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Hyperinnervation of Arrested Granule Cells Produced by the Transplantation of Monoamine-Containing Neurons into the Fourth Ventricle of Rat*

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Summary. An attempt to learn whether chemically specific neurons affect the sequence of cerebellar development was made by transplanting ectopic tissues rich in monoamines adjacent to the early developing cerebellum of neonatal rat. Three types of brainstem grafts were used: (1) ventral midline raphe region, (2) inferior olivary region, (3) locus coeruleus region. When transplanted into the fourth ventricle of host animals, neurons from the transplant sprout axons into the host cerebellar parenchyma producing changes in cerebellar cytoarchitecture. The changes produced by the three types of brain grafts were investigated with conventional light and electron microscopy. Autoradiography with tritiated serotonin (^{3}H-5HT) and norepinephrine (^{3}H-NE) and immunocytochemistry using antibodies raised against serotonin allowed identification of the chemical specificity of the process. The three fundamental changes caused by the transplants were folial malformation, arrest of migration of external granule cells, and disruption of the Purkinje cell monolayer. By intraventricular infusion of ^{3}H-5HT and immunocytochemistry with antibodies raised against serotonin, an extraordinarily rich serotonin innervation was detected within or around the foci of arrested granule cells after transplantation with raphe-rich tissue. In addition to an increase in the number of parallel fibers that accumulate ^{3}H-5HT, numerous glomerulus-like structures were observed within the foci. After transplantation with locus coeruleus fragments, intraventricular infusion of ^{3}H-NE demonstrated some increase of labeled fibers inside the foci of arrested granule cells, but the extent of the increase of NE fibers was less marked than the increase in 5-HT fibers. Conventional electron mi-

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croscopic study revealed numerous synaptic formations within the arrested granule cell foci. Terminals containing large granular vesicles were seen, which resemble serotonin nerve terminals previously described (Chan-Palay, 1975, 1977).

Thus ectopic neuronal tissues rich in monoamine neurons survive after transplantation into the fourth ventricle of neonatal rats, can disrupt cerebellar development, and sprout axons that hyperinnervate foci of neurons in disarray, in a pattern reminiscent of the normal innervation.

Key words: Transplant – Monoamine neurons – Cerebellar development – Serotonin – Norepinephrine.

Introduction

The normal developmental sequence of neurons and neuroglia in the nervous system has nowhere been more extensively investigated than in the cerebellar cortex. Numerous authors have described the events that culminate in the cellular arrangements of the molecular, Purkinje cell, and granular layers (Miale and Sidman, 1961; Fujita et al., 1966; Fujita, 1967; Mugnaini and Forstrønen, 1967; Rakic, 1971; Altman, 1972a, b, c). Of these descriptions, the original work of Ramón y Cajal (1911) still remains at the forefront. Proliferation of cells in the external granular layer is followed by the extension of transverse axonal processes and cell migration. The granule cells migrate past the Purkinje cells into the presumptive internal granular layer where synapses are formed with mossy fibers and other axons. Chan-Palay (1972) recorded the presence of foci of arrested external granule cells in the molecular layer of the cerebellar cortex in normal adult animals. Electron microscopy revealed synaptic formations between these arrested granule cells and incoming mossy fibers that had grown outwards from the confines of the internal granular layer to reach the arrested cells stranded in the upper molecular layer. It is generally agreed that the developmental sequence of cell proliferation, migration, maturation, and synapse formation is carefully programmed. In the light of the observations recounted, it was concluded (Chan-Palay, 1972; Palay and Chan-Palay, 1974) that the extension of the mossy fibers compensated for the nonmigration of granule cells. These observations indicate that specific cell-to-cell signals ensure that appropriate partners for a particular connection may locate each other, even if a mistake occurs in the program.

The present studies go several steps to explore the relations of timing and of chemical specificity in the development of the cerebellum.

The transplantation of central and peripheral neuronal tissue into the mammalian brain has been used by many groups in an attempt to examine regenerative and plastic changes in the central nervous system (CNS). Extensive studies by Björklund et al. have focused on the changes in monoaminergic systems after the transplantation of peripheral non-neuronal tissue (Björklund and Stenevi, 1971; Svendgaard et al., 1975), superior cervical ganglion, and CNS fragments (Björklund et al., 1975, 1976; Björklund and Stenevi, 1979; Björklund

et al., 1976) into various brain sites in the rat. Interrupted monoamine neurons of host central nervous system display regenerative properties and can innervate iris transplants. Transplanted monoamine-containing neurons from the superior cervical ganglion or CNS also survive and innervate the host CNS. Using intraocular transplants, Olson et al., 1979 (Olson and Seiger, 1972, 1974, 1975; Seiger and Olson, 1975, 1977a b, c) have shown that monoamine-containing cells transplanted into the anterior chamber of the eye grow and mature and innervate target tissues in a manner similar to that occurring during normal development (Olson et al., 1979).

The cerebellum is one of the established targets for monoamine neurons. The function of the cerebellar monoamine innervation is not yet clear. One possible role of monoamine neurons may be to influence the time of differentiation of prospective serotonin target cells during embryogenesis (Lauder and Bloom, 1974; Lauder and Krebs, 1978). The depth of knowledge of cerebellar development makes it a favorable system for examining changes caused by axonal regeneration of monoamine neuron transplants. Rosenstein and Brightman (1978, 1979) have reported that adult superior ganglion transplanted into the fourth ventricle survives and can cause observable foci of arrested granule cells in the cerebellum.

