Derangement of Purkinje Cells in the Rat Cerebellum Following Prenatal Exposure to X-Irradiation: Decreased Reelin Level Is a Possible Cause

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Abstract. It has been reported that prenatal X-irradiation of rats during the late gestation period causes heterotopic Purkinje cells in the internal granular layer (IGL) of the abnormally foliated cerebellum. The present study was designed to demonstrate the process of X-ray-induced derangement of Purkinje cells and their surrounding cells. In addition, the expression of some morphoregulatory molecules was examined to determine which molecules are involved in the abnormal pattern of Purkinje cells. Pregnant rats (n = 22) were exposed to 2.5 Gy X-radiation on gestation day 21 and the cerebellum of progeny was examined histologically and by immunohistochemistry to identify Purkinje and Bergmann cells. At 12 h after exposure, extensive cell death was observed in the external granular layer (EGL). By postnatal day (P) 9, while Purkinje cells with well-developed dendrites aligned underneath the EGL in the control cerebellum, Purkinje cells with shorter and abnormally oriented dendrites failed to align and remained in the heterotopic location in the IGL. Bergmann cells and their fibers were also disoriented but later recovered in their proper position. Abnormal folia developed in the irradiated rats. Using immunohistochemistry, we next examined the levels of the neural cell adhesion molecule (NCAM), fibronectin, tenascin, and Reelin. Among them, only the level of Reelin was affected significantly. Reelin decreased strikingly in the premigratory zone of the EGL and IGL in the irradiated cerebellum on P1, and the decrease continued until P9. Decreased Reelin expression was demonstrated quantitatively by Northern blot analysis and the correlation between the mRNA and protein levels was well presented. The expression of *reelin* mRNA decreased significantly by irradiation from P0, being almost one third of the level in controls on P4, and tended to recover up to P9. It is thus indicated that X-irradiation causes a marked decrease in the level of Reelin at the critical stage for the alignment of Purkinje cells. Since Reelin has been shown to play an important role in the migration of neural cells, it is suggested that the decrease in Reelin by X-irradiation is an important factor for the derangement of Purkinje cells.

Key Words: Cerebellum; Extracellular matrix protein; Foliation; Purkinje cell; Radiation; Reelin.

INTRODUCTION

It has previously been reported that prenatal exposure to X-ray on gestation days 19–21 causes heterotopic Purkinje cells in the internal granular layer and white matter of the abnormally foliated cerebellum in the rat (1–3). Because the studies concerned sections of the mature cerebellum stained with hematoxylin and eosin or cresyl violet, accurate identification of Purkinje cells or detailed examination of cellular morphology was not possible. Moreover, the process of heterotopic Purkinje cell formation in X-irradiated rats has not been studied (1–3). It also remains to unclear whether heterotopic Purkinje cells result from direct effects of X-irradiation on immature Purkinje cells, or from alteration of the developing milieu that prevent their normal migratory behavior. Therefore, the present study was undertaken to demonstrate the process of derangement of Purkinje cells and their surrounding cells caused by X-radiation employing immunohistochemistry.

In addition, the present study aimed to elucidate a possible factor(s) to induce heterotopic Purkinje cells and abnormal cerebellar foliation of the rat following prenatal exposure to X-radiation. For this purpose we examined the expression of morphoregulatory molecules by immunohistochemistry since their functions have been considered essential for the neuronal migration and cellular arrangement of the brain (4). The neural cell adhesion molecule (NCAM) is a well-known integral membrane glycoprotein involved in cell migration and direction of axonal outgrowth (5). This molecule is known to be expressed in the granule cells and Purkinje cells in the developing cerebellar cortex of the mouse (4, 6). Tenascin influences the processes of neuron-glial adhesion and cell migration at critical developmental stages of the nervous tissue (4, 7). In addition, Reelin has been suggested to play an important role in the migration of Purkinje cells. Miyata et al demonstrated that anti-Reelin antibody, CR-50, interferes with the formation of the Purkinje cell layer (8). The derangement of Purkinje cells was also demonstrated in reeler cerebellum in which no CR-50 immunoreactivity is detectable (8–10).

Our present results show that the level of Reelin is specifically reduced in the external and internal granular layers of irradiated rats in the period when immature Purkinje cells are in the process of alignment. It is therefore suggested that the decrease in Reelin is the possible cause
of the derangement of Purkinje cells in the irradiated cerebellum. The mechanism of abnormal foliation of the irradiated cerebellum is also discussed.

