], whereas the same procedure done in an adult rodent reveals that callosal connections are now concentrated at the border of cortical areas 17 and 18 but are no longer abundant in the centers of these zones[98]. These patterns can be altered to some extent by experience[24,49] or by removing one eye soon after birth[52,61,96]. However, not every region in every species exhibits the diffuse to discrete transition; some connections may be discrete from their inception[8].

How is the BALB cortical organization altered when there is no CC or a very small CC? Total absence of the CC eliminates direct interhemispheric connections between most cortical regions. The callosal axons do not find an alternative route via the anterior commissure (AC)[53,85] and the size of the AC is not altered by absence of the CC[85]. The acallosal BALB mouse is not a reversion to the marsupial pattern wherein transcortical axons from the visual cortex cross via an enlarged AC[31]. Rather, putative CC axons divert to form a *novel* adult structure, the longitudinal bundle of Probst. In the Probst bundle of the ddN mouse strain there is an orderly correspondence between the area from which axons originate and their location in the longitudinal bundle[58], although the course of individual fibers appears 'tortuous and convoluted' within a general region of the bundle[57]. Axons from the Probst bundle do not enter the AC in this strain[57], either. When the CC is completely absent, the topographic pattern of ipsilateral cortical connections does not appear to be markedly abnormal in either BALB[27,53] or ddN[58], and quantitative analysis will probably be required to detect more subtle changes. Whether the putative CC axons that never cross to the opposite hemisphere form functional synapses is not yet known. No electrophysiological studies of acallosal mice have been reported.

The situation is quite different when absence of the CC is caused by prenatal gamma radiation, which destroys many of the cortical neurons that would have given rise to CC axons[64]. In this case there is no Probst bundle. Partial absence of the CC allows close examination of interhemispheric connections. It might be expected that certain cortical regions would lack all CC connections because the sparse innervation simply could not compete, or that the remaining projections would be quite diffuse. Careful tracing of pathways in BALB mice reveals that neither pattern occurs[53]. On the contrary, when the CC is very small, the topographic distribution of cortical connections shows the patchiness typical of the normal adult, but the density of interhemispheric connections is substantially reduced. This occurs despite the exceptionally long and disordered path of the axons from visual cortex to the opposite side. In the ddN mouse with a very small CC just dorsal to the HC, axons sometimes enter the Probst bundle on one side, exit the bundle and cross over the HC, then enter the bundle on the opposite side, and again leave it to reach their destination in a homotopic site in lateral cortex[58]. This evidence supports the notion that factors guiding growth of CC axons over long distances are distinct from those shaping the pattern of synapses at the destination.

c) *Behavioral consequences of absent CC*

It would be instructive to run a large number of BALB mice through a battery of tests that may be sensitive to interhemispheric communication and then process them histologically to find out which had no CC. The behavioral testing would necessarily be blind with respect to CC status because the acallosal mice have no obvious behavioral deficits. Two particular problems arise in the study of the role of the CC in BALB, however.

First, the anatomical results of Olavarria et al.[53], strongly suggest that behavioral deficits in mice with even a very small CC will be minimal. If there is going to be a substantial change in behavior, it will likely appear in mice with total absence of the CC. If a sample of mice has none or very few with total CC absence, a non-significant correlation between CC size and a measure of behavior

will be inconclusive. In some BALB substrains total CC absence occurs in only 2 or 3% of mice[84], which makes for a dreadfully inefficient experiment. The occurrence of severe defects in BALB/cWah1 should greatly aid behavioral studies (see fig. 2) because a sample of 100 mice should yield at least 20 of each extreme, making the test reasonably powerful.

Ward and co-workers[92] reported a significant correlation between area of the CC and the strength of paw preference in 129/J mice but no significant relation among BALB/cCF mice. Unfortunately, not one of the 35 BALB mice had complete absence of the CC, so further work will be needed before any firm conclusions can be drawn from a non-significant correlation.

