Weaver Mutant Mouse Cerebellum: Defective Neuronal Migration Secondary to Abnormality of Bergmann Glia

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ABSTRACT

Previous work showed that in cerebella of mice homozygous for the autosomal mutation weapon, wv, most postmitotic granule cell neurons die during the first 2 weeks after birth close to their site of genesis in the external granular layer. Analysis of the less severely affected heterozygotes by electron microscopy and autoradiography indicates that granule cell death occurs several days after cell genesis and is secondary to failure of their somas to migrate across the molecular layer to the granular layer. This migration defect in turn appears secondary to a hitherto unrecognized disorder of Bergmann glial cells, the cells that normally guide the young neurons in their migration. In +/wv cerebella, Bergmann glial processes are enlarged and irregular in caliber, electronlucent, and often vacuolated; in wv/wv, Bergmann cell processes are almost absent. The primary genetic abnormality remains undefined, but the gene dosage effect, here recognized at a cellular level for the first time in a mammalian neurological mutant, suggests that even though neuronal death serves as the most prominent and clinically relevant phenotypic expression, the Bergmann glial abnormality may actually be closer to the primary cellular target of the wv genetic locus.

The cerebellar cortex has been the most intensively analyzed region of adult brains (1–3), and it receives comparable special emphasis during development (4). In the immature cerebellum, precursors of granule cell neurons proliferate on the external surface. The postmitotic daughter cell then undergoes a highly ordered series of transformations in shape as its soma migrates inward to the granular layer, while a T-shaped axon remains behind in the molecular layer where it contacts the growing dendrites of earlier-formed Purkinje cells (5-7). These cell relationships are essentially identical in mice (8, 9), rats (10), rhesus monkeys (6, 7), and man (11).

Recent electronmicroscopic analysis of monkey cerebellum has demonstrated that, during their entire translocation across the molecular layer during late developmental stages, young granule cell neurons are directly apposed to rectilinear, vertically oriented Bergmann glial fibers that span the entire width of the layer (6). The newly generated granule cell contacts the distal part of the Bergmann glial fiber soon after its final division or earlier; then the vertical part of the axon maintains the contact even after the granule cell soma has passed deep to the Bergmann and Purkinje cell somas and into the granular layer. (The term "contact" is used to designate side by side apposition, with the electron dense components of the surface membranes of the two cells separated by an about 180-Å space usually seen in epithelia; no other cellular processes intervene.)

Several observations suggest that this cell interrelationship is necessary for the granule cell to attain its normal position and establish its synaptic contacts (6). First, if numerous sections through a migrating granule cell are examined, the neuron–glial contact is always seen. Second, the young granule cell establishes no such relationship with any of the myriad other processes that it encounters during its passage across the complex developing molecular layer. Third, a similar relationship is seen at late stages of cerebral development in fetal monkeys, when young neurons migrating outward from their sites of genesis near the ventricular wall to the distant cortical plate are apposed during their entire migration to radially-arranged glial fiber guides (12, 13).

In both cerebellum and cerebrum these oriented glial fibers perhaps impose radial constraints and provide guidelines facilitating migration of young neurons, especially at late developmental stages when the migration route is densely packed with cell processes, many of which have already established synaptic connections (6).

Weaver (wv), a mutant mouse with an abnormal cerebellum, provides a critical test of the possible developmental significance of this neuron–glia relationship. The mutation occurred in and is maintained in the C57BL/6J strain. It is expressed as an autosomal recessive by the behavioral criteria of severe ataxia, hypotonia, and fine tremor (14, 15). Homozygous affected mice rarely survive more than 1 month after birth. Heterozygous mice are often slightly smaller in body size than normal, but do not show abnormal behavior (16).

Cerebella of 3-week-old or older wv/wv mice are drastically reduced in size. The most dramatic histological abnormality is virtual absence of the granule cell population (15). In addition, the molecular layer is attenuated, and the Purkinje cells are crowded, somewhat irregularly oriented, and moderately reduced in volume. The deficit of granule cells is accounted for by cell death in the external granular layer during the first 2 weeks after birth.

Rezai and Yoon (16) established that cerebellar development is partially affected in weaver heterozygotes, both on the inbred genetic background and where the gene was outcrossed to other backgrounds. The major defect is an abnormality of postmitotic granule cell migration. By autoradiography after injection of [3H]thymidine, they found that in the heterozygote, external granule cells proliferate normally but the daughter cells move more slowly than normal across the molecular layer, some of them never reaching the granular layer. They confirmed earlier observations that extensive cell death occurs in the external granular layer of homozygous weavers (15) and suggested, by extrapolation from their findings in heterozygotes, that cell death is a consequence of migration failure rather than the reverse.

