Effects of a Hybrid Maternal Environment on Brain Growth and Corpus Callosum Defects of Inbred BALB/c Mice: A Study Using Ovarian Grafting

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Abstract:
The corpus callosum of many but not all BALB/c mice is either abnormally small or absent. The basis for the defect is hereditary, but the proportion of mice expressing the anomaly can be modified by changing the early environment. The present study investigated the effects of an F₁ hybrid maternal environment, which is known to promote better fetal development than an inbred environment, on body and brain growth in BALB/c and on the incidence of the corpus callosal defect. Ovarian follicle cells from BALB/cWah (albino) mice were grafted into pigmented F₁ hybrid, BALB/c Wah, and pigmented BALB female hosts of comparable age which were subsequently mated to BALB/cWah males. Groups of unoperated BALB/cWah and pigmented BALB dams were included as controls. Data from 111 litters were analyzed. Results showed that BALB mice with F₁ mothers had heavier bodies at birth, weaning, and 100 days of age and heavier brains at 100 days than those with either grafted or ungrafted BALB mothers. The effects at birth were evident across all litter sizes (2 to 11), but those at weaning and 100 days were seen only in litters of more than 5 or 6. Neither the F₁ hybrid maternal environment nor the grafting procedure itself appeared to influence the incidence or expression of the corpus callosal defect. About 22% of the offspring from each of the three groups (ungrafted BALB, grafted BALB, and grafted F₁) showed a defective corpus callosum.

Abbreviations: AC—anterior commissure, CC—corpus callosum, HC—hippocampal commissure, GB—grafted BALB mother, GF₁—grafted hybrid mother, UGB—ungrafted BALB mother.

Article:

INTRODUCTION

The BALB/c inbred mouse strain exhibits defects of the corpus callosum (CC) such that in 15 to 30% of the animals the midplane area of the structure is severely reduced or, in a few cases, nonexistent (22). The basis for the CC defect is clearly hereditary, but differences in the frequency of BALB mice with defective CC occur among laboratories (22) and can be produced by concurrent pregnancy and lactation (23). Because critical events in the formation of the CC occur before and shortly after birth (18, 26, 27), it is likely that variations in the early maternal environment influence expression of the CC defects.

It may be significant that the maternal environment of inbred BALB/c mice is inferior to that of hybrid mice. BALB dams suffer an inordinately high number of fetal resorptions and, after birth, often neglect their pups for several hours, which may in some cases result in lasting effects on brain growth (28). On the other hand, the F₁ hybrid mice from BALB×C57 crosses have fewer resorptions, produce larger litters, and provide better postnatal care than do BALB mice. Furthermore, F₁ mice mated to BALB yield far fewer offspring with a defective CC than would result from a single gene effect (24). Although this could occur because of polygenic inheritance, it might also reflect a superior hybrid maternal environment which promotes better brain growth. (25).
The present study investigated the effects of this superior F₁ maternal environment on the body and brain growth and CC size of BALB/c mice. For the experimental group, BALB/c (albino) ovaries were grafted into F₁ hybrid dams which were subsequently mated with BALB/c males in order to produce inbred BALB mice having an F₁ hybrid maternal environment. These BALB mice were then compared with those whose BALB mother had been grafted with BALB ovaries and to those whose BALB mother had not had the operation. It is well known that pieces of host ovarian tissue accidently left behind during the delicate surgical procedure can produce viable ova (14). Therefore, in order that albino offspring from the F₁ dams could be positively identified as being of graft origin, pigmented BALB mice and C57BL/6J mice were used to produce the F₁ hybrid hosts. Consequently, all albino offspring were derived from the grafted BALB ovary, whereas those from any remaining host ovary were pigmented and therefore were not used in the analyses.

