Fetal Rat Cerebellar Fragment Transplantation into Adult Rat Forebrain Lesion Cavities

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SUMMARY

Fragments of fetal rat cerebellar tissue were grafted into forebrain cortical lesion cavities of adult rats. After a survival ranging from 12-151 days, no graft was found to fill the cavity completely. Large neurons, occasionally grouped into nests, were identifiable from the 22nd day. Myelinated graft fibers, first seen at 32 days, failed to enter host brain. Although grafts demonstrated elements resembling cerebellar tissue, overall organization did not resemble that of the cerebellum.

KEY WORDS

cerebellum, rat, CNS transplantation, fetal, myelin

Transplantation of fetal and neonatal mammalian cerebellar tissue into mammalian host brain has been successfully accomplished by numerous investigators. In addition to homotopic transplantation of cerebellar donor tissue to host cerebellum /2,3/, cerebellar tissue has been heterotopically grafted to a variety of sites, including cerebral cortex. Specifically, fetal rat cerebellum has been grafted into adult occipital-hippocampal cortical cavities overlying the superior colliculus /1/, adult occipital-retrosplenial and parietal-hippocampal cortical cavities /6/, as well as intraparenchymally or superficially to the neonatal neocortex near the coronal suture /8/. In all of the above cases, the grafts develop features typical of cerebellum, including the development of a trilaminar cortex /1,6/, deep nuclei /6/, glomeruli, synapses, and a spectrum of cell types that are characteristic of normal cerebellar cortex /1/.

In an attempt to investigate further the developmental capabilities of heterotopically placed fetal cerebellar grafts, we transplanted fetal rat cerebellar fragments into forebrain lesion cavities of adult rats. The resulting grafts exhibited elements that were suggestive of cerebellum, but lacked the overall organizational features typical of cerebellum.

Both donors and recipients were members of an inbred colony of Long-Evans rats. Four rats, aged 2.5 to 7.5 months at the time of transplantation, acted as hosts. All surgery was performed under sterile conditions, with the host rat maintained at an acceptable plane of surgical anesthesia by intraperitoneal chloral hydrate (420 mg/kg in sterile saline). A craniotomy was performed over the right forebrain, the dura resected, and the right rostral motor cortex (areas

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Fr1, Fr2, and Fr3) /7/ removed by vacuum aspiration. The lesion cavity was transcortical, quadrilateral, and bounded by the following coordinates /7/: bregma 2.4, lateral 1.4-4, and bregma 0.6, lateral 1.4-2.4. A graft of cerebellar tissue was placed into the lesion cavity within thirty minutes after aspiration. Graft tissue was obtained from pregnant rats on the fifteenth day of gestation, with the first full day following impregnation counted as day 1. The pregnant donor rat was anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg), a midline abdominal incision was performed to expose the uterus, and a fetus was removed and immediately submerged in Hank's balanced salt solution (Sigma, modified to be calcium and magnesium free) maintained at 21-23°C. The developing cranium and soft tissues overlying the dorsal hindbrain were incised and reflected. One or two fragments of developing cerebellar tissue were dissected free with iris scissors and transferred into the lesion cavity with a pipette or spatula. The entire graft harvesting process required approximately five minutes per fetus. The total volume of tissue grafted into each host ranged from 10 to 50 μl. Upon completion of the tissue transfer, the balance of the lesion cavity was packed with gelfoam (Upjohn), the skin sutured, and the rat allowed to recover with free access to food and water.

On postoperative (PO) days 12, 22, 32, and 151 the hosts were perfused transcardially with 200-500 ml of 0.9% saline followed by 300-500 ml of 10% neutral buffered formalin (NBF). The brains were removed and fixed in 10% NBF for a minimum of two months before processing. The PO day 151 host brain was infiltrated with a solution of 30% sucrose in 10% NBF and sectioned frozen on a sliding microtome. All other brains were dehydrated through a graded series of butyl alcohol, embedded in paraffin, and sectioned on a sliding microtome. All brains were serially sectioned at 30-35 μm in the coronal plane; adjacent serial sections from each subject and through the entire extent of the graft and lesion cavity were alternately stained with cresyl violet (for Nissl substance) or Weil (for myelin) and examined microscopically.