The present study was undertaken to seek evidence for a possible abnormal developmental interaction between granule cell neurons and Bergmann glia that might underlie the migration disorder. C57BL/6J+/wv mice were mated and pro-
duced wild-type, heterozygous, and homozygous affected offspring in the expected 1:2:1 Mendelian ratios. Since the homozygous mutants die by about 3 weeks of age on the inbred genetic background, one older wv/wv male from a cross with noninbred mice was studied. In another series, the ovaries from a wv/wv female were transplanted to a normal histocompatible recipient, who was mated to a wild-type (+/+ ) male and produced a litter of 100% +/+ offspring. In both series, mice were killed and the brains were fixed by cardiac perfusion for 15-25 min with a formaldehyde-glutaraldehyde mixture in phosphate buffer (6, 7). Brains were examined at intervals between 3 and 21 days after birth, when cerebellar granule cells are forming and migrating, and at 3 months of age. Each brain was removed and bisected in the midsagittal plane. Three to four sagittal slices across one side of the cerebellum were processed for electronmicroscopic analysis; the other half of the brain was impregnated by a modification of the rapid Golgi method (6, 7). In a third series, progeny from several additional matings of C57BL/6J-+/wv mice were injected once each with 5 μCi/g of [3H]-thymidine between 3 and 21 days after birth and were killed 1 hr and either 1, 2, 5, or 10 days later; sections of cerebellum were processed for autoradiography (17).

Up to the third day after birth animals show no differences in cerebellar size, gross morphology, or cytoarchitectonic organization. As noted previously (16), the differences between normal, heterozygous, and homozygous animals appear at the fourth day, the age when granule cells begin to migrate inward from the external granular layer. Specimens taken at 10 days, when granule cell proliferation and migration reaches a peak (8), demonstrate the developmental defect vividly (Fig. 1). The cerebellar cortex in homozygous wild-type (+/+ ) animals (Fig. 1A) shows granule cells descending through the molecular layer along straight, vertically oriented Bergmann glial fibers and undergoing the same complex morphogenetic transformations that were reconstructed three-dimensionally for developing monkey cerebellar cortex (6). In 10-day old heterozygous (+/+ ) littersmates the gross size of the cerebellum is reduced by 5-10%. Cytologically it is clearly distinguishable from normal (Fig. 1B). The external granular layer is somewhat disarranged and occasionally wider than in control specimens. Purkinje cells are less strictly aligned, the granular layer is much thinner than in the control, and the molecular layer is narrower but contains about a 3-fold excess of cell bodies per unit volume (Fig. 1B). Electronmicroscopic examination establishes that these cell bodies belong to young granule cells, although the oval or round shape of many of them is a departure from the usual bipolar form of cell bodies that are in transit across the developing molecular layer. Those cells that do show typical somas appear to be properly aligned with normal Bergmann glial fibers (Fig. 2A). However, many of the cells that have anomalous shapes lie contiguous with abnormal glial processes. Others appear to have lost their direct contact with a glial cell and are in various stages of degeneration (Fig. 1B). The increased number of granule cell somas and the rounded, rather than bipolar, shapes are consistent with a delay in translocation across the molecular layer, as found by Rezai and Yoon (16).

A dramatic finding in +/+ mice is an alteration in many Bergmann glial cells. The cell bodies and proximal segments of the cytoplasmic processes in the molecular layer appear normal, but the distal half of the Bergmann fibers are often 2- to 4-times larger in diameter than normal and show varicose, irregular contours (Figs. 1B and 2B). The enlarged