A second problem is posed by the reliability of the behavioral tests, evident when an animal is tested twice with the same apparatus. If the reliability is relatively low, as is sometimes the case for simple tests for mice[55], a real correlation between brain structure and a behavioral process may not be apparent to the researcher because of measurement error. This has special relevance for BALB mice because the strain lacks genetic variability. Reliability of a test is not a property of the apparatus and procedure in themselves; it also depends strongly upon the magnitude of true individual differences in the population being studied. If the population contains large genetic variation, the range of individual differences will be relatively wide. On the other hand, for an inbred strain reared in a carefully controlled laboratory situation, stable individual differences ought to be rather small, and much of the variation in behavior may reflect transitory reactions to the test and day-to-day fluctuations that engender measurement error. If so, very large samples will be required to detect a real correlation amidst the noise.

d) *The origins of incomplete penetrance*

Studies of anatomical and behavioral sequelae of failure to form a CC will leave one very intriguing question unanswered. Why is it that one fetus suffers permanent absence while its littermate recovers completely? In terms of the z score of abnormality, why are some BALB fetuses below -5.0 and others are in the normal range above -2.0? Some would reply that, because BALB has no genetic variation, all individual differences in the CC must therefore arise from small differences in the uterine environment. On the other hand, there are reasons to believe a third source of individual differences exists within the organism proper and cannot be attributed to either genetic or environmental sources[69, 83], although this third source may very well interact with these variations. Several kinds of sporadic malformations give the appearance of randomness[39, 44]. Perhaps minor fluctuations in the configuration of cells at the time of a critical bifurcating process in development can create the appearance of chance[21].

Of course, the uterine environment is not uniform, and local variations *could* induce severe CC defects in certain fetuses. For example, the uterine location of male and female fetuses relative to one another creates a heterogeneity of the hormonal environment which can affect early endocrine maturation and later reproductive behavior in adult rodents[73]. The spatial distribution of males and females in the uterus is itself random[17] because of the nature of meiosis in spermatogenesis and subsequent fertilization of ova. Thus, it will be difficult to distinguish between a random pattern of defects occasioned by processes internal to the embryo and a similar pattern produced in response to randomly distributed features of the prenatal environment. Defects of the CC provide a favorable situation for assessing these alternatives.

In a sample of 52 BALB/cWah litters observed at 17.5 days of gestation, there was no clear correlation between severity of the retardation of the CC + HC index and any measure of the uterine environment, and the spatial distribution of severely and mildly abnormal fetuses did not depart significantly from randomness[5]. It may be objected that crucial features of the environment were not measured, but this possibility must be weighed against evidence that rather large experimental changes in the prenatal environment have no effect on the severity of adult CC defects[4, 89, 90] and that other influences increase the severity to some extent but never produce even close to 100% of mice with total absence of the CC[78, 80]. Either there must be really potent variations in the uterine environment which remain undetected, or the origin of the individual variation in BALB is not located in the uterine environment.

Conclusions

The BALB mouse can provide useful information about the ontogeny and cortical organization of the mammalian brain which cannot be obtained from humans. Already the discovery of dynamic processes of recovery from a severe prenatal defect in BALB has led to a new interpretation of variable degrees of the defect in adult humans. The notion that arrested midline development causes partial CC absence was originally derived from comparisons of brain anatomy of abnormal adult cases with the sequence of ontogeny in normal human fetuses. However, direct observation of mouse fetuses destined to be abnormal as adults invites a fresh look at the human data. The presence of the Probst bundle in adult mice and humans is now seen as a symptom of a prenatal defect in the substrates of axon guidance near midline rather than in the axons themselves, which appear to reach the interhemispheric fissure on schedule. The data for CC ontogeny in BALB demonstrate very clearly that genetically identical organisms can have radically different outcomes of development, depending upon the precise circumstances in each embryo and fetus. These findings

contradict the simple idea that the genes provide a 'blueprint' for brain structure. The origins of the variable outcomes are not known for sure, but available facts suggest there may be a third source of individual differences in brain structure which is neither genetic nor environmental. If so, the BALB brain will contribute greatly to the growth of theory in the behavioral and brain sciences.