METHOD

Animals. Mice of the strains BALB/CWah2 and C57BL/6J, both highly inbred and genetically homogenous, were all bred and raised in our laboratory using full sibling mating pairs. Their origins and conditions of rearing are described elsewhere (29). BALB mice homozygous for the normal allele at the albinism locus (+/+), were produced just prior to the start of the study by mating congenic BALB mice heterozygous for albinism (+/c) and selecting from among the pigmented offspring by test crossing with albino (c/c) mice to confirm the lack of the albinism allele. The congenic BALB mice resulted from an original cross in 1974 of a BALB/cJ female with a pigmented C57BL/6J male followed by backcrosses of pigmented offspring to BALB/cJ for four generations and then to BALB/cCvah for a further 15 generations. Hereafter, the following abbreviations are used: B(c/c)—albino BALB/cCvah2; B(+/c)—pigmented BALB/cCvah heterozygous for the wild allele; B(+/+)—pigmented BALB, homozygous for the wild allele; C(+/+)—pigmented C57BL/6J; BC(+/+), CB(+/+)—reciprocal F₁ hybrids resulting from crosses between C(+/+). and B(+/+).

Ovarian Grafting. The surgical procedure was essentially that used by Jones and Krohn (7), with only minor adaptations. Half ovaries rather than whole ovaries were grafted because Stevens and others have reported that grafting half an ovary, rather than a whole ovary or a quarter of an ovary, results in a larger yield of pups (15, 20). A typical operating session involved the B(c/c) donor and both an inbred (B(c/c) or B(+/+)) and a hybrid (CB(+/+)) or BC(+/+)) host. The donor was killed with an overdose of sodium pentobarbital and the host animals were anesthetized with either Avertin (2,2,2-tribromoethanol, dose 250 mg/kg body weight) or sodium pentobarbital (80 to 90 mg/kg body weight). Donors and hosts were between 6 and 11 weeks of age at the time of their operations. Grafted dams recovered for 2 weeks after the surgery prior to being placed with a male. In total, 27 F₁ hybrid dams (BC(+/+)) and 27 BALB (B(c/c) and B(+/+)) were grafted with B(c/c) ovaries. The design also included unoperated control BALB dams (B(c/c) and B(+/+)).

Matings. With the exception of the unoperated B(+/+) controls which were mated to B(+/+) males, all females were mated to B(c/c) males. Female were group-housed two to four per cage with a male, isolated when visibly pregnant, and thereafter checked twice daily until delivery had occurred. Pups were weighed to the nearest milligram as soon after birth as possible. Weaning occurred at between 29 and 31 days after birth, at which time the pups were weighed to the nearest 0.1 g and thereafter were housed with siblings of the same sex.

Histology and Fiber Tract Measurement. At between 99 and 102 days after birth each mouse was weighed, anesthetized with an overdose of sodium pentobarbital, and perfused intracardially with 0.9% saline followed by buffered 10% Formalin. Brains were extracted and stored in Formalin for 1 week prior to being trimmed to a standard configuration (25) and weighed to the nearest milligram. The cerebellum was then removed and weighed to the nearest 0.1 mg. Five 33-μm serial frozen sections were cut from each left hemisphere starting at midplane and sections were wet-mounted using Aquamount. Midplane tracings of the CC, the anterior commissure (AC), and the hippocampal commissure (HC) were made using a Leitz tracing device at a magnification of 40x. The fiber tract areas were determined with a Hipad digitizing tablet and the digitizing morphometry subprogram of the Bioquant software package (R & M Biometrics, Nashville, Tennessee).
RESULTS

The ovarian grafting technique proved to be extremely successful. Of the 54 mice that were grafted, 50 became pregnant and gave birth to at least one litter. Furthermore, of the 324 offspring which could be positively identified using the pigmentation marker (that is, all offspring from grafted dams except grafted B(c/c)) as having been derived from either graft or host ovarian tissue, only five or about 1.5% were pigmented and derived from the remaining host ovarian tissue.

Preliminary analysis of body and brain data revealed that there were no significant \( (P > 0.05) \) differences between BALB offspring produced by BC and CB dams or between pigmented and nonpigmented control group off-spring (both grafted and ungrafted). Therefore, data were pooled for analysis to the following three groups: (i) UGB—ungrafted BALB mother, (ii) GB—grafted BALB mother, and (iii) GF\(_1\)—grafted hybrid mother.