![Fig. 1. Schematic drawing of the developing cerebellar cortex in 10-day-old wild-type (A), heterozygous (B), and homozygous weaver (C) littersmates. To highlight the main effects of the wv allele, the drawing has been grossly simplified by omitting dendrites and axons of Purkinje and granule cell neurons, and omitting entirely the other cell classes and afferent axons of the cerebellar cortex. (A) In normal mice, cells migrate (arrows) from the external granular layer (EG) to the granular layer (G) along straight, radially oriented Bergmann glia (black cells) whose cytoplasmic processes span the entire thickness of the molecular layer (M). (B) In heterozygous mice, a smaller than normal number of granule cell somas becomes successively translocated. Many are oval or round and lie in contact with thickened and irregular Bergmann glial fibers; some of these granule cells remain permanently in the molecular layer or degenerate (fragmented cells). (C) In homozygous affected weavers, almost no granule cells descend deep to the Purkinje cell bodies and, instead, most of them degenerate at the border between the external granular layer and the molecular layer.](image-url)
Fig. 2. Electronmicrographs of comparable regions of cerebellar cortex from weaver heterozygotes (+/w) (A–E) and homozygotes (w/w) (F) at various ages. (A) Bipolar-shaped part of a migrating cell (MC) passing inward (arrows) from the external granular layer (EGL) across the molecular layer (ML); through the whole length of the picture this young granule cell neuron is closely apposed to a Bergmann glial process (BG) and appears to ignore the other cell processes that cross its path (asterisk); 10-day-old heterozygous mouse. (B) Purkinje cell dendrite (PD) penetrates the external granular layer (EGL), although they never do so in normal specimens; the dendrite lies adjacent to an enlarged Bergmann glial fiber (BG) and terminates among glial endfeet (EF). Attenuated glial cytoplasmic processes separate the Purkinje and other cell processes from the external surface; 11-day-old heterozygous animal. (C) Abnormally positioned Purkinje dendrites (PD) in a heterozygous animal are identified by several normal features, such as multivesicular bodies (arrow), synapses on dendritic shafts (crossed arrow), and dendritic spines (asterisk); however, in areas such as this where granule cell axons are missing, the dendritic spines are devoid of synaptic contacts. (D) Many of the Bergmann glial fibers (BG) in heterozygous animals are enlarged and are composed of unusually electronlucent cytoplasm with numerous empty vacuoles (V). (E) The vacuolization (V) is more severe in
profiles are electronlucent and contain numerous empty vacuoles of various sizes (Fig. 2D). Purkinje cell dendrites are closely entwined with some of these altered glial fibers and penetrate the full thickness of the external granular layer (Fig. 2B, C). The Purkinje cells develop numerous dendritic spines despite the lack of contact with granule cell axons. Dendritic spines not only form in the absence of the usual presynaptic element, but they are then maintained indefinitely in the absence of any direct synaptic input.

Cerebella of 10-day-old we/we littermates are about one-quarter to one-third the normal size, i.e., about the size of normal 3-day cerebella. Cytoarchitecturally they are very abnormal. The external granular layer is indistinct. Postmitotic granule cells are concentrated in irregular vertical stacks in which many cells, particularly the deeper ones, are degenerating (Figs. 1C and 2F). Purkinje cells form a layer several rows thick, and there are virtually no granule cells in the usual zone below them (Fig. 1C). Radial Bergmann gli fibers are very rare, though not absent. Whereas in the normal cerebellum at this age the endfeet of Bergmann fibers form an almost continuous sheet at the external surface, the reduced number of Bergmann fibers in the homozygous affected weavers leaves large areas without an external glial coat. The radial alignment of migrating granule cells along glial fibers, an intercellular relationship so frequently encountered in ultrathin sagittal sections of the developing +/+ or +/+ we specimens, has not been observed in extensive sampling of the medial parts of the we/we cerebellum.

At subsequent developmental stages, cerebellar cytoarchitecture in the heterozygotes somewhat improves. Many of the granule cells do eventually succeed in crossing the molecular layer and reach the granular layer. However, some granule cells remain permanently in the molecular layer, and the granular layer never attains normal thickness. In homozygous affected mice, abnormalities become even more severe at later developmental stages. Most of the postmitotic granule cells that accumulate just below the proliferating cells in the external granular layer degenerate and completely disappear. Only a few granule cells, particularly in the most lateral parts of the cerebellar hemispheres, descend among the multilayered Purkinje cell somas. Those granule cells that do survive in the heterozygous and homozygous mutants form morphologically normal synaptic connections with mossy fibers, Golgi II neurons, and spines of Purkinje cell dendrites.

Our autoradiographic studies, confirming and amplyfying those of Rezai and Yoon (16), illustrate further the dramatic disturbance of cell migration. One hour after [3H]thymidine injection on P9 (Postnatal day 9) or P13, the labeling index (percent labeled cells) in the external granular layer is normal or increased in +/+ and we/we. (The apparently increased labeling index in the homozygous weaver probably reflects increased death of unlabeled cells.) 2 Days after injection on P10, somas of heavily labeled cells are in transit across the molecular layer in +/+ animals, but lie predominantly in the deepest zone of the external granular layer in we/we mice. 5 Days after labeling on P9 or P10, most of the heavily labeled cell bodies in the normal cerebellum have entered the granular layer as described earlier (8); in the heterozygote, by contrast, about half or more still lie in the molecular layer, whereas in the we/we cerebellum, only rare labeled cells have left the external granular layer. At 10-12 days after injection on P10 to P12, labeled cells again lie mainly in the granular layer in the +/+ cerebellum and are in the molecular and granular layers in +/+ we, while few labeled cells persist in the we/we specimens. Heterozygotes at 10 and 11 days after injection show a lower percentage of their labeled cells in the granular layer than wild-type animals at 5 days after injection.