Acknowledgments. Much of the research on the BALB mouse reviewed in this paper was supported by grants from the Natural Sciences and Engineering Research Council of Canada to the author as well as NSERC scholarships to Barbara Bulman-Fleming, Bryan Cassells, Jill Lyons, and Glenna Smith. Invaluable technical assistance was provided for several studies by Kathryn Blom and Walter Zagaja. Secretarial assistance from Frances Rowe of the University of Alberta for preparation of this paper is appreciated.

1 Bailey, D. W., Substrains of the BALB/c strain, in: Handbook on Genetically Standardized JAX Mice, p. 2.5. Eds H.-J. Heiniger and J. J. Dorey. Jackson Laboratory, Bar Harbor, Maine 1980.
2 Bailey, D. W., Recombinant inbred strains and bilineal congenic strains, in: The Mouse in Biomedical Research, vol. 1, pp. 223–229. Eds H. L. Foster, J. D. Small and J. G. Fox. Academic Press, New York 1981.
3 Berrebi, A. S., Fitch, R. H., Ralphe, D. L., Denenberg, J. O., Friedrick, V. L. Jr, and Denenberg, V. H., Corpus callosum: region-specific effects of sex, early experience and age. Brain Res. *438* (1988) 216–224.
4 Bulman-Fleming, B., and Wahlsten, D., Effects of a hybrid maternal environment on brain growth and corpus callosum defects of inbred BALB/c mice: A study using ovarian grafting. Exp. Neurol. *99* (1988) 636–646.
5 Bulman-Felming, B., and Wahlsten, D., Severity of corpus callosum defects in two substrains of BALB/cWah mice in relation to uterine location of fetuses. Soc. Neurosci. Abstr. *14* (1988) 827.
6 Cassells, S. B., Hereditary influences on the morphology of anterior commissure and columns of fornix in *Mus musculus*. Ph.D. dissertation, University of Waterloo 1988.
7 Cassells, B., Wainwright, P., and Blom, K., Heredity and alcohol-induced brain anomalies: Effects of alcohol on anomalous prenatal development of the corpus callosum and anterior commissure in BALB/c and C57BL/6 mice. Exp. Neurol. *95* (1987) 587–604.
8 Dehay, C., Kennedy, H., Bullier, J., and Berland, M., Absence of interhemispheric connections of area 17 during development in the monkey. Nature *331* (1988) 348–350.
9 Dennis, M., Language in a congenitally acallosal brain. Brain Lang. *12* (1981) 33–53.
10 Easter, S. S. Jr, Purves, D., Rakic, P., and Spitzer, N. C., The changing view of neural specificity. Science *230* (1985) 507–511.
11 Edelman, G. M., Neural Darwinism. Basic Books, New York 1987.
12 Elberger, A. J., The functional role of the corpus callosum in the developing visual system: a review. Progr. Neurobiol. *18* (1982) 15–79.
13 Elberger, A. J., The existence of a separate, brief critical period for the corpus callosum to affect visual development. Behav. Brain Res. *11* (1984) 223–231.
14 Elberger, A. J., Visual function alterations resulting from neonatal corpus callosum section are linked to primary visual cortex. Soc. Neurosci. Abstr. *14* (1988) 1112.
15 Elberger, A. J., and Smith, E. L. III, The critical period for corpus callosum section to affect cortical binocularity. Exp. Brain Res. *57* (1985) 213–223.
16 Elias, M. F., Dupree, M., and Eleftheriou, B. E., Differences in spatial discrimination reversal learning between two inbred mouse strains following specific amygdaloid lesions. J. comp. Physiol. Psychol. *83* (1973) 149–156.
17 Endo, A., Goto, T., and Sakai, N., Distribution by sex of mouse fetuses in the intrauterine postion. Gamete Res. *16* (1987) 79–82.
18 Festing, M. F. W., Genetic contamination of laboratory animal colonies; An increasingly serious problem. Int. Lab. Anim. Resource News *25* (1982) 6–10.
19 Glas, P., Onderzoek naar de Vroege Ontwikkeling van de Commissuren in het mediane gebied van het Telencephalon bij de Witte Muis, pp. 68–75. Drukkerij van Denderen B. V., Groningen 1975.
20 Geoffroy, G., Lassonde, M., Delisle, F., and Décarie, M., Corpus callosotomy for control of intractable epilepsy in children. Neurology *33* (1983) 891–897.
21 Grebogi, C., Ott, E., and Yorke, J. A., Chaos, strange attractors, and fractal basin boundaries in nonlinear dynamics. Science *238* (1987) 632–638.