In each of the four hosts, the lesion cavity and graft tissue were readily identifiable. In every case, the graft failed to fill the entire lesion cavity. Total graft volume increased with the duration of the post-transplantation period. On PO day 12, the graft was a single fragment (volume 0.22 mm^3) suspended within a meshwork of strands composed of fibrovascular and reactive inflammatory tissue (Fig. 1A). On PO day 22, the single graft fragment (volume 0.43 mm^3) was intimately opposed to the lateral lesion cavity wall, but connected by fibrovascular reactive tissue strands medially and occasionally ventrally (Fig. 1B). On PO day 32, the graft was present in multiple fragments (total volume 0.93 mm^3), with each fragment suspended in a manner similar to the PO day 12 graft (Fig. 1C). On PO day 151, the graft constituted a single, continuous tissue mass (volume 1.68 mm^3). Graft parenchyma was fused with the medial and lateral lesion cavity walls (Fig. 1D); a sizeable cystic space, however, persisted between the floor of the lesion cavity and the ventral surface of this graft (Fig. 2). Fibrovascular reactive tissue was sparse and closely adherent to the cavity walls. None of the grafts demonstrated evidence of florid rejection, and vascular channels were present within graft tissue at all survival periods.

The cytologic diversity and degree of cellular organization within the graft increased with the postoperative survival time. On PO day 12, the majority of cells were small to medium size (4-10 μm diameter) and intensely stained, with a high nuclear-to-cytoplasmic ratio. Cell shape ranged from round to fusiform, and no cell was observed to possess a distinct nucleus, nucleolus, peripheral rim of cytoplasm, and Nissl substance. Cell population density within the PO day 12 graft was variable, but laminar organizations and obvious groupings were lacking.

The types of cells found within the three long survival grafts were diverse and included a wide range of sizes (4-28 μm diameter), shapes, and staining characteristics. The density of cellular packing within the PO days 22, 32, and 151 grafts was highly variable, and ranged from hypo- to iso- to hyperdense relative to surrounding host cortex. From PO day 22 onward, cells arranged in nests were seen scattered throughout the grafts. A cell nest was defined as a grouping of closely packed, relatively large cells (8-22 μm diameter) with neuronal characteristics (distinct