These studies investigate the effect of transplants of tissues rich in monoamine-containing neurons upon the development of the cerebellum. When grafts of selected brainstem areas (Yamamoto-Yoshida and Chan-Palay, 1979) are transplanted into the fourth ventricle, serotonin and norepinephrine-containing neurons sprout axons into the host cerebellar parenchyma and produce changes in cerebellar cytoarchitecture. The neurons and processes involved in these developmental changes and their chemical nature are investigated using conventional light and electron microscopy, autoradiography with tritiated serotonin and norepinephrine, and immunocytochemistry with antibodies raised against serotonin.

Material and Methods

Procedure and Transplantation. Fifty pairs of newborn rats (Sprague-Dawley, Charles River Breeding Laboratories) were used as hosts and donors in these experiments. The results reported here include data from thirty-five of these experiments. The rats that were to receive grafts were anesthetized with diethyl ether, the dorsal neck skin cut, and neck muscles over the occipital bone removed to permit incision of the dura between cerebellum and brainstem. Transplant grafts of the brainstem (1–2 mm) were obtained as coronal slices and placed on agar or sterilized glass slides covered with saline. Under stereoscopic microscopic observation specific regions (see below) of the brainstem were dissected. Brain tissue was then inserted into the space between the cerebellum and the brainstem of the host animal by means of a sterile glass rod or fine forceps as described previously (Rosenstein and Brightman, 1978), with bleeding kept to a minimum. After the operation, the skin was sutured and the wound cleaned with antiseptic solutions. Newborn rats were then returned to the mother. After varying survival times (see below), operated rats were perfused and examined histologically. The origin of brain grafts, the ages of donor and host animals, and post-operative survival times are listed in Table 1.

Brain grafts were obtained from three different portions of the midbrain. In the first group of 15 rats, midline brainstem was dissected into a fragment 1 mm wide and 2–3 mm long whose rostro-caudal boundaries were the rostral edge of the inferior olive and the pontine decussation

(Fig. 1b). This serotonin-rich fragment included neurons of raphe pallidus, raphe magnus, and raphe obscurus. We attempted to avoid inclusion of inferior olivary neurons wherever possible. From a second group of eleven rats (Fig. 1a) the inferior olivary nucleus was dissected unilaterally. This explant contained non-monoamine inferior olivary neurons, some sertotonin olivary neurons, serotonin neurons within the nucleus paragigantocellularis lateralis (Andrezik and Chan-Palay, 1977; Chan-Palay, 1977, 1978), which has been classified as group B-2 by Dahlström and Fuxe (1964) as well as the catecholamine cells of group A-1. In the third group of four animals, grafts were obtained from tissue underlying the fourth ventricle in the midline caudal to the inferior colliculus (Fig. 1c). These tissues contained norepinephrine cells of the locus coeruleus and/or serotonin cell groups of the raphe dorsalis.

Ages of Host and Donor Animals (see Table 1). Since it is well recognized that the cerebellum develops postnatally, with neonatal differentiation essentially completed at 21 days after birth (Ramón y Cajal, 1911), it was necessary to use host and donor animals in the early postnatal period. In all cases, 4–10 day old rats were used as hosts. Animals of various ages served as donors: 4 day old (5 cases), 5 day old (1 case), 6 day old (8 cases), 10 day old (5 cases), 60 day old (5 cases), 120 day old (4 cases), and 180 day old (3 cases).

Survival Time (see Table 1). The cerebella of host animals were examined after various post-natal survival times: 26 days (2 cases), 28 days (4 cases), 49 days (4 cases), 60 days (4 cases), 90 days (6 cases), 120 days (7 cases), 180 days (8 cases).

Control Experiments. In control experiments, fragments of liver approximating the size of the brain grafts or gelfoam soaked in 10^{-6} M serotonin (5-HT) solution were transplanted separately into the fourth ventricle of five 6-day old rats.

Tissue Preparation

Histology. All animals were perfused intracardially, after various survival times, with 1% glutaraldehyde and 1% formaldehyde in 0.12 M phosphate buffer (pH 7.3). On a freezing microtome or in a cryostat, the cerebellum was cut into serial coronal or sagittal sections, 40 µm thick, and stained with cresyl violet for light microscope observation.

Autoradiography. Six rats were infused intraventricularly with tritiated serotonin (^{3}H-5HT) and four rats were infused with tritiated norepinephrine (^{3}H-Ne). Twenty-four h and 0.5 h before infusion, rats were treated with a monoamine oxidase inhibitor (pargyline HCl, Sigma, 50 mg/kg body weight). Animals were anesthetized with chloral hydrate (35%, 0.1 ml per 100 mg body weight) and a cannula was inserted into the left lateral ventricle according to stereotaxic coordinates (A+5, L+2 to a depth of 3.0 mm from the surface, De Groot, 1959), ^{3}H-5HT creatinine sulphate (New England Nuclear, 10.8 Ci/mmol specific activity), 10^{-5}–10^{-6} M in sterile saline, or ^{3}H-norepinephrine (New England Nuclear, 10 Ci/mmol specific activity), 10^{-5} M in sterile saline, was introduced in a total volume of approximately 50–80 µl over a three-hour period. Animals were then perfused with aldehydes. Light microscope autoradiograms were prepared from frozen, 40 µm thick serial coronal or sagittal sections of the cerebellum. Each section was mounted on chrome alum gelatin-coated glass slides, dipped in Kodak NTB-2 emulsion diluted 1:1, and exposed for 2–6 weeks. Sections were developed with D-19 (18° C, 4 min) and lightly stained with thionine. Labelled fibers were examined with a light microscope and compared to those of normal cerebellar sections.