22 Hankin, M. H., and Silver, J., Mechanisms of axonal guidance, in: Developmental Biology, vol. 2, pp. 565–604. Ed. L. W. Browder. Plenum, New York 1986.
23 Herrup, K., Role of staggerer gene in determining cell number in cerebellar cortex. I. Granule cell death is an indirect consequence of staggerer gene action. Devl Brain Res. *11* (1983) 267–274.
24 Innocenti, G. M., General organization of callosal connections in the cerebral cortex, in: Cerebral Cortex, vol. 5, pp. 291–353. Eds E. G. Jones and A. Peters. Plenum, New York 1986.
25 Inoue, Y., Mikoshiba, K., Yokoyama, M., Inoue, K., Terashima, T., Nomura, T., and Tsukada, Y., Alteration of the primary pattern of central myelin in a chimaeric environment – study of shiverer ↔ wild-type chimaeras. Devl Brain Res. *26* (1986) 239–247.
26 Ivy, G. O., Akers, R. M., and Killackey, H. P., Differential distribution of callosal projection neurons in the neonatal and adult rat. Brain Res. *173* (1979) 532–537.
27 Ivy, G. O., and Killackey, H. P., Anomalies of the corpus callosum in the mouse (BALB/cJ). Anat. Rec. *199* (1981) 124.
28 Jeeves, M. A., and Silver, P. H., The formation of finger grip during prehension in an acallosal patient. Neuropsychologia *26* (1988) 153–159.
29 Jeeves, M. A., and Temple, C. M., A further study of language function in callosal agenesis. Brain Lang. *32* (1987) 325–335.
30 Jeret, J. S., Serur, D., Wisniewski, K. E., and Lubin, R. A., Clinicopathological findings associated with agenesis of the corpus callosum. Brain Devl. *9* (1987) 255–264.
31 Johnson, J. I. Jr, Central nervous system of marsupials, in: The Biology of Marsupials, pp. 157–278. Ed. D. Hunsaker II. Academic Press, New York 1977.
32 Juraska, J. M., and Meyer, M., Environmental, but not sex, differences exist in the gross size of the rat corpus callosum. Soc. Neurosci. Abstr. *11* (1985) 528.
33 Kahan, B., Auerbach, R., Alter, B. J., and Bach, F. H., Histocompatibility and isoenzyme differences in commercially supplied 'BALB/c' mice. Science *217* (1982) 379–381.
34 Katz, M. J., Lasek, R. J., and Silver, J., Ontophyletics of the nervous system: Development of the corpus callosum and evolution of axon tracts, Proc. natl Acad. Sci. USA *80* (1983) 5936–5940.
35 Keeler, C. E., Absence of the corpus callosum as a Mendelizing character in the house mouse. Proc. natl Acad. Sci. USA *19* (1933) 609–611.
36 Keeler, C. E., Mouse News Letter No. 1 (1949) 3.
37 King, L. S., Hereditary defects of the corpus callosum in the mouse, *Mus musculus*. J. comp. Neurol. *64* (1936) 337–363.
38 Koppel, H., and Innocenti, G. M., Is there a genuine exuberancy of callosal projections in development? A quantitative electron microscopic study in the cat. Neurosci. Lett. *41* (1983) 33–40.
39 Kurnit, D. M., Layton, W. M., and Matthysse, S., Genetics, chance, and morphogenesis. Am. J. hum. Genet. *41* (1987) 979–995.
40 Lai, M., and Lewis, P. D., Effects of undernutrition on myelination in rat corpus callosum. J. comp. Neurol. *193* (1980) 973–982.
41 LaMantia, A.-S., and Rakic, P., The number, size, myelination, and regional variation of axons in the corpus callosum and anterior commissure of the developing rhesus monkey. Soc. Neurosci. Abstr. *10* (1984) 1081.
42 Land, P. W., and Lund, R. D., Development of the rat's uncrossed retinotectal pathway and its relation to plasticity studies. Science *205* (1979) 698–700.
43 Larbrisseau, A., Vanasse, M., Brochu, P., and Jasmin, G., The Andermann syndrome: agenesis of the corpus callosum associated with mental retardation and progressive sensorimotor neuronopathy. Can. J. neurol. Sci. *11* (1984) 257–261.
44 Layton, W. M. Jr, Random determination of a developmental process. Reversal of normal visceral asymmetry in the mouse. J. Hered. *67* (1976) 336–338.
45 Lent, R., Neuroanatomical effects of neonatal transection of the corpus callosum in hamsters. J. comp. Neurol. *223* (1984) 548–555.
46 Loeser, J. D., and Alvord, E. C. Jr, Agenesis of the corpus callosum. Brain *91* (1968) 553–570.
47 Looney, G. A., and Elberger, A. J., Myelination of the corpus callosum in the cat: Time course, topography, and functional implications. J. comp. Neurol. *248* (1986) 336–347.