**Corpus Callosum.** Figure 1 shows the frequency distributions of CC areas for males and females in the three groups. Considering a CC area of less than 0.8 mm\(^2\) as abnormal (24), \( \chi^2 \) tests revealed no significant group differences in the proportion of abnormally small CC areas for either males or females (all \( P > 0.05 \)). The frequencies of abnormal CC were 20.4, 25.2, and 21.1\% for groups UGB, GB, and GF\(_1\), respectively.

However, for males, a Kruskal-Wallis analysis of variance on ranks for the three groups was significant \( (\chi^2 = 13.26, P = 0.001) \). Mann-Whitney \( U \) tests revealed no difference between the two grafted groups, GB and GF\(_1\), but the pooled grafted groups had higher ranks than the ungrafted group UGB \( (z = -3.38, P = 0.001, \text{one-tailed}) \). For females, the Kruskal-Wallis ANOVA was not significant \( (\chi^2 = 5.82, P = 0.055) \), but Mann-Whitney \( U \) tests indicated that the ranks of GF\(_1\) mice were slightly higher than the UGB and GB groups \( (z = -2.14, P = 0.016, \text{one-tailed}) \). Thus, there were no apparent effects of maternal environment on the proportion with an abnormal CC but there were small effects on rank ordering of CC size. This pattern occurred because of small group differences in the normal range (CC area of 0.8 mm\(^2\) or more), as shown by the arrows indicating group means in Fig. 1. Pooling males and females in this range and using one-way analysis of variance on the three groups, planned comparisons indicated that mice from F\(_1\) hybrid mothers had a larger mean CC area than the combined UGB and GB controls \( (t = 4.9, \text{df} = 547, P < 0.001) \), whereas GB offspring had slightly greater CC size than did the UGB \( (t = 2.2, \text{df} = 547, P = 0.014) \).

![Fig. 1. Frequency distribution of corpus callosum (CC) areas. Dashed lines indicate criterion for abnormally small CC. White arrows indicate mean CC area for each group and sex within the normal size range (\(>0.8 \text{ mm}^2\)). Abbreviations: N—number of individuals of each sex in each group, UGB—offspring from ungrafted BALB dams, GB—offspring from grafted BALB dams, GF\(_1\)—offspring from grafted F\(_1\) hybrid dams.](image)

Evidently the hybrid maternal environment increased CC size only for those mice with a CC sufficiently large to be in the normal range. Further analysis revealed that this increase in CC size resulted from an increase in the size of the whole brain.
Body and Brain Weights. Analysis of body weight and brain weight data was restricted to those 111 litters which consisted of at least one member of each sex. As a result of pup mortality, litter sizes at birth and at weaning were not always the same (see Table 1). For the birth weight data the litter size at birth was the appropriate measure, whereas for the analysis of weaning and 100-day data, litter size at weaning was used. Body and brain sizes were larger for the GF$_1$ group than for the UGB or GB groups, but the grafted dams had smaller litters than the ungrafted dams (see Table 1). Because there is a strong negative relationship between litter size and body and brain weights for mice (13, 29), it was necessary to adjust the data statistically for litter size differences using multiple regression. Analysis was done of mean values separately for males and females in each litter, weighting each mean according to the number of mice on which it was based such that the sum of the weights equaled the number of litters (19). Group mean values adjusted to a common litter size of six using multiple regression are shown in Table 2.

Figure 2 presents least-squares regression lines which show the relationship between litter size and body weight at birth, weaning, and 100 days of age. At birth, there were no differences between the two BALB groups (UGB and GB) when litter size effects were taken into account. There was, however, a highly significant difference between the pooled BALB group, (grafted or ungrafted BALB mother) and GF$_1$ ($P < 0.0001$). As seen from Fig. 2a, there was no interaction between group membership and litter size at birth. In other words, BALB pups benefited from an F$_1$ hybrid prenatal maternal environment to an equivalent extent across all litter sizes. At weaning (Fig. 2b) there was no significant difference between groups UGB and GB but a highly significant difference between them and the GF$_1$ group for both males and females (both $P < 0.0001$), and there were significant interactions between group and litter size for both sexes (all $P < 0.005$). The 100-day body weight data for females (Fig. 2c) revealed no significant difference between groups UGB and GB and a highly significant difference between their combination and the GF$_1$ group ($P < 0.0001$), but no significant interaction ($P > 0.05$). For males, there was a small difference between UGB and GB (0.04 < $P < 0.05$) and a significant interaction between these two groups and litter size ($P < 0.01$). However, inspection of the data revealed that equations including these effects were not biologically realistic. Furthermore, no difference between UGB and GB had been evident at birth and weaning, when males were in fact living in the maternal environment. For presentation in Fig. 2c, data from males of groups UGB and GB were combined to form group B, which was then tested against group GF1. Males with a hybrid mother were heavier than those whose mother was a BALB ($P < 0.0001$) and the effect tended to be larger for bigger litters ($P < 0.04$).

