

NSR 00256

**Short Communications****Reorganization of cerebellar cell suspension  
transplanted into the weaver mutant cerebellum and  
immunohistochemical detection of synaptic formation****Shinichi Kohsaka<sup>1</sup>, Hideichi Takayama<sup>2</sup>, Tetsufumi Ueda<sup>3</sup>, Shigeo Toya<sup>2</sup>  
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(Received 29 March 1988; Accepted 2 June 1988)

**Key words:** Transplantation; Weaver mutant mouse; Cerebellum; Synapsin I; Granule cell**SUMMARY**

Dissociated cells prepared from the cerebellar primordia of normal 15-day mouse embryos were grafted into the cerebellum of 1-month-old weaver mutant mice which are characterized by degeneration of cerebellar granule cells during the early postnatal period. The growth of the grafted cells was investigated at 1 month after the operation. Implanted cells were highly developed to form a large mass of tissue in the host cerebellar folia. Histological examination revealed that a trilaminar cortical structure was partially developed in certain areas of the grafted tissue. The implanted granule-like cells were labeled with [<sup>3</sup>H]thymidine which was injected into the host, suggesting that the granule-like cells actively proliferate in the host cerebellum after the transplantation. In this area, strong immunoreactivity with synapsin I was detected indicating that the dissociated granule cells of the cerebellar primordia are able to develop a synaptic organization in the weaver mouse cerebellum.

Transplantation of neural cells has been used extensively as a viable approach to studying the development and plasticity of the neural cells in the central nervous system<sup>1,6,7</sup>. To investigate the behavior of the implanted neuronal cells, it is of advantage to use a mutant mouse which is deficient in a single class of neuronal cells. For example, Sotelo et al.<sup>11</sup> transplanted the dissociated cerebellar cells of 12-day mouse embryos into the cerebellum of Purkinje cell degeneration (pcd) mutant mice, and examined the behavior of the implanted Purkinje cells. The weaver mutant mouse is characterized by degeneration of cerebellar granule cells in the early postnatal days<sup>8,10</sup>. Thus the weaver mutant mouse can be used as a useful tool to investigate the behavior of the cerebellar granule cells following the transplantation. Previous studies from our laboratory demonstrated that the cerebellar cortical trilaminar structures were organized by solid embryonic cerebellar tissue transplanted into the weaver mutant cerebellum<sup>12</sup>. In the present study, dissociated cerebellar cells from normal embryonic mice were transplanted into

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1-month-old weaver mutant mice, and histological and immunohistochemical examinations were performed with special reference to the reorganization of the dissociated cerebellar cells and the synaptic formation.

Heterozygotes of the weaver mutant mice (B6CBA/F1) were obtained from the Jackson Laboratory (Bar Harbor, U.S.A.). Homozygous weaver mutants were obtained by mating the heterozygotes. Timed pregnant CBA/JNCij mice were purchased from Nippon Bio-supply Center (Tokyo, Japan). The cerebellar primordia were dissected from 15-day embryonic normal mice and were incubated with 0.01% trypsin and 0.001% DNase I in  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free phosphate-buffered saline for 30 min at 37°C. The tissues were then passed through stainless steel mesh (150  $\mu\text{m}$ ) to complete the dissociation of the cerebellar cells. The dissociated cells were harvested by low-speed centrifugation and resuspended in Eagle's minimum essential medium (MEM) at a density of  $10^5$  cells/ $\mu\text{l}$ . Four-week-old weaver mutant mice were anesthetized with ether and ketamine hydrochloride and held in a stereotaxic apparatus with ear bars. Under aseptic conditions, a small incision was made in the cranium to expose the surface of the cerebellar vermis. A Hamilton syringe was lowered to a depth of 1.0 mm from the surface and 5  $\mu\text{l}$  of MEM containing dissociated cells was slowly injected into the cerebellar vermis. To label the dividing cells, 3 injections of [ $^3\text{H}$ ]thymidine (100  $\mu\text{Ci}$ ) were intraperitoneally administered into the host mouse at 8, 10 and 12 days after the transplantation. The recipient mice were sacrificed 4 weeks after the transplantation. The brains were embedded in paraffin and cut sagittally into 6  $\mu\text{m}$  thick sections for histological and immunohistochemical analyses. The deparaffinated sections were immunohistochemically stained with synapsin I antiserum according to the method described previously<sup>12</sup>. Some sections were dipped in NR-M2 emulsion (Konishiroku Co., Tokyo) for autoradiographic analysis. In the present study, 4 weaver mutant mice were operated and successful graftings were achieved in 3 cases.