48 Métin, C., Godement, P., and Imbert, M., The primary visual cortex in the mosue: receptive field properties and functional organization. Exp. Brain Res. *69* (1988) 594–612.
49 Milleret, C., and Buser, P., Réorganisation des connexions interhémisphériques calleuses chez le chat adulte: effets de l'occlusion monoculaire après chiasmotomie, C. r. Acad. Sci. Paris. Sér. III *305* (1987) 325–330.
50 Milner, A. D., Jeeves, M. A., Silver, P. H., Lines, C. R., and Wilson, J., Reaction times to lateralised visual stimuli in callosal agenesis: stimulus and response factors. Neuropsychologia *23* (1985) 323–331.
51 Mouse News Letter No. 77 (1987) 9.
52 Olavarria, J., Malach, R., and Van Sluyters, R. C., Development of visual callosal connections in neonatally enucleated rats. J. comp. Neurol. *260* (1987) 321–348.
53 Olavarria, J., Serra-Oller, M. M., Yee, K. T., and Van Sluyters, R. C., Topography of interhemispheric connections in neocortex of mice with congenital deficiencies of the callosal commissure. J. comp. Neurol. *270* (1988) 575–590.
54 O'Rahilly, R., Muller, F., Hutchins, G. M., and Moore, G. W., Computer ranking of the sequence of appearance of 100 features of the brain and related structures in staged human embryos during the first 5 weeks of development. Am. J. Anat. *171* (1984) 243–257.
55 Ossenkopp, K.-P., Macrae, L. K., and Teskey, G. C., Automated multivariate measurement of spontaneous motor activity in mice: Time coruse and reliabilities of the behavioural measures. Pharmac. Biochem. Behav. *27* (1987) 565–568.
56 Ozaki, H. S., Murakami, T. H., Toyoshima, T., and Shimada, M., Agenesis of the corpus callosum in ddN strain mouse associated with unusual facial appearance (flat face). Neurosci. Res. *1* (1984) 81–87.
57 Ozaki, H. S., Murakami, T. H., Toyoshima, T., and Shimada, M., The fibers which leave the Probst's longitudinal bundle seen in the brain of an acallosal mouse: a study with the horseradish peroxidase technique. Brain Res. *400* (1987) 239–246.
58 Ozaki, H. S., and Shimada, M., The fibers which course within the Probst's longitudinal bundle seen in the brain of a congenitally acallosal mouse: a study with the horseradish peroxidase technique. Brain Res. *441* (1988) 5–14.
59 Rakic, P., Organizing principles for development of primate cerebral cortex, in: Organizing Principles of Neural Development, pp. 21–48. Ed. S. C. Sharma. Plenum, New York 1984.
60 Rakic, P., and Yakovlev, P. I., Development of the corpus callosum and cavum septi in man. J. comp. Neurol. *132* (1968) 45–72.
61 Rhoades, R. W., and DellaCroce, D. D., Neonatal enucleation induces an asymmetric pattern of visual callosal connections in hamsters. Brain Res. *202* (1980) 189–195.
62 Roderick, T. H., Wimer, R. E., Wimer, C. C., and Schwartzkroin, P. A., Genetic and phenotypic variation in weight of brain and spinal cord between inbred strains of mice. Brain Res. *64* (1973) 345–353.