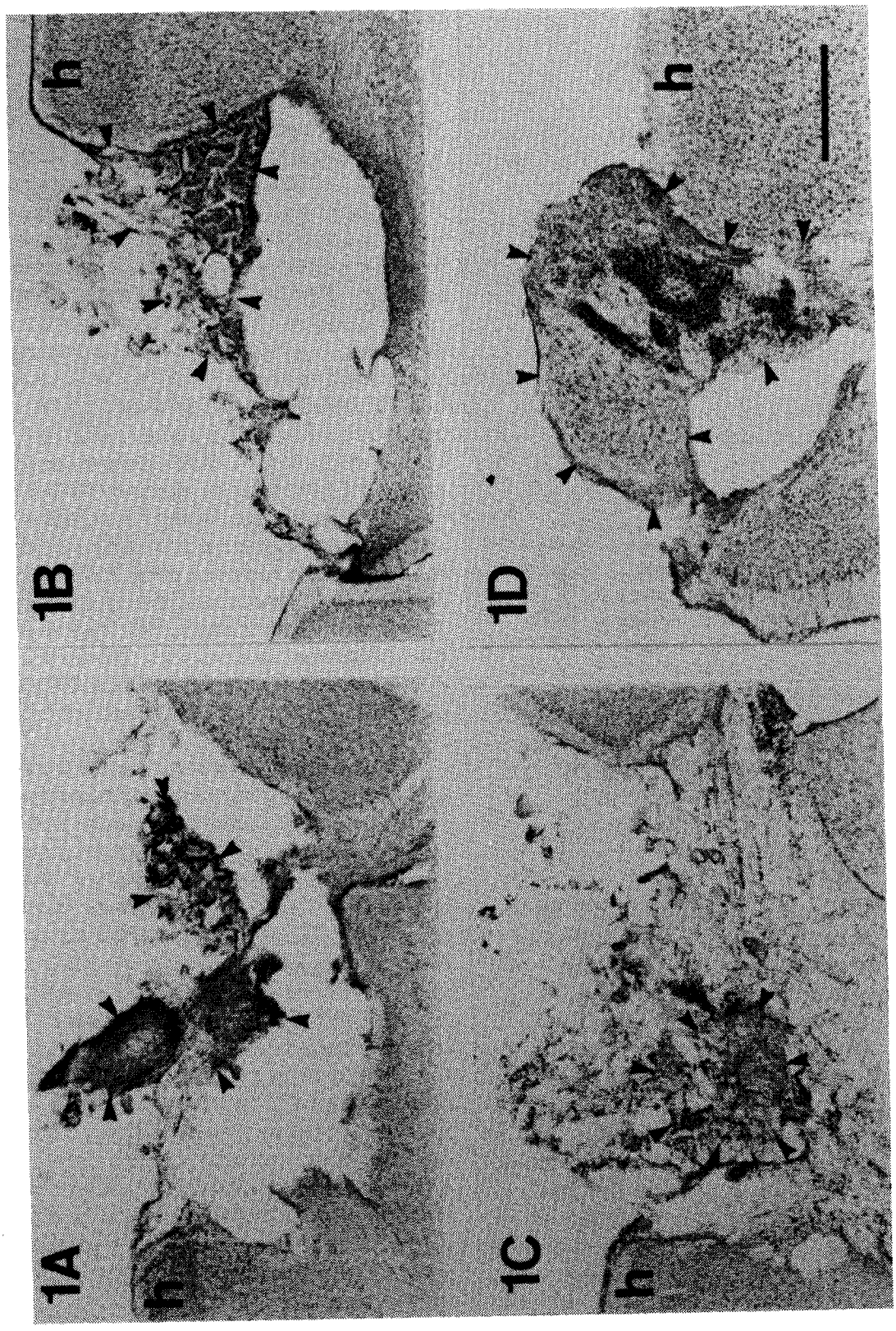

Fig. 1: Overview of grafts within lesion cavities. 1A: PO day 12. 1B: PO day 22. 1C: PO day 32. 1D: PO day 151. Arrowheads indicate graft boundaries, h superficial host cortex lateral to lesion cavity. Cresyl violet; scale bar for all = 500 μm.

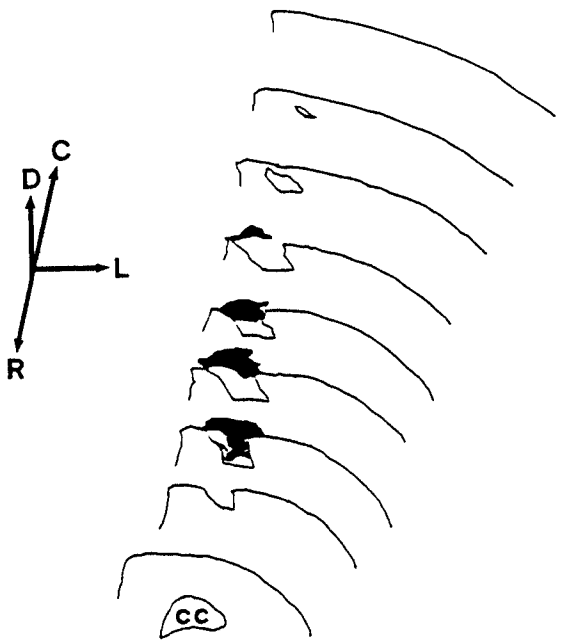

Fig. 2: Serial coronal forebrain reconstruction (perspective view) of the PO day 151 graft and lesion cavity. The graft failed to fill the entire lesion cavity and remained extra-parenchymal; such findings were typical of every graft. Graft is depicted in solid black, CC indicates corpus callosum, D dorsal, L lateral, and C caudal. Inter-section distance = 140 μm. Computer reconstruction using Cellmate III quantitation and image display system.

nucleus, nucleolus, and Nissl substance); nest size was found to range between 40 and 200 μm in the longest dimension (Fig. 3). Total number of spatially separate nests per graft varied from 1 on PO day 22, 3 on PO day 32, and 11 on PO day 151. Putative neurons, however, were also observed to be independent of cell nests. On PO day 151, in addition to cell nests, separate clusters of densely packed, small (4-8 μm diameter), intensely stained cells that resembled cerebellar granule cells were seen in the graft. Organizational features characteristic of mature cerebellum, including a trilaminar cortical appearance and folial arrangements, were not seen in grafts of any age.