Immunohistochemistry. The cerebella from the brains of two cases with midline medullary raphe transplants of tissues from 10-day old donors into 10-day old host animals were used in these studies. After a 60-day survival period, the animals were treated with a monoamine oxidase inhibitor (pargyline hydrochloride, 50 mg/kg b.w. in saline, i.p.) at 5 h prior and with 6-hydroxytryptamine (6-HT, Astra Läkmedel, 0.2 mg/kg in saline, i.v.) 30 min prior to perfusion. The animals were anesthetized with diethyl ether and given artificial respiration through a tracheotomy tube with a mixture of 95% oxygen and 5% carbon dioxide. The blood was washed out with cold Ca^{++}-free

Tyrode's buffer (50 ml at 4° C) and perfused with cold fixative (4% formaldehyde in 0.12 M phosphate buffer, pH 7.4, 500 ml, 4° C) for 20 min. Throughout the perfusion the animal was immersed in ice. Following this the brain was dissected from the skull and soaked in fresh fixative for 2 h. After fixation the cerebellum was dissected out and soaked in 0.05 M tris buffer with 5% sucrose at pH 7.6 for 18 h prior to sectioning on a Dittes cryostat. Serial 10 μm thick sections were mounted onto slides coated with chrome alum-gelatin. The sections were treated with antibodies raised against serotonin conjugated to bovine serum albumin (Steinbusch et al., 1978). The antibodies were diluted 1:500 with phosphate buffered saline, applied to the tissue sections for up to 72 h at 4° C under coverslips. The tissues were rinsed in tris buffer, reacted with goat anti-rabbit immunoglobulin conjugated to fluorescein isothiocyanate diluted 1:150 in buffer, for 30 min at 37° C. The sections were rinsed well, coverslipped in buffer glycerol (3:1 v/v) and observed in a Zeiss Universal microscope equipped with high pressure mercury epifluorescence illumination. Fibers with specific immunoreactivity were readily identified.

Electron Microscopy. The brains from four cases provided material for electron microscopy. After perfusion with fixative (1% glutaraldehyde and 1% paraformaldehyde in 0.12 M phosphate buffer, pH 7.3), the cerebellum was cut into small pieces and processed for electron microscopy after postfixation in 2% osmium tetroxide, dehydration with methanol, and embedding in epoxy resin (see Palay and Chan-Palay, 1974). Serial semithin (1 μm) sections were cut, stained with toluidine blue and examined under a light microscope. Regions where arrested granule cells were observed were trimmed and thin-sectioned in serial order, stained with 3% uranyl acetate (4 min) and 0.1% lead citrate (2 min).

Results

When monoamine-rich brainstem tissue was transplanted into the fourth ventricle, some degree of disruption in the development of the cerebellum was observed in all cases, regardless of the precise source of the transplanted tissue, the survival period, or the age of host or donor. Three fundamental changes were observed: folial malformation, arrest of migration of external granule cells, and disruption of the Purkinje cell layer. These results are summarized in Table 1. The developmental abnormalities elicited by grafts of central nervous tissue differed from those elicited by control transplants of liver or gelfoam. Nonnervous tissue grafts produced only folial malformation, but did not cause the arrest of granule cell migration or disarray of Purkinje cells.

Cerebellar folial malformation and dural thickening and fibrosis were obvious. These changes included uneven width of a folium and/or unevenness of the surface of that folium. In a few cases, a small fibrous mass was attached to the affected part (Fig. 2a, *arrow*). In most of the cases, malformation was restricted to the posterior vermis, especially lobules IX or VIII; however, in two of the most severely affected cases, the abnormality extended up to the anterior hemisphere of the cerebellum (Fig. 2b, c, d). Disruption of folia was observed in all of the cases, with uneven widths within any one folium (Fig. 4a, *arrows*). The width of individual granular, molecular, and Purkinje cell layers was also uneven.

In all 30 experimental cases, the disruption of folia was associated with granule cell arrest and the normal laminar architecture of the cerebellum was disrupted. In the molecular layer, numerous granule cells were seen, some of which were clustered together into small islands at the pial surfaces of two

Table 1.