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of litters</th>
<th>At birth*</th>
<th>At weaning*</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGB</td>
<td>39</td>
<td>7.38</td>
<td>2.55</td>
</tr>
<tr>
<td>GB</td>
<td>38</td>
<td>6.07</td>
<td>2.62</td>
</tr>
<tr>
<td>GF$_1$</td>
<td>34</td>
<td>5.76</td>
<td>2.62</td>
</tr>
</tbody>
</table>

* Including pups dead at birth.

UGB—ungrafted BALB mother, GB—grafted BALB mother, GF$_1$—grafted hybrid mother.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Brain weight (mg)</th>
<th>Cerebellum weight (mg)</th>
<th>Anterior commissure area (mm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>UGB</td>
<td>25.9</td>
<td>23.1</td>
<td>492.3</td>
<td>503.3</td>
</tr>
<tr>
<td>GB</td>
<td>26.7</td>
<td>23.0</td>
<td>494.8</td>
<td>502.1</td>
</tr>
<tr>
<td>GF$_1$</td>
<td>27.4</td>
<td>23.5</td>
<td>498.4</td>
<td>508.9</td>
</tr>
<tr>
<td>SDR</td>
<td>1.1</td>
<td>1.0</td>
<td>9.0</td>
<td>8.5</td>
</tr>
</tbody>
</table>

* See Table 1 for abbreviations.

Standard deviation of residuals derived from multiple regression using litter means separately for each sex. The degrees of freedom for residuals are 105 in all cases.
Values for 100-day brain weight (shown in Fig. 2d) and cerebellum weight also declined as a linear function of litter size. Comparing groups equated for litter size (Table 1), whole brains and cerebella of BALB mice were heavier when they experienced an F₁ hybrid maternal environment, and the effect was larger for larger litters. For all groups there was a strong positive relationship between brain weight and body weight (Pearson r ranging from 0.60 to 0.89), such that a multiple regression analysis of litter mean brain weight revealed no significant effects of group membership or interaction when litter mean body weight was included in the equation. Thus, there was no maternal environmental effect on brain weight apart from that mediated by whole body size. For the range of data in the present study, the correlations obtained from the use of the allometric formula, brain weight = a(body weight)^b, were no different from those obtained from a simple linear regression of brain weight on body weight.

**Corpus Callosum Area vs Brain Weight.** Because the hybrid maternal environment produced larger mice, it might be expected that CC size would also be larger. However, for the entire sample of 707 BALB mice this did not occur. When multiple regression was used to adjust for effects of group and sex, the semipartial correlation between CC area and brain weight (r = 0.045) was not significant (P > 0.05). The principal reason for this rather surprising result was the lack of difference in brain weight for mice with a very small CC and a normal CC, a fact which was noted previously (22). On the other hand, a similar analysis of the 550 mice with CC area of 0.8 mm² or more revealed a modest (r = 0.176) but highly significant (P <0.0001) linear semipartial correlation between CC area and brain weight. It may seem counter-intuitive from a statistical point of view that restricting the range of one variable would yield a much larger correlation, but this makes good sense neurologically. Mice with an unusually small CC are fundamentally different from their normal littermates. The growth of their CC is not sensitive to the hybrid maternal environment, whereas for their siblings in the normal range a hybrid mother yields larger brains and a modest increase in CC size.