There was no evidence of intragraft myelin production on PO days 12 or 22. On PO day 32, however, discrete myelin staining of sparse solitary fibers was seen within the caudal core regions of the graft, but none of these fibers were found to exit the graft. On PO day 151, extensive myelination was present throughout the entire graft. The myelinated fibers were arranged in varying degrees of compactness, from tightly bundled fascicles to tangled collections of randomly oriented fibers (Fig. 4A). Heterogeneity of fiber arrangement was often evident within a single coronal section. The trajectories of the assorted bundles of myelinated fibers were highly variable. Most of the interface regions, composed of graft tissue and connecting the core of the graft to the walls of the lesion cavity, were heavily myelinated. In the majority of cases, the myelinated fibers travelling within an interface region terminated either before reaching the graft-host interface, or abruptly at the interface (Fig. 4B). In the remaining cases, wisps of myelinated fibers travelled into the indeterminate boundary zone between graft and host, but none could be followed into the definitive host brain. The appearance of intragraft myelin resembled that of normal host myelin. None of the grafts demonstrated examples of myelinated fibers originating within host brain and travelling into a core region of graft.

Transplantation of fetal cerebellar fragments into adult frontal cortical lesion cavities resulted in viable grafts containing mature elements but lacking the overall anatomic arrangement or characteristic cytoarchitecture of the normal cerebellum. Cells with neuronal features were present by PO day 22, and a proportion resembled normal cerebellar elements such as granule and Purkinje cells. In focal areas within a graft, cells formed aggregates, but never developed into recognizable folial or deep nuclear structures. Myelin production was extensive by PO day 151, and the myelin distribution was highly variable, without well-defined distinct fiber tracts. These results suggest that although elements contained within grafts appeared similar to cerebellar tissue on a focal level, the grafts were unable to develop in an organized fashion into a structure that overall resembled normal cerebellum.