Case	Age of host/donor (postnatal days)	Survival time in days	Folium disruption	Lobule	Arrested granule cells	Purkinje cell dislocation	Hyperinnervation by monoamine fibers
1 VR	5/5	90	+	Vermis IX, V, VI Hemisphere IV Deep Nuclei	++	++	
2 VR	6/6	90	+	Vermis VI, VII	++	+	
3 VR	6/6	90	+	Vermis VIII, IX	+	+	
4 VR	6/6	90	+	Vermis IX	+	+	
5 VR	6/6	90	+	Vermis VII, VIII, IX	+	+	
6 VR	6/6	90	+	Vermis IX	+	+	++5HT (^{3}H-5HT)
7 VR	6/6	49	+	Vermis IX	+	−	
8 VR	10/10	60	+	Vermis IX	+	−	++5HT (5HT-Ab)
9 VR	10/10	60	+	Vermis IX	+	−	+5HT (5HT-Ab)
10 VR	6/4	28	+	Vermis IX	+	−	
11 VR	6/4	28	+	Vermis IX	+	−	
12 VR	6/4	28	+	Vermis IX	+	−	
13 VR	6/4	28	+	Vermis IX	+	−	
14 VR	6/6	26	+	Vermis IX	+	−	
15 VR	6/6	26	+	Vermis IX	+	−	
16 IO	7/120	49	++	Vermis IX	+	−	
17 IO	6/60	49	+	Vermis IX, X	+	−	
18 IO	6/60	49	+	Vermis IX	+	−	
19 IO	6/60	180	+	Vermis IX (bc)	+	+	
20 IO	6/60	180	+	Vermis IX (b)	+	+	
21 IO	6/60	180	+	Vermis IX (c)	+	+	
22 IO	6/120	180	+	Vermis IX	+	−	+5HT (^{3}H-5HT)
23 IO	6/180	180	+	Vermis IX, X	+	+	+NE (^{3}H-NE)
24 IO	6/180	180	+	Vermis IX	+	+	(+) 5HT (^{3}H-5HT)
25 IO	6/120	180	+	Vermis IX	+	+	NE (^{3}H-NE)
26 IO	6/120	180	+	Vermis IX	+	+	NE (^{3}H-NE)
27 LC	10/10	120	+	Vermis IX	+	−	5HT (^{3}H-5HT)
28 LC	10/10	120	+	Vermis IX, X	+	−	
29 LC	10/10	120	+	Vermis IX	+	−	5HT (^{3}H-5HT)
30 LC	10/10	120	+	Vermis IX	+	−	NE (^{3}H-NE)
31 Liver	6/6	120	+	Vermis IX	−	−	
32 Liver	6/4	120	+	Vermis IX	−	−	
33 Liver	6/6	120	+	Vermis IX	−	−	
34 5HT-gel	6/	60	+	Vermis IX	−	−	
35 5HT-gel	6/	60	+	Vermis IX	−	−	

VR = Ventral midline medullary raphe; IO = Inferior olivary complex; LC = Locus coeruleus and dorsal raphe; − = No change; + = Focus of abnormality less than 2 mm; ++ = Focus of abnormality greater than 2 mm; Ab = antibody

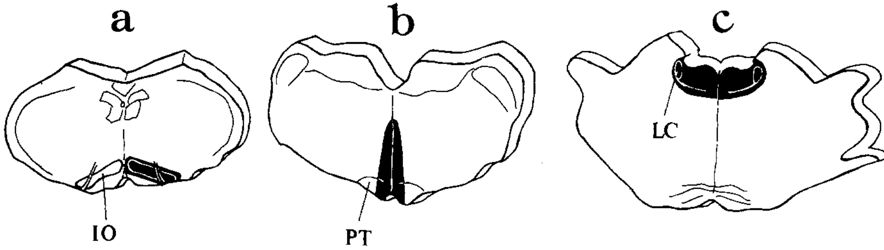


Fig. 1a–c. Diagrams to illustrate the origins of tissue transplants from brainstems of host animals. **a** Unilateral inferior olivary region *(IO)*; **b** Ventral midline medullary raphe (*PT* pyramidal tract); **c** Floor of the fourth ventricle including locus coeruleus *(LC)* and dorsal raphe

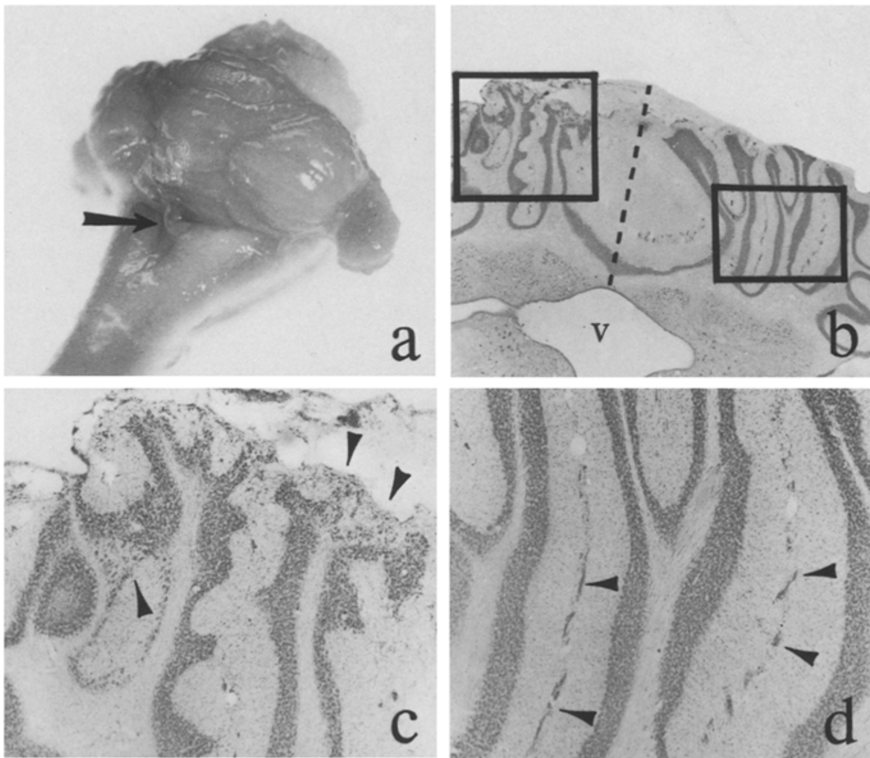


Fig. 2a–d. Photomicrographs to illustrate the cerebellar abnormalities produced by brainstem transplants into the fourth ventricle. **a** Inferior olivary transplant, 6-day old host, 120-day old donor, 180 days survival. Dorsal view of the cerebellum and brainstem showing residual transplant mass *(arrow)* attached to the caudal vermal surface of the cerebellum. ×2. **b** Ventral raphe transplant, 5-day old host, 5-day old donor, 90 days survival. Low magnification photomicrograph of a coronal section through the cerebellum in which the fourth ventricle *(V)* and the midline *(dotted line)* are indicated. Malformations in folial lobulation *(box on left)* and ectopic foci of undescended granule cells *(box on right)* are present. These areas are reproduced in Fig. 2c and d, respectively. **c** High magnification of affected area shown in Fig. 2b. There are considerable areas of displaced Purkinje cells *(arrows)* and the normal folial architecture is disrupted. ×27. **d** Numerous foci of arrested granule cells are present at the juxtaposition of the pial surface in the folia illustrated *(arrows)*. ×27