The interval between cell generation and cell death was estimated by recording the percentage of pyknotic nuclei labeled in +/+ we and we/we specimens killed at various times after a single injection of [3H]thymidine on P9, 10, 11, or 12.

some areas and is accompanied by degeneration of the Bergmann fiber shafts (BG) in the molecular layer (ML) and endfeet (EF) spreading at the external surface of the cerebellum; 3-week heterozygous mouse. (F) Completely disarranged external granular layer in 10-day-old homozygous animal. This field shows one of the few Bergmann glial fibr (BG) present in the homozygous weaver cerebellum and also illustrates a representative Purkinje dendrite (PD) abnormally penetrating the markedly reduced external granular layer. Superimposed on the dendrite section are numerous profiles of dendritic spines lacking synaptic inputs. Degenerating cells (DC) are usually concentrated, as here, at the inner border of the external granular layer, where postmitotic granule cells have become stacked up through failure of their somas to translocate across the molecular layer.
1 Hr, 1 day, and 2 days after injection pyknotic nuclei are unlabeled, whereas 5 days after injection up to 10% of the pyknotic nuclei in both +/+w and w/w specimens are labeled; at 10–12 days almost all pyknotic nuclei again are unlabeled. Thus, cell death occurs in the mutant several days after cell genesis on the average, i.e., at a time when cell migration is already completed in normal mice. The failure of migration, then, precedes cell death and, as already suggested (16), may be a causal antecedent.

It is more difficult to establish whether the neuron migration disorder is traceable to the Bergmann glial defect or vice versa. The two are clearly proportional, in that (i) granule cell migration is slowed along abnormal glial fibers in heterozygotes and fails completely where Bergmann fibers are absent in homozygotes, and (ii) the defects parallel each other regionally within a given cerebellum.

We would argue from principles of pathology that the glial change is the cause of neuronal death rather than the reverse. If the Bergmann glial fibers were enlarging in response to granule cell death, one would expect them to contain the increased concentration of cytoplasmic fibrils characteristic of reactive glial cells (18), but instead they appear electronlucent and vacuolated (Fig. 2). Also, reactive glial cells persist indefinitely whereas many of the Bergmann cells in the mutant cerebellum die. A further argument against a nonspecific glial reaction is that other mutants, staggerer and reeler, on the C57BL background show increased death of young granule cells but do not display the weaver-type of Bergmann glial abnormality (ref. 19, and unpublished observations). Our speculations on the probable causal sequences in phenotypic expression are summarized in Fig. 3.

Comparison with other mutants also gives insight into a possible reason why the nonmigrating granule cells die instead of merely persisting near their site of genesis in the external granular layer. In staggerer, the granule cell—Bergmann glial relationship appears normal and migration proceeds properly, but the Purkinje cells fail to form dendritic spines, the granule cells fail to make synapses on Purkinje cells (though they do synapse on their other usual targets), and the granule cells secondarily die (Landis and Sidman, in preparation). In reeler, many populations of neurons are malpositioned, but only cerebellar granule cells are significantly reduced in number (14, 15); this reduction results in part from a decrease in granule cell genesis, but it appears in addition that those granule cells die that fail to contact Purkinje cells (Rakic and Sidman, unpublished observations). In another mutant, nervous, Purkinje cells die several weeks after birth, and in lateral areas of the cerebellum where the Purkinje loss is most severe, granule cells disappear secondarily (20). These data and our present findings in weaver, while clearly incomplete, are consistent with the view that young granule cells may be unusual among neurons in depending for their very survival upon establishment of effective contacts with Purkinje cells.

The causal chain suggested in Fig. 3 is deliberately ambiguous at the left side. The primary action and target cell of the wv gene are unknown. The gene dosage effect, i.e., the early expression of a Bergmann glial abnormality in heterozygotes and the near absence of these cells in homozygotes, implies that this phenotypic expression is likely to be reasonably close to the direct action of the wv genetic locus. Weaver is the first mammalian neurological mutant to show a dosage effect at the cellular level.

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