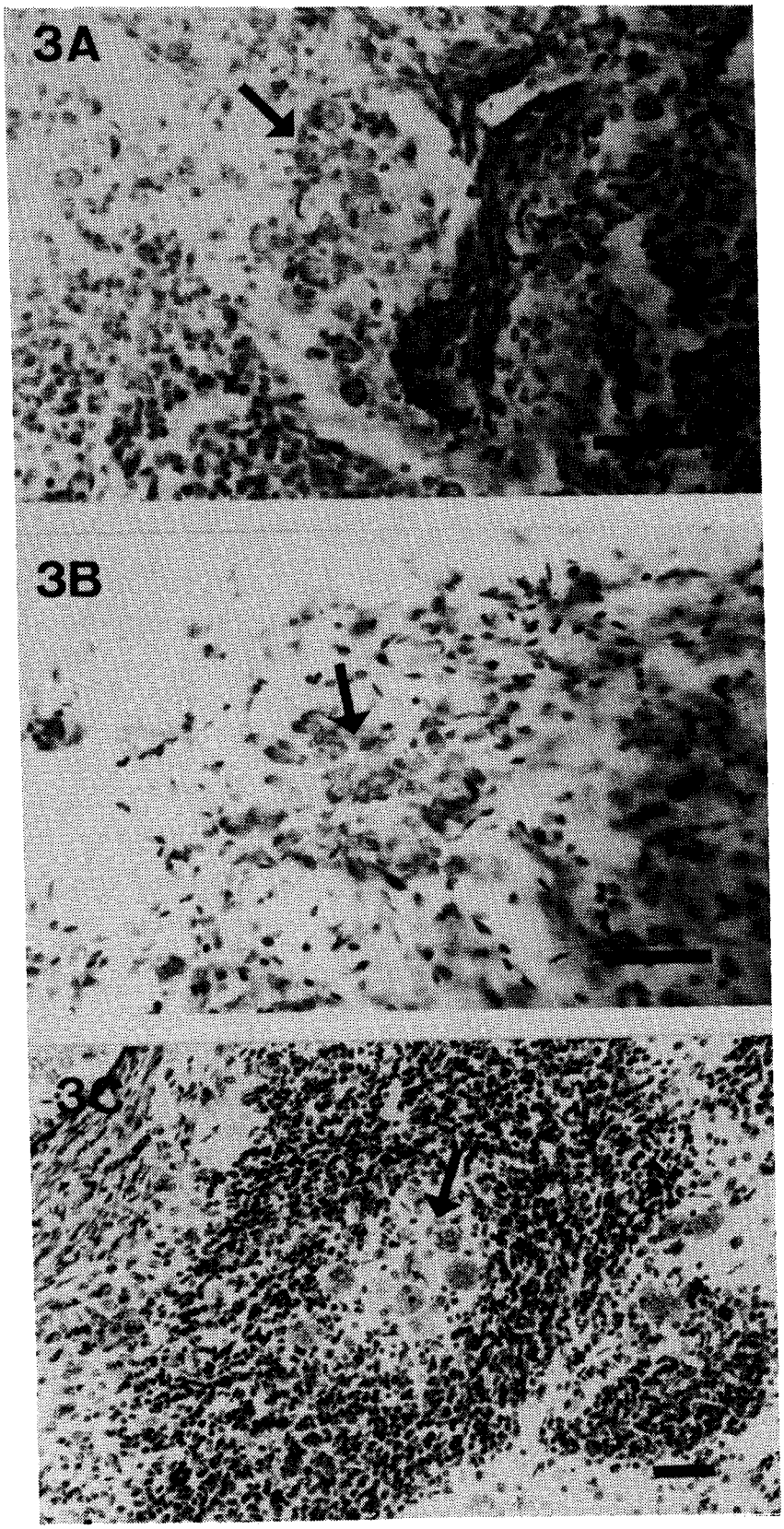

Fig. 3: Cell nests (arrows) were present within grafts beginning on PO day 22. 3A: PO day 22. 3B: PO day 32. 3C: PO day 151. Cresyl violet; scale bar = 50 μm.