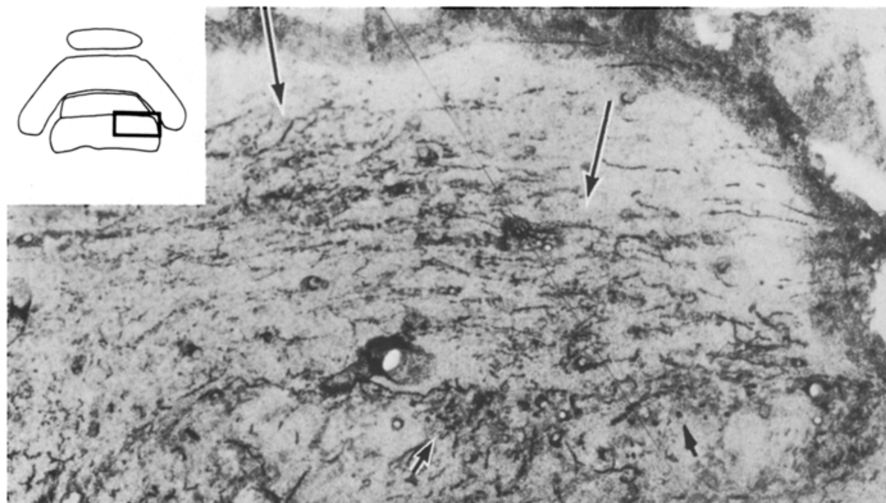


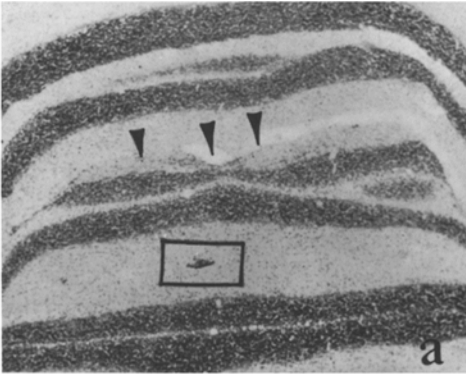
Fig. 3. Autoradiographic demonstration of serotonin fibers in the malformed cerebellar cortex after ^{3}H-5HT intraventricular infusion. The photomicrograph shows numerous labeled varicose fibers *(arrows)* running parallel to the length of the folium and numerous glomerulus-like structures *(small arrows)* in the regions of ectopic masses of undescended granule cells. Inset diagram indicates position in cortex from which the illustration was derived. ×80. Ventral medullary raphe transplant, 6-day old host, 6-day old donor, 90-day survival

juxtaposing molecular layers (Fig. 2d, *arrow*), while others were distributed diffusely. The width and configuration of granule cell layers were also irregular.

In four cases severe and in 8 cases moderate disruption of the Purkinje cell layer was observed. Purkinje cell layer disarray was always found in association with arrested granule cells. Purkinje cells were distributed in multiple laminae or dislocated towards the molecular layer (Fig. 2c). Foci of dislocated Purkinje cells formed circular masses devoid of granule cells (Fig. 2c).

The locations of the major folial and cellular changes observed were related to the placement of the transplants. In most instances the affected regions of cerebellum directly overlay the transplant in vermal lobule IX and to a lesser extent the neighboring folia. In a few cases, effects were seen more distantly in vermal lobules IV and V.