63 Roderick, T. H., Langley, S. H., and Leiter, E. H., Some unusual genetic characteristics of BALB/c and evidence for genetic variation among BALB/c substrains, in: The BALB/c Mouse. Genetics and Immunology, pp. 9–18. Ed. M. Potter. Springer-Verlag, Berlin 1985.
64 Schmidt, S. L., and Lent, R., Effects of prenatal irradiation on the development of cerebral cortex and corpus callosum of the mouse. J. comp. Neurol. *264* (1987) 193–204.
65 Serur, D., Jeret, J. S., and Wisniewski, K., Agenesis of the corpus callosum: Clinical, neuroradiological and cytogenetic studies. Neuropediatrics *19* (1988) 87–91.
66 Silver, J., Lorenz, S. E., Wahlsten, D., and Coughlin, J., Axonal guidance during development of the great cerebral commissures: Descriptive and experimental studies, in vivo, on the role of preformed glial pathways. J. comp. Neurol. *210* (1982) 10–29.
67 Sperry, R. W., Plasticity of neural maturation. Devl Biol. Suppl. 2 (1968) 306–327.
68 Sperry, R., Some effects of disconnecting the cerebral hemispheres. Science *217* (1982) 1223–1226.
69 Spudich, J. L., and Koshland, D. E. Jr, Non-genetic individuality: chance in the single cell. Nature *262* (1976) 467–471.
70 Stanfield, B. B., Postanatal reorganization of cortical projections. The role of collateral elimination. Trends Neurosci. *7* (1984) 37–41.
71 Sturrock, R. R., Myelination of the mouse corpus callosum. Neuropath. appl. Neurobiol. *6* (1980) 415–420.
72 Taylor, B. A., Genetic relationships between inbred strains of mice. J. Hered. *63* (1972) 83–86.
73 vom Saal, F. S., The intrauterine position phenomenon: Effects on physiology, aggressive behavior and population dynamics in house mice, in: Biological Perspectives on Aggression, pp. 135–179. Eds K. Flannelly, R. Blanchard and D. Blanchard. Alan R. Liss, New York 1984.
74 Wahlsten, D., Heritable aspects of anomalous myelinated fibre tracts in the forebrain of the laboratory mouse. Brain Res. *68* (1974) 1–18.
75 Wahlsten, D., A developmental time scale for postnatal changes in brain and behavior of B6D2F_2 mice. Brain Res. *72* (1974) 251–264.
76 Wahlsten, D., Heredity and brain structure, in: Genetics, Environment and Intelligence, pp. 93–115. Ed. A. Oliverio. Elsevier, Amsterdam 1977.
77 Wahlsten, D., Pre-natal schedule of appearance of mouse brain commissures. Devl Brain Res. *1* (1981) 461–473.
78 Wahlsten, D., Deficiency of corpus callosum varies with strain and supplier of the mice. Brain Res. *239* (1982) 329–347.
79 Wahlsten, D., Mode of inheritance of deficient corpus callosum in mice. J. Hered. *73* (1982) 281–285.
80 Wahlsten, D., Mice in utero while their mother is lactating suffer higher frequency of deficient corpus callosum. Devl Brain Res. *5* (1982) 354–357.