At the 4th week after the transplantation, the development of the implanted cells was examined by hematoxylin eosin staining. A large mass of tissue was detected in the host cerebellum (Fig. 1a). At higher magnification (Fig. 1b), many granule-like cells were seen to be clustered, forming a granule cell layer intermingled with large neurons (possibly Purkinje cells and neurons of the deep cerebellar nuclei). Some of the Purkinje-like cells were aligned regularly between the molecular layer and the granule-like cell layer like a normal trilaminar structure. Autoradiography of the adjacent section revealed that most of the granule-like cells were labeled with [ $^3\text{H}$ ]thymidine (Fig. 1c, d). Since [ $^3\text{H}$ ]thymidine was injected at 8, 10 and 12 days after transplantation of the dissociated cells from 15-day embryonic cerebella, the labeled granule-like cells seemed to have originated in the implanted donor cells and actively proliferated in the host cerebellum taking the normal time course of postnatal proliferation. It was also speculated that the granule-like cells had migrated to the appropriate positions in relation to those of the Purkinje cells as discussed by Ezerman and Kromer<sup>4</sup> who had also demonstrated that dissociated cells from 13-day embryonic rat cerebellum develop a trilaminar structure after transplantation into the intracerephalic cavity of adult rats.

The synaptic organization in the area where the trilaminar organization was detected was investigated immunohistochemically by using an antiserum to Synapsin I. Synapsin I is a phosphoprotein which is specific to the membranes of synaptic vesicles<sup>2,3,13</sup>. Dolphin and Greengard<sup>5</sup> reported that the amount of Synapsin I is markedly reduced in the weaver mutant cerebellum. As reported previously<sup>12</sup>, the Synapsin I immunoreactivity was greatly diminished in the weaver mouse cerebellum, and was only sparsely distributed in the whole cerebellum (Fig. 2a). However, intense immunoreactivity was observed in the