Fig. 4a–h. Immunocytochemical demonstration by reaction with antibodies against serotonin of the hyperinnervation of ectopic granule cell foci by serotonin-like fibers. Figure 4**a** shows a low magnification (×30) of the cerebellum with a focus of undescended granule cells illustrated in serial sections in Fig. 4**b–h.** Fig. 4e shows a portion of normal cerebellar cortex with the standard amount of innervation by serotonin fibers after reaction with antibodies against serotonin. The remaining figures 4b–h are arranged in pairs, b and f, c and g, d and h, showing serial sections through three adjacent levels. The illustrations on the left of each pair, Fig. b, c, and d, show the cells of the focus stained by thionin whereas the illustrations on the right of each pair, Fig. f, g and h, show the same areas respectively after immunohistochemical reactions with 5-HT antibodies in the focus, and the characteristic glomerulus-like and varicose nature of these serotonin axons. ×280. Ventral medullary raphe transplant, 10-day old donor, 10-day old host, 60-day survival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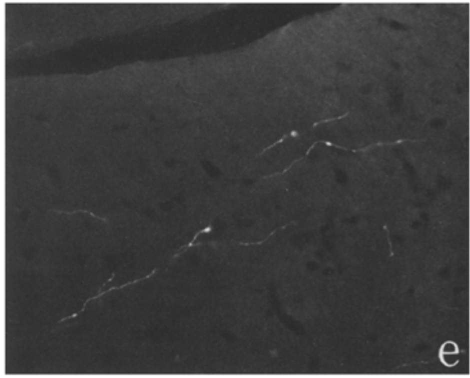
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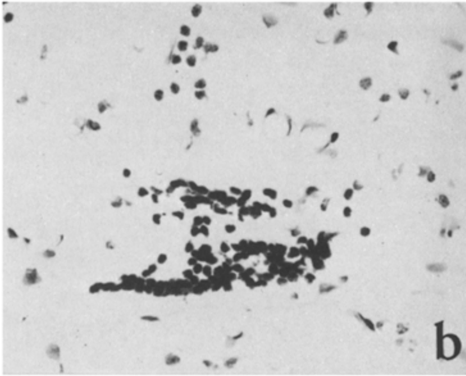
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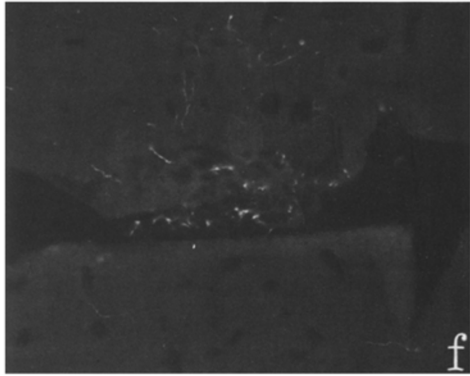
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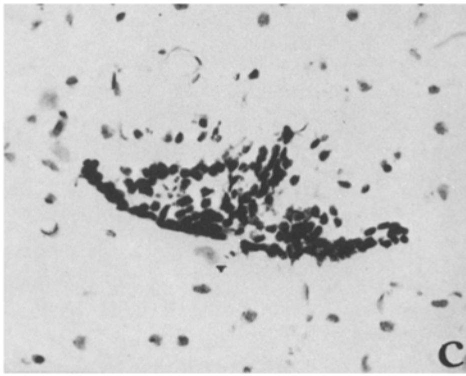
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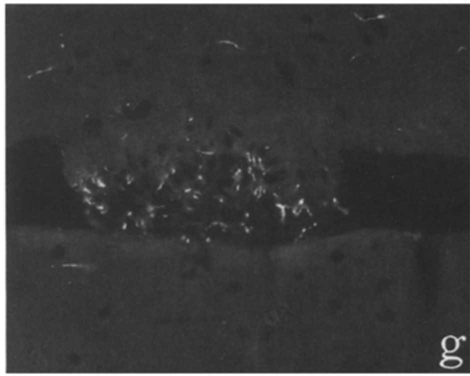
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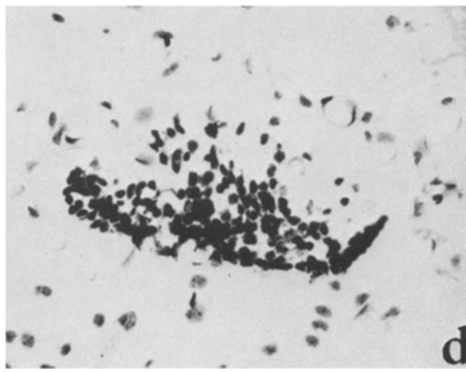
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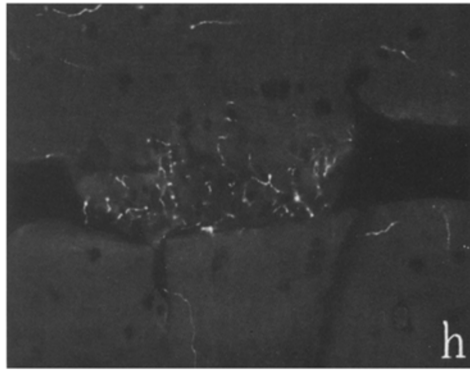
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Autoradiography. ^{3}H-5HT intraventricular infusions followed by autoradiography were used to investigate the extent to which serotonin fibers from the transplant might be involved in the cerebellar developmental anomalies described above. Previous studies have shown that specific identification of serotonin neurons and processes is possible with this method (Chan-Palay, 1975, 1977). With this technique, in normal cerebellum, some parallel fibers in the molecular layer and mossy fiber glomeruli in the granular layer are labeled. In the present experiments an extraordinarily rich serotonin innervation was present within or around the arrested granule cell foci (Fig. 3). In addition to the increase in parallel fibers that accumulate ^{3}H-5HT, numerous glomerulus-like structures were observed within the foci of arrested granule cells in the molecular layer. An increase in labeling of glomerular structures was also seen in the granular layer. In some foci, labeled varicose 5-HT fibers were observed running parallel to arrested granule cells.

Immunohistochemistry with Antibody Against 5-HT. Figure 4a–d show serial sections through one focus of arrested granule cells and Fig. 4f–h show darkfield microscopic pictures of the same sections after indirect immunofluorescence. A rich plexus of fluorescent axons is present and in higher density than is found in the normal cerebellar cortex (Fig. 4e). Hyperinnervation by serotonin fibers occurred in the region of arrested granule cells.

Autoradiography after ^{3}H-norepinephrine Intraventricular Infusion. Following intraventricular infusion of ^{3}H-NE the labeling pattern was different from that observed following infusion of ^{3}H-5HT. In particular, no glomeruli were labeled by ^{3}H-NE. Labeled fibers appeared to run close to Purkinje cell somata, climbing directly to the molecular layer. Some parallel fibers were also labeled. In host animals, some increase of labeled fibers inside the foci of arrested granule cells was detectable; however, the increase of NE fibers is less extensive than the increase in 5HT fibers.