**Anterior Commissure.** For both males and females, multiple regression of litter mean AC areas (see Table 2) on group, litter size, and their interaction indicated that mice from the GF₁ group had significantly larger values than those from groups UGB and GB combined (males, P < 0.01, females, P < 0.02). However, when brain weight was included in the equation, there were no significant group or interaction effects, which indicated that the F₁ hybrid maternal environment effect on AC was mediated by its effect on whole brain size.

**Hippocampal Commissure.** There were four animals in which the HC area was very severely reduced, a phenomenon noticed elsewhere (21), and which is always accompanied by a complete lack of CC at midline. These four extreme cases were omitted from the HC analyses. Because there was no significant correlation
between HC area and brain weight or litter size, analysis of variance was done on individual scores. Mean HC area for the six conditions of group and sex clustered in a narrow range from 0.31 to 0.32 mm\(^2\) with standard deviations of about 0.028. There were no significant effects of group, sex, or their interaction (\(P > 0.05\)).

**DISCUSSION**

Although the hybrid maternal environment was superior to that of the inbred BALB strain for overall body and brain growth, it had no effect on the incidence of the CC defect in 13ALB/c mice. However, within the normal range of CC areas, animals with hybrid mothers had a larger CC than those whose mothers were BALB. There is probably a critical CC size which must be achieved by birth in order for subsequent CC growth to show maternal environment effects.

In mice, pioneering CC fibers reach midline and cross into the contralateral hemisphere before birth (18, 27). Although the crossing of these first fibers is greatly delayed in BALB relative to normal controls that never exhibit the defect, by 19 days postconception (E19) most BALB fetuses have some fibers that have crossed the midplane (27). In fact, the proportion of animals having very small or no CC at E19 is very similar to that observed in adult BALB mice. Axons which have not crossed the midplane before birth instead remain in the ipsilateral cortex and grow longitudinally to form a Probst bundle (18, 27). In fetuses where vanguard CC fibers cross very late in gestation, a fraction enter the contralateral cortex, but the majority remain ipsilateral. Thus, the temporal organization of the direction and growth of these vanguard fibers is crucial in determining the size of the adult structure. If, at birth, enough axons have traversed midplane such that axons arriving later can fasciculate along them to produce a substantial adult structure, postnatal CC growth will then be influenced by many of the same factors which affect whole brain growth, such as a hybrid maternal environment. It is interesting that mice with a CC size in the normal range and a BALB mother had, on average, a slightly larger CC area if the mother was grafted, presumably because grafted ovaries yielded smaller litters and, hence, slightly larger brains. Therefore, the larger CC size in the normal range produced by the hybrid maternal environment as well as by the grafting procedure itself was probably a reflection of the modest association (\(r = 0.18\)) between CC size and brain size. Both a hybrid mother and smaller litter size yield mice with larger brains and consequently a larger CC, provided the CC is sufficiently large at birth to be sensitive to the family environment during its postnatal growth.

In the present study no attempt was made to distinguish experimentally between prenatal and postnatal environment effects. The critical period of I CC formation occurs prenatally (18, 27), but most of the growth of the CC occurs postnatally (26). Results from another study using ovarian grafting and surrogate fostering to separate pre- and postnatal factors, suggest that prenatal uterine effects on growth are apparent at birth but dissipate by 100 days of age, when postnatal factors have greater influence (3).

Maternal effects on growth (1, 10, 17), number of vertebrae (12), fetal malformations (8), and behavior (4, 11) in mice, as well as on pupation behavior in *Drosophila* (2) have been observed. Prenatal uterine environment and postnatal care as well as cytoplasmic influences are generally subsumed under the term maternal environment effects. In the absence of surgical manipulations or fostering, a complicated series of reciprocal crosses must be done in order to distinguish between the former two and the latter influence (25). The technique of ovarian grafting in mice, first introduced by Robertson in 1940 (16), has been used in studies concerning ageing (5, 9) and reproduction (6). Although more challenging than the related procedure of fertilized embryo transfer, ovarian grafting has the considerable advantage of yielding more than one litter per operated dam.

**REFERENCES**


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