MATERIALS AND METHODS

Animal Experiments

Rats from Std:Wistar/st were housed in an air-conditioned room (21 ± 1°C) with relative humidity of 50 ± 10% under an alternating 12 h light/dark schedule, and were fed standard food (CE-2, CLEA, Japan) and water ad libitum.

Pregnant rats on GD-21 (plug day = GD-0) were exposed to a whole body X-irradiation at a dose of 2.5 Gy at 7 a.m., in accordance with the previous experiment (3). Radiation factors were 140 kVp, 5 mA, 0.5 mm Cu + 0.5 mm Al added filtration, and 104.0 ± 0.5 mGy/min exposure rate. To achieve a homogeneous dose distribution, the rats were placed in individual plastic cages, and the cages were rotated at 4 rpm on a turntable during exposure. Control pregnant rats were treated in the same manner except for exposure to X-irradiation. Both control and irradiated groups were allowed to give birth and rear their litters. They delivered neonates usually 5–8 h after exposure to X-irradiation (postnatal day 0, P0). The following experiments were repeated at least 3 times, and 34 litters of animals in total (22 irradiated, 12 controls) were used.

Conventional Histology and Immunohistochemistry of IP3 Receptor, NCAM, Fibronectin, and Tenascin

Pups of both groups were deeply anesthetized with diethyl ether and perfused transcendally with 4% formaldehyde. Their brains were then removed and postfixed with the same solution overnight. Brains were obtained 6 h and 12 h after exposure, on consecutive days from P1 to P16. Three or 4 pups were used for each time interval. After fixation the cerebella were dissected, dehydrated, embedded in paraffin, and serially sectioned in a mid-sagittal plane at 5-µm thickness. They were either stained with hematoxylin and eosin or subjected to immunohistochemistry for inositol 1,4,5-triphosphate (IP3) receptor, NCAM, fibronectin, and tenascin.

For immunohistochemistry, 2 or 3 sections for each cerebellum were selected from the serial sections, dewaxed, and sequentially incubated as follows: (1) in 0.6% H2O2 methanol for 5 min; (2) in 0.1 M phosphate-buffered saline (PBS), pH 7.0, containing 0.3% Triton X-100 for 30 min; (3) in 10% normal goat serum at room temperature for 10 min; (4) in anti-IP3 receptor antibody, 4C11 (11), diluted 1:100 in PBS, at 4°C overnight; (5) in biotinylated universal secondary antibody (Vector Laboratories, Burlingame, CA) diluted 1:50 in PBS for 1 h. The sections were rinsed in PBS, preincubated with 0.3% triton X-100, 3% bovine serum albumin (BSA) and 5% normal goat serum at 1:50, and then incubated with anti-Reelin antibody, CR-50 (13), diluted 1:10 in blocking solution (normal horse IgG) overnight. After incubation with the primary antibody, the sections were washed with PBS and reacted with biotin conjugated secondary antibody (anti-mouse IgG) diluted 1:250 in PBS for 1 h, then incubated with fluorescein isothiocyanate (FITC)-conjugated streptavidin (Biomedica, Foster City, CA) diluted 1:50 in PBS for 1 h. Images were taken with a fluorescence microscope (Axioskop 2, Zeiss, Germany). For the negative control, the first antibody was omitted during the labeling procedure.

To detect Bergmann cells and fibers, the sections adjacent to those treated immunohistochemically for Reelin were immunostained with anti-S-100 protein (IBL, Shizuoka, Japan, diluted 1:1,000) at 4°C overnight using the same procedure as immunofluorescence with anti-Reelin antibody.

Northern Blot Analysis

For Northern blot analysis of reelin mRNA, total RNA was extracted from the cerebellum by the method of acid-guanidinium-phenol-chloroform (14). The amount of total RNA was determined by the absorbance at 260 nm. Fifteen microgram of total RNA from each sample was subjected to Northern blot analysis under the protocol described previously (15). Mouse reelin cDNA fragment (a gift from Dr. T. Saitoh) was labeled with [32P]dCTP (specific activity, 111 TBq/mmol; New England Nuclear, Boston, MA) by the Random Primer Labeling Kit (Boehringer Mannheim, Mannheim, Germany) and used as a probe for hybridization. The density of the bands for reelin mRNA was analyzed by the Molecular Imager System (GS-363, Bio-Rad Laboratories, Hercules CA). Autoradiography was performed by exposing the membrane to Kodak X-AR (Eastman Kodak, Rochester, NY) film for 3 days. Membranes were reprobed with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (16) to monitor the equal delivery and the integrity of RNA.