Electron Microscopic Observation. Figure 5 inset shows a light microscopic picture of a single arrested granule cell focus. In this case arrested cells were located at the edge of the molecular layer. The outline of the folium is not smooth, and arrested cells can be seen protruding from the normal cerebellar parenchyma. Figure 5 is an electron micrograph taken from a thin section in the region of the arrested granule cells. One of the arrested granule cells can be seen on the right lower edge of the picture. Numerous synapses are detectable. Glomerulus-like structures were observed (Fig. 5), containing large granular vesicles, small clear round vesicles, and pleomorphic vesicles (see Chan-Palay, 1977). Other presynaptic terminals containing large granular vesicles, pleomorphic vesicles, and terminals with only small round vesicles were also observed in the neuropil. In the normal rat cerebellum, EM study of the arrested granule cell foci showed that myelinated fibers ascending through the molecular layer and entering a focus terminate in this neuropil as the central components of glomerular formations between the granule cells. The terminals contain large clusters of round synaptic vesicles, 300–460 Å in diameter, similar to those

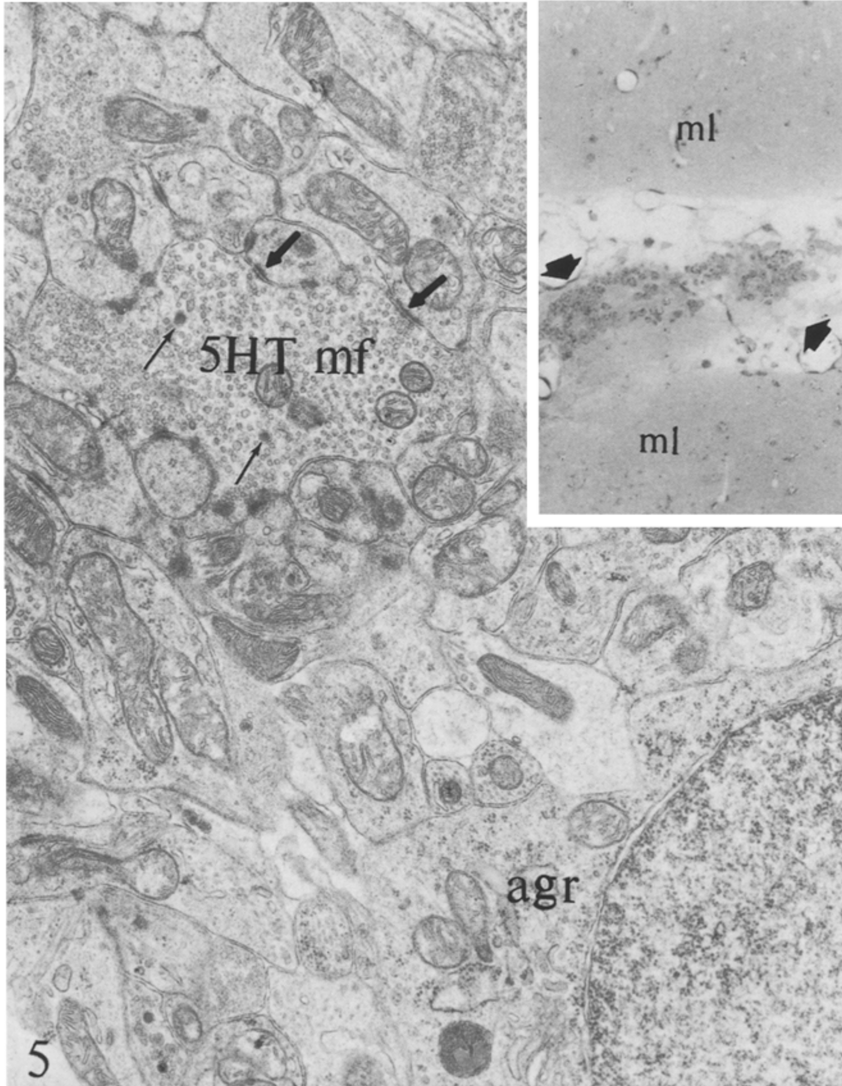

Fig. 5. Electron microscopic demonstration of neuronal entities in a focus of undescended granule cells produced by transplant of ventral medullary raphe from a 6-day old donor into a 6-day old host after 28-day survival. In the inset a low magnification photomicrograph shows the focus of undescended granule cells *(arrows)* sandwiched between the molecular layers *(ml)* of two adjacent folia. ×230. The electron micrograph from a thin section adjacent to the area in the inset shows one arrested granule cell *(agr)* surrounded by numerous synaptic profiles. A prominent glomerulus-like structure (5-HT mf) resembles serotonin-containing mossy fiber glomeruli (previously identified by ^{3}H-5HT electron microscopic autoradiography; see Chan-Palay, 1975, 1977). The large axonal varicosity contains numerous small clear vesicles of round and pleomorphic shape as well as several large dense core vesicles *(small arrows)*. The axon has numerous synapses with dendritic profiles *(arrows)*. ×12,400