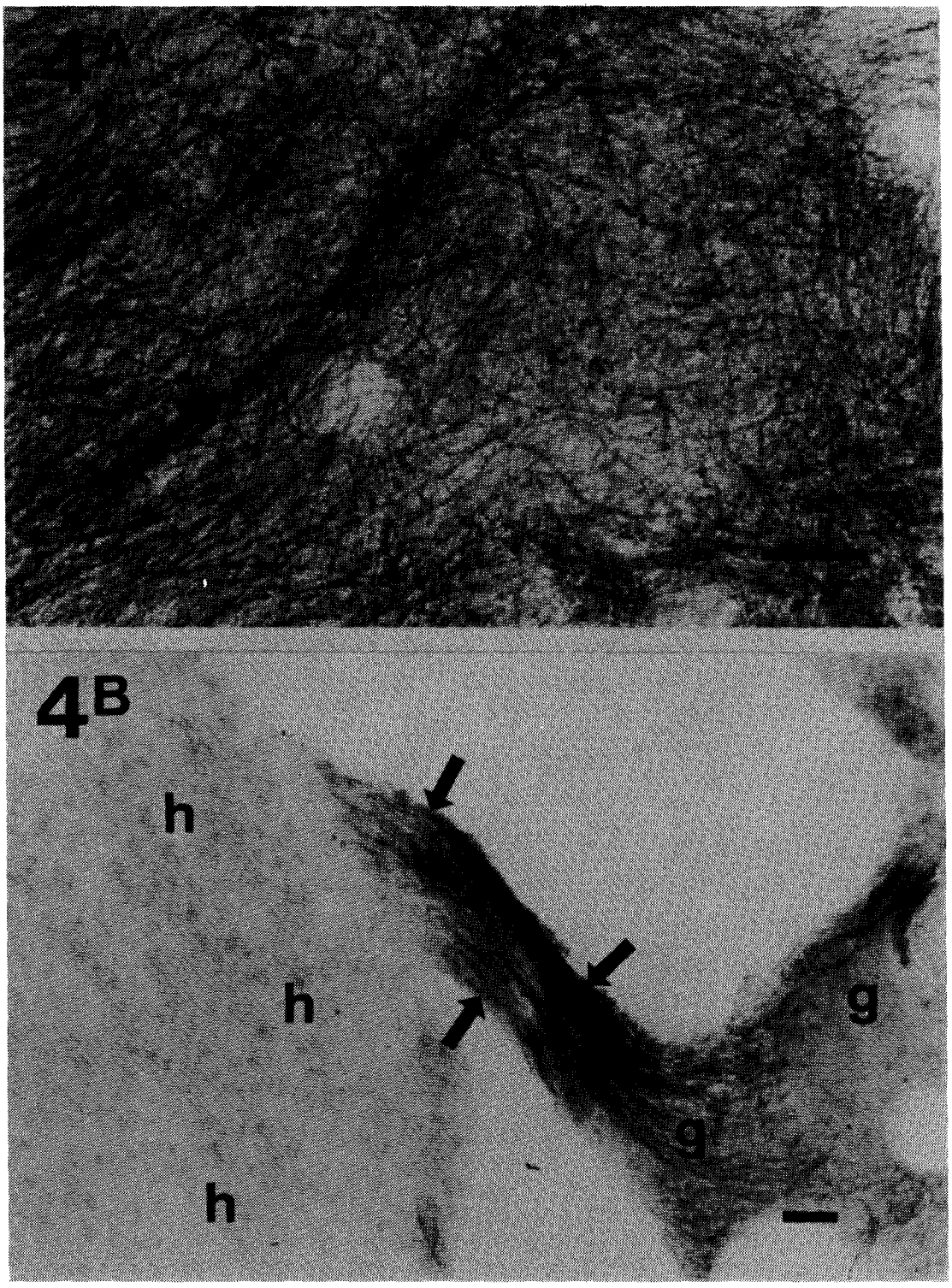

Fig. 4: The PO day 151 graft demonstrated extensive myelin production. 4A: Myelinated graft parenchyma. 4B: Myelinated fibers (arrows) exiting graft (g) abruptly terminate at the graft-host interface. h indicates host cortex. Weil; scale bar = 50 μm.

No graft proliferated and developed to the point of completely filling the cortical lesion cavity, and all grafts developed in extraparenchymal sites. While the short PO survival grafts (less than 32 days) may not have had sufficient time to proliferate, the long survival grafts (32 and 151 days) demonstrated large regions of cell-free space. Furthermore, myelinated fibers originating within the grafts were never found definitively to enter host parenchyma. In some cases these fibers followed a course which curved near the graft-host boundary before returning back toward the central graft region.

Previous reports of cerebellar fragment grafts placed into adult cerebral cortical lesion cavities described instances of extraparenchymal growth /1,6/ and failure of graft fibers to enter the adult host brain /1/. The current study complements existing reports concerning heterotopic transplantation in adult hosts by having examined a previously unreported combination consisting of a fetal cerebellar fragment graft transplanted to a forebrain cortical lesion cavity. Although the degree of similarity between grafted tissue and native cerebellum was less than that reported by others /1,4-6,8/, the small sample size does not allow one to draw definitive conclusions concerning the observed differences at this time.

REFERENCES

1. Alvarado-Mallart RM, Sotelo C. Differentiation of cerebellar anlage heterotopically transplanted to adult rat brain: a light and electron microscopic study. J Comp Neurol 1982; 212: 247-267.
2. Das GD. Transplantation of cerebellar tissue in the cerebellum of neonate rabbits. Brain Res 1973; 50: 170-173.
3. Das GD, Altman J. Studies on the transplantation of developing neural tissue in the mammalian brain. I. Transplantation of cerebellar slabs into the cerebellum of neonate rats. Brain Res 1972; 38: 233-249.
4. Ezerman EB. Survival and development of embryonic and postnatal cerebellum transplanted into adult rat hosts: effect of growth as explants in culture prior to transplantation. Dev Brain Res 1988; 41: 253-261.
5. Ezerman E, Kromer LF. Development and neuronal organization of dissociated and reaggregated embryonic cerebellum after intracephalic transplantation to adult rodent recipients. Dev Brain Res 1985; 23: 287-292.
6. Kromer LF, Bjorklund A, Stenevi U. Intracephalic embryonic neural implants in the adult rat brain. I. Growth and mature organization of brainstem, cerebellar, and hippocampal implants. J Comp Neurol 1983; 218: 433-459.
7. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates, 2nd edn. Sydney: Academic Press, 1986; plates 9-19.
8. Wells J, McAllister JP. The development of cerebellar primordia transplanted to the neocortex of the rat. Dev Brain Res 1982; 4: 167-179.