Deficient corpus callosum in hybrids between ddN and three other abnormal mouse strains

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Abstract:
The mouse strain ddN from Japan was crossed with three other inbred strains prone to absence of the corpus callosum (BALB/cWahl, I/LnJ and 129/ReJ), and at least one brain with abnormally small corpus callosum was observed in offspring from each F_1 hybrid cross. Data for several polymorphic protein markers revealed that the four strains are not closely related genetically. Nevertheless, they share common genetic causes of an absent corpus callosum, which helps to understand why anatomical studies of ddN and BALB/c have yielded similar results. The hippocampal commissure is abnormally small in I/LnJ mice and the anterior commissure is often malformed in BALB/c mice, but both commissures in hybrids were normal, which suggests a different genetic basis for these defects and the absent corpus callosum.
Key words: Allelism; Hippocampal commissure; Anterior commissure; Neurogenetics; Mon guidance; Protein polymorphism

Article:

Neuroanotomical tract tracing has been done in two mouse strains prone to absence of the corpus callosum. In the strain BALB/c, the topographical pattern of corti-cocortical connections is remarkably normal, although reduced in density, when the corpus callosum (CC) is abnormally small, and there is no dramatic alteration of ipsilateral projections when it is absent [6]. Similarly, in the strain ddN the ipsilateral pattern appears normal when there is no CC [7]. Both strains form a longitudinal Probst bluidle when the CC axons fail to cross to the opposite hemisphere [3, 8], and in neither strain do CC axons appear to reroute through the anterior commissure [6, 8].

The question thus arises whether these two albino strains show similar neural defects because they are recently descended from a common ancestor. Alternatively, the defects may have different genetic causes, just as there are several distinct loci in mice which produce deafness or obesity. The callosal defect shows recessive inheritance in crosses with normal strains [4, 14]. If two recessive neurological mutations result in similar phenotypes because they are alleles at the same locus, then an F_1 hybrid cross should also exhibit the abnormal phenotype, as occurred with the tottering (*tg*) and leaner (now tg^{la}) genes [II] and the dreher (dr) and shaker short-tail (now dr^{sst}) genes [15]. The F_1 hybrids should be anatomically normal if the recessive mutations are at different loci, because the hybrid will be heterozygous at both loci. Normal F_1 hybrids resulted when the genetically acallosal strains BALB/cWahl and 129/ReJ were crossed [5].

The inbred ddN mice were obtained from the Kagawa Medical School, Kagawa, Japan, and then mated with BALB/cWahl at the University of Alberta. They were also crossed with the inbred strains C57BL/6J, 1/LnJ and 129/ReJ obtained from the Jackson Laboratory, Bar Harbor, ME. The ddN strain had been maintained at Kagawa with brother-sister inbreeding for 35 generations after it was obtained from Okayama University. BALB/c was started in 1913 by H. Bagg in the U.S.A., whereas ddN descended from outbred Deutschland-Densenbyo *(dd)* mice shipped from Germany to Japan before 1920 [2].

The C57 mice and their F_1 hybrids with abnormal strains never show severe deficiency or total absence of the CC [4, 12-14]. The approximate frequency of absent or abnormally small CC is 100%, 70%, 55% and 8% for the strains 1/LnJ, 129/ReJ, BALB/cWah 1 and ddN, respectively [4, 5, 8, 12, 13, plus unpublished data]. Quantitative standards for the normal range of CC sizes have been established for adult [13] and weanling mice [5], which makes it possible to assert with confidence that a single individual is abnormal. Even one abnormal CC in a cross with ddN would provide strong evidence of common genetic causes. On the other hand, failure to observe any animals with abnormal CC would be conclusive evidence of lack of allelism only when sample size is quite large.