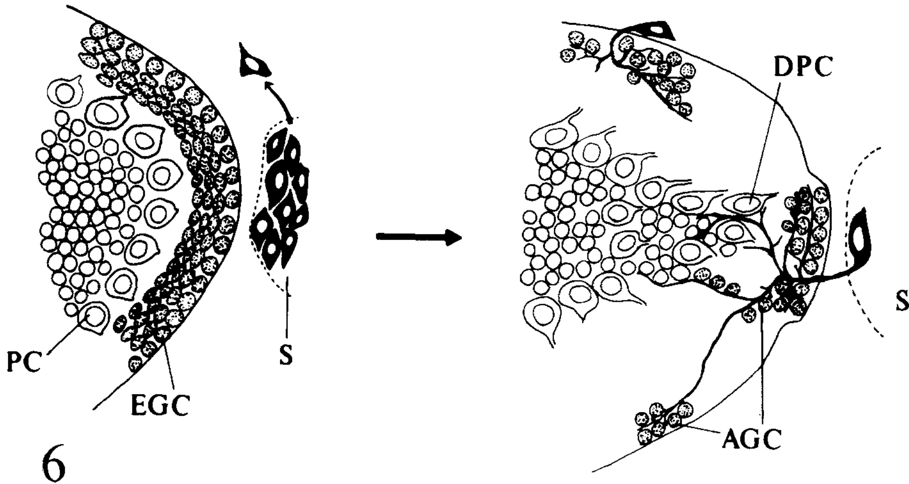

Fig. 6. Diagram to summarize the events subsequent to transplantation of serotonin neurons *(S)* into the vicinity of the cerebellum. The developing cerebellar cortex with prominent external granule cell layer *(EGC)* and developing Purkinje cells *(PC)* lies adjacent to the transplant. Neurons *(S)* in the transplant survive, migrate, and sprout axons to hyperinnervate the cerebellar cortex. Malformations of the developing cerebellum due to these events include disruption of the Purkinje cell layer *(DPC)*, foci of arrested granule cells *(AGC)* and gross malformation of the cerebellar cortical folia

found in mossy fiber rosettes (Chan-Palay, 1972). Synaptic vesicles larger than 460 Å were not observed. In the transplanted host animal, the terminals containing large granular vesicles (900 Å) were seen more frequently than in the arrested granule cell foci of the normal animal. Although it is likely that some of these terminals are monoamine-containing, conclusive evidence awaits the results of electron microscopic studies with immunocytochemistry or autoradiographic techniques.

Discussion

The results of the experiments detailed above show that transplantation of defined fragments of brainstem tissues rich in monoamines from a young postnatal donor rat into the fourth ventricle of another young postnatal recipient rat can induce developmental malformations in the cerebellum. These include gross disruption of folial and cellular architecture as well as distortion in cortical lamination and multiple foci of undescended granule cells. The locations and severity of these lesions are related directly to the proximity of the transplant. Control experiments with liver or gelfoam as transplants instead of brainstem tissues indicate that whereas folial malformations occur, the disturbances in cellular architecture and foci of undescended granule cells do not occur. A question could be raised as to whether or not these results could have been due to mechanical trauma induced at the time of surgery. We attempted to minimize the disruption of donor tissue as well as that of the host animal

during surgery and it is unlikely that the results are due solely to mechanical disruption. Folial changes however might be induced by long-term pressure of tissue fragments against a developing cerebellum.

The question remains as to whether or not chemical stimuli from the presence of an unusual mass of 5-HT- or NE-containing neurons can induce the developmental changes detailed above. The present studies indicate that transplants of 5-HT-rich or NE-rich tissues can induce focal hyperinnervation of the cerebellar cortex, which is more dramatic with serotonin than with norepinephrine. One is tempted to regard these hyperinnervating axons as functional since numerous synaptic contacts occur between them and other neural elements.

In recent studies, adult rat sympathetic ganglia were transplanted into the fourth ventricle of young postnatal rats (Rosenstein and Brightman, 1978, 1979). When superior cervical ganglia (SCG) from newborn rats are used, the transplants did not survive; only adult SCG produced successful results. The residual tissue was recognizable histologically even after several months of post-implantation survival. In the present experiments, although monoamine-containing fibers within the affected regions of the cerebellum were detectable, the transplant residues were difficult to locate regardless of the age of animals used. The conditions for survival of transplanted tissue in adult brain have been well described (Björklund and Stenevi, 1979; Svendgaard et al., 1975). According to these authors, the age of the donor animal, the site of the transplantation, and sterile conditions during the operation seem to be the most important factors. Embryonic monoamine neurons have been reported to grow better than those obtained from postnatal animals. However, since the cerebellum is a postnatally developing organ, the experiments described here were planned for newborn animals. Probably the major reason for our difficulties in locating the transplant residua as single masses may be the fact that the tissues are fragile and the transplant may dissociate during surgery, resulting in a number of smaller masses which, though effective as chemical inducers of developmental abnormalities, may be difficult to locate in histological preparations.

The present studies indicate that the cellular, laminar and architectural integrity of the developing cerebellum can be disrupted by the presence of an ectopic mass of central nervous tissue transplanted to the fourth ventricle of a young postnatal rat. When the transplants originate from a region rich in serotonin neurons the overlying cerebellar cortex contains foci of undescended granule cells hyperinnervated by serotonin axons. Similarly, when transplants originate from regions rich in catecholamine neurons there is a hyperinnervation by the processes of these cells. The source of this hyperinnervation was not ascertained in the present study. One possibility is that it results from a proliferation of collateral sprouts in the endogenous monoamine fiber plexus of the host cerebellar cortex. Alternatively, it could arise from a small number of surviving cells in the transplant that invade and ramify profusely in the ectopic granule cell foci, as diagrammed in Fig. 6 (Björklund and Stenevi, 1979). The well-documented capacity for such proliferation of the regenerating monoamine fiber plexus makes the latter possibility most attractive.

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