81 Wahlsten, D., Growth of the mouse corpus callosum. Devl Brain Res. *15* (1984) 59–67.
82 Wahlsten, D., Defects of the fetal forebrain in mice with hereditary agenesis of the corpus callosum. J. comp. Neurol. *262* (1987) 227–241.
83 Wahlsten, D., Three sources of individual differences. Can. Psychol. *28*(1987) No. 2a Abstr. 640.
84 Wahlsten, D., Deficiency of the corpus callosum: Incomplete penetrance and substrain differentiation in BALB/c mice. J. Neurogenet. *5* (1989) 61–76.
85 Wahlsten, D., and Jones, G. B., Structural changes in brains of mice with agenesis of the corpus callosum. Soc. Neurosci. Abstr. *9* (1983) 494.
86 Wahlsten, D., Lyons, J. P., and Zagaja, W., Shaker short-tail, a spontaneous neurological mutant in the mosue. J. Hered. *74* (1983) 421–425.
87 Wahlsten, D., and Smith, G., Inheritance of retarded forebrain commissure development in fetal mice: Results from classical crosses and recombinant inbred strains, J. Hered. *80* (1989) 11–16.
88 Wahlsten, D., and Wainwright, P., Application of a morphological time scale to hereditary differences in prenatal mouse development. J. Embryol. exp. Morph. *42* (1977) 79–92.
89 Wainwright, P., and Fritz, G., Effect of moderate prenatal ethanol exposure on postnatal brain and behavioral development in BALB/c mice. Exp. Neurol. *89* (1985) 237–249.
90 Wainwright, P., and Gagnon, M., Effects of fasting during gestation on brain development in BALB/c mice. Exp. Neurol. *85* (1984) 223–228.
91 Ward, R., Genetic polymorphisms and additive genetic models. Behav. Genet. *15* (1985) 537–548.
92 Ward, R., Tremblay, L., and Lassonde, M., The relationship between callosal variation and lateralization in mice is genotype-dependent. Brain Res. *424* (1987) 84–88.
93 Wiggins, R. C., Myelination: a critical stage in development. Neurotoxicology *7* (1986) 103–120.
94 Wimer, R. E., Mouse News Letter No. 33 (1965) 32.
95 Wise, S. P., and Jones, E. G., The organization and postnatal development of the commissural projection of the rat somatic sensory cortex. J. comp. Neurol. *168* (1976) 313–344.
96 Wree, A., Angenendt, H.-W., and Zilles, K., The size of the zone of origin of callosal afferents projecting to the primary visual cortex contralateral to the remaining eye in rats monocularly enucleated at different postnatal ages. Anat. Embryol. *174* (1986) 91–96.
97 Wyllie, E., Corpus callosotomy for intractable generalized epilepsy. J. Pediatr. *113* (1988) 255–261.
98 Yorke, C. H., and Caviness, V. S., Jr, Interhemispheric neocortical connections of the corpus callosum in the normal mouse: A study based on anterograde and retrograde methods. J. comp. Neurol. *164* (1975) 233–246.
99 Zaki, W., Le processus dégénératif au cours du développement du corps calleux. Arch. Anat. Microsc. Morphol. exp. *74* (1985) 133–149.

0014-4754/89/090828-11$1.50 + 0.20/0