The hybrid offspring were perfused with 10% neutral buffered formalin at 27-29 days after birth, a time when the CC is close to the adult size and any severe abnormalities are permanent. Serial frozen sagittal sections were cut at 25 μm and stained with either Sudan Black B for phospholipids or Schmued's gold chloride for myelin [10], and the midsagittal section was measured for cross-sectional areas of forebrain commissures using the Sigma Scan program (Jandel Scientific). Care was taken to distinguish between the corpus callosum and nearby tracts such as the longitudinal striae, the dorsal commissure of the fornix and the fornix superior. At this age with these methods, any CC area less than 0.55 mm^2 would be considered abnormal. Blood and kidney samples from BALB/cWahl, C57BL/6J and ddN mice were sent to Charles River Laboratories to determine genotype at 11 loci on 9 chromosomes *(Idh-1, Pep-3,Car-2,Gpd-1, Pgm1, Ldr-1, Gpi-1, Hbb, Es-1, Mod-1, Es-3).*

As shown in Fig. 1, 1/4 of ddN, 1/6 of ddN × BALB/c, 4/9 of ddN × I/Ln and 2/26 of ddN × 129 mice had abnormally small CC. The larger sample of ddN x 129 mice was collected because there were no severely abnormal offspring in the first three litters. Each cross with an abnormal strain had at least one brain with CC area less than 0.25 mm^2. Such a severe abnormality is strong evidence of common genetic causes between ddN and the other three abnormal strains. Anatomically, the small fragment of the CC seen in hybrids was dorsal to the hippocampal commissure and was virtually the same as the defect seen in BALB/c mice [12, 13].

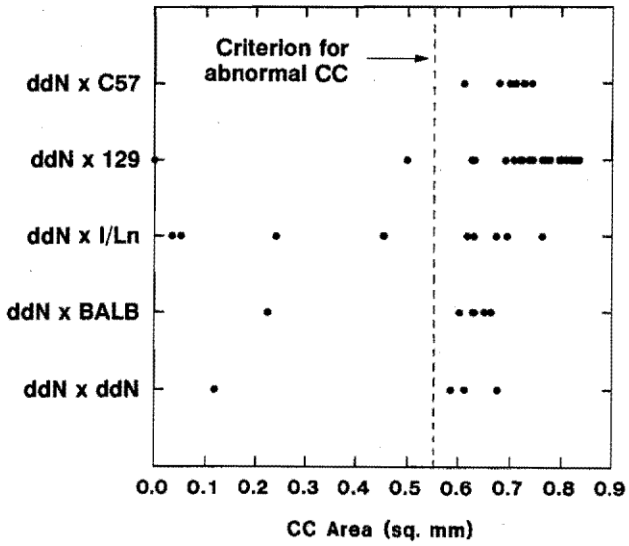

Fig. 1. Cross-sectional area of the corpus callosum at the midsagittal plane for 28-day-old mice of the ddN strain and crosses with four other inbred strains. Any brain with area less than 0.55 mm^2 was considered definitely abnormal [5].

Two other forebrain defects are noteworthy in certain of these strains. I/LnJ often shows greatly reduced hippocampal commissure [5] as a result of the same midline deficit which impedes crossing of CC axons in the embryo, and BALB/c sometimes suffers a collision between the columns of the fornix and the anterior commisure [1, 5, 12] which causes the anterior commissure to form separate bundles. Neither of these defects appeared in the ddN mice or hybrid crosses with ddN. This supports the claim that the defects of the corpus callosum and the anterior commissure are genetically distinct [1,5] and that the defect of the hippocampal commissure, although related to CC formation in the embryo, is a more severe midline anomaly than deficiency or absence of the CC [5].

Genotypes at the 11 biochemical loci revealed that ddN differs from BALB/cWahl at 4 loci, 129/J at 4 loci [9], I/LnJ at 4 loci [9] and C57BL/6J at 8 loci. The strains C57BL/6J, BALB/cWahl, I/LnJ and 129/J also differ from each other at 30% or more of a larger sample of loci [9]. These facts indicate that ddN is genetically well differentiated from all three abnormal strains but suffers absent CC for similar genetic reasons. The ddN strain is not simply a substrain of BALB/c separated recently from the ancestral line.

Absence of the corpus callosum is not a simple single-locus Mendelian trait. BALB/c, 129 and I/LnJ differ from C57BL/6J at two or more relevant loci [4, 5. 13], whereas BALB/c and 129 differ from each other by atleast two relevant loci [5]. Therefore, occurrence of CC defects in F_1 hybrids between affected strains does not by itself not prove allelism at each relevant locus, but it does demonstrate allelism at a majority of loci. Final confirmation of strain relations will require the major genes responsible for these defects of forebrain commissure development to be identified and mapped. This demonstration of allelism with the distantly related ddN strain provides encouraging evidence that the genetic architecture of the callosal defect is relatively simple.

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