RESULTS

Cell Death and Recovery of the External Granular Layer (EGL) after X-Irradiation

Because heterotopic Purkinje cells and foliar abnormality of the cerebellum have been reported to be more prominent in the anterior lobes (1), the effect of X-radiation on the cerebellum was examined in sagittal sections of the anterior and dorsal vermis (anterior to the fissura secunda). Figure 1 shows the early histological events...
Fig. 1. Cell death and recovery of the EGL after X-irradiation. The vermis in newborn rats prenatally exposed to X-irradiation (right column) and sham-exposed controls (left column). A, B: 12 h after exposure (P0), C, D: 48 h after exposure (P1), E, F: P4. EGL: external granular layer, PCL: Purkinje cells layer. The surface area of the control cerebellum was occupied by 5- to 9-cell-thick EGL (A, C, E). Extensive cell death appeared in the EGL 12 h after X-irradiation (B). A small number of granule cells remained (arrowheads) in the EGL 48 h after X-irradiation (D). Recovery of EGL had occurred by P4 and a layer was seen on the surface area (arrowheads) of the irradiated cerebellum (F). Hematoxylin and eosin stain. Scale bars A, B = 40 μm, and C, D, E, F = 20 μm.
Fig. 2. Arrangement of Purkinje cells in cerebella prenatally exposed to X-irradiation (right column) and sham-exposed controls (left column). A, B: P4, C, D: P9, E, F: P16. EGL: external granular layer, PC: Purkinje cells, ML: molecular layer, IGL: internal granular layer. Purkinje cells in the control on P4 aligned with apical cones directing toward the pial surface (A). Purkinje cells were deranged forming a 2- to 7-cell-thick layer and some apical cones had opposite directions (arrows) in the irradiated rat on P4 (B). Purkinje cells in the control on P9 were developing dendrites (C). The Purkinje cell layer of the irradiated
Fig. 3. Bergmann cells and their fibers in cerebella prenatally exposed to X-irradiation (right column) and sham-exposed controls (left column). A, B: P4, C, D: P9. BC: Bergmann cells, BF: Bergmann fibers, asterisks: Purkinje cells. Bergmann cell bodies of control rats on P4 and P9 were seen beneath the Purkinje cells, extending their fibers into the EGL (A, C). Bergmann cell bodies were below the scattered Purkinje cells and some were close to the pial surface (arrows) in the irradiated rat on P4 (B). Bergmann cells were seen beneath the Purkinje cell layer, extending their fibers into the EGL in the irradiated rat on P9. Bergmann cell bodies were not seen in the EGL (D). Immunofluorescence stain for S-100 protein. Scale bars = 30 μm.

rat on P9 remained in 2- to 3-cell-thick, and many cells were heterotopically located in the IGL. They had randomly directed short and thick stem dendrites with few branches (D). Dense dendritic arborization of Purkinje cells was seen in the ML of P16 control (E). Purkinje cells formed dense dendritic arborization in the ML of the irradiated rat on P16. Some part of the Purkinje cell layer was still 2- to 3-cell-thick, and some cells remained in the IGL with randomly directed dendrites (F). Immunohistochemistry for IP3 receptor. Scale bars A, B = 25 μm; C, D = 150 μm, and E, F = 75 μm.
after exposure. By 12 h after exposure, extensive cell death and destruction of the EGL became apparent (Fig. 1B), whereas the surface area of the control cerebellum was occupied by 5- to 9-cell-thick EGL (Fig. 1A). There were no findings of Purkinje cell death, suggesting that Purkinje cells are resistant to X-radiation. By 48 h after exposure, all cell debris had disappeared and a small number of surviving granule cells formed clumps together in places, while in other places granule cells were almost missing in the surface (Fig. 1D). By P4 the EGL started to recover. The recovering EGL showed variable thickness (Fig. 1F); a 1- to 4-cell-thick EGL was present in some regions, while the surface of the cerebellum was still devoid of these cells in others.

Heterotopic Purkinje Cells in the Irradiated Cerebellum

Figure 2 shows the development of Purkinje cells after exposure. By P4 the majority of Purkinje cells in the control formed a monolayer with apical cones directing toward the pial surface of the cerebellum (Fig. 2A). In X-irradiated rats, the arrangement of Purkinje cells was irregular, forming a 2- to 7-cell-thick layer with random orientation of the apical cones (Fig. 2B). Purkinje cells in the control on P9 were well aligned with developing dendritic arbors toward the pial surface (Fig. 2C). In contrast, as shown in Figure 2D, Purkinje cells in the irradiated cerebellum remained in a 2- to 3-cell-thick layer. Moreover, many Purkinje cells were heterotopically located in the internal granular layer (IGL). They also exhibited morphological abnormalities: short and thick stem dendrites with few branches and