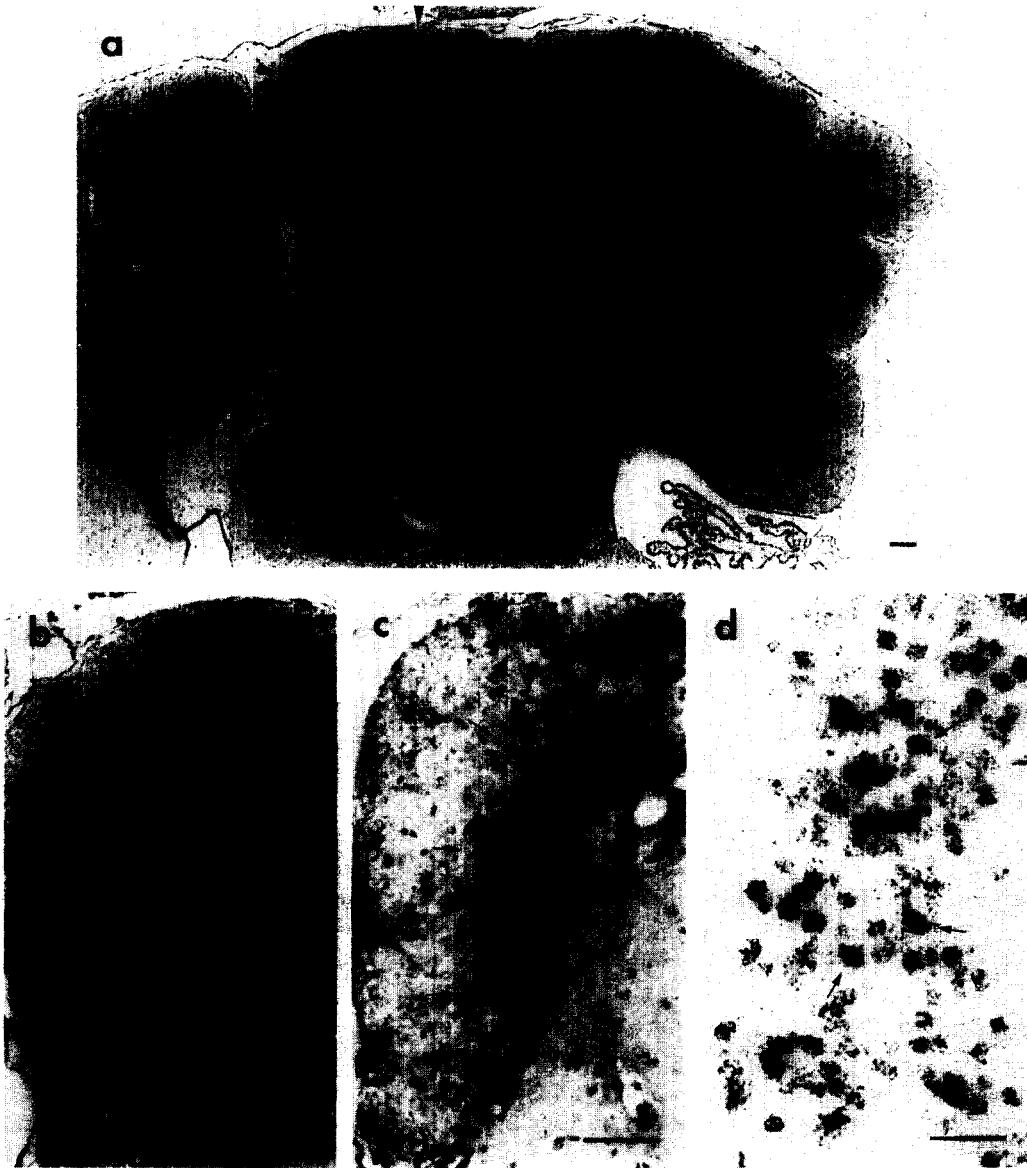


Fig. 1. Sections from the cerebellum of a weaver mutant mouse, stained with hematoxylin eosin, at the 4th week after the transplantation. a: a large mass of grafted tissue is observed in the deeper part of one folium of the recipient mouse as indicated by arrowheads. b: higher magnification of the same section. G, granule-like cells; P, Purkinje-like cells; M, molecular layer. c: autoradiography of the section adjacent to that in Fig. 1b. Many granule-like cells are labelled with [ $^3\text{H}$ ]thymidine as indicated by arrows. Scale = 50  $\mu\text{m}$ . d: higher magnification of the same section of Fig. 1c. The labelled cells were indicated by arrows. Scale = 20  $\mu\text{m}$ .

molecular layer adjacent to the cluster of granule-like cells (Fig. 2b). These findings may indicate that synaptic contacts may develop between the implanted granule-like cells and other types of neurons including Purkinje cells. It is unclear at the moment whether the Purkinje cells had originated in the host or the donor cerebellum.

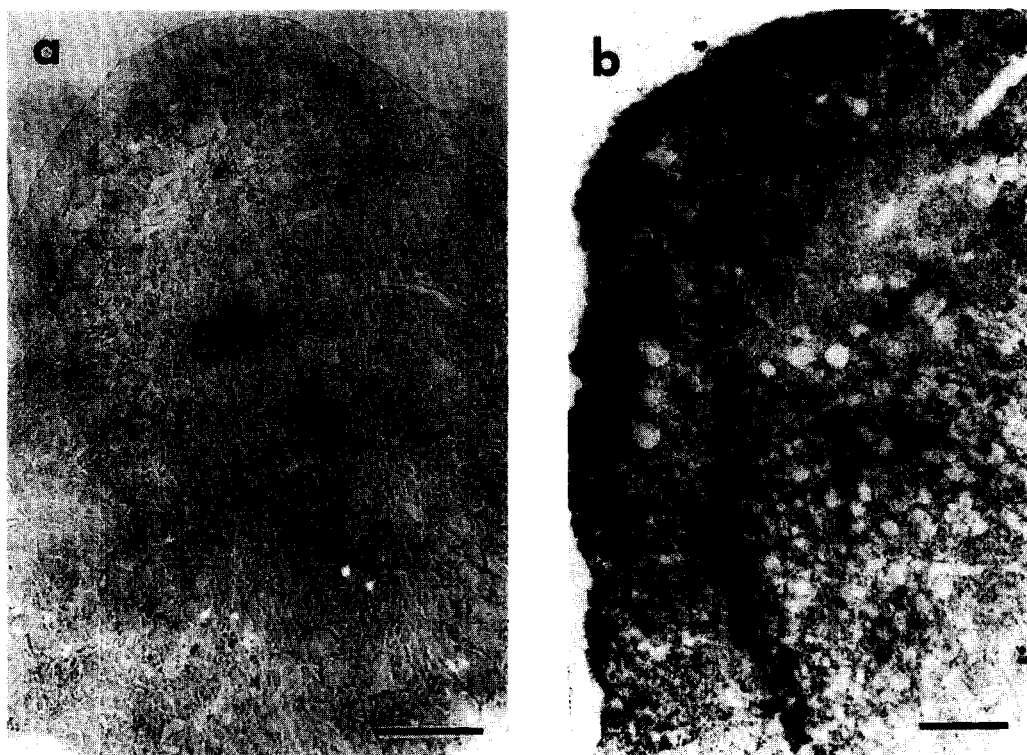


Fig. 2. Sections from the cerebellum of a weaver mouse, immunohistochemically stained with antiserum to synapsin I. a: sections from a weaver mutant mouse not receiving transplantation. b: Section adjacent to that in Fig. 1b. Scale = 50  $\mu$ m.

The present study demonstrated that implanted dissociated cerebellar cells are able to survive and partially develop a trilaminar organization in the weaver cerebellum. It also demonstrated that implanted granule-like cells proliferate over the proper time period and migrate in the developing tissue and make synaptic contacts with the neuronal cells. The mechanisms of the migration and their sorting out are not clear at the moment. Do the granule cells go down into the right position once they reach the subpial space, or do they just migrate from the inside to the outer surface? To clarify these issues, further studies including electron microscopic analysis are needed. Finally, the results may suggest that the degeneration of the granule cells in the weaver mutant cerebellum is not associated with certain diffusible factors.

#### ACKNOWLEDGEMENT

This work was partially supported by a Grant-in-Aid from the Japanese Ministry of Education, Science and Culture (No. 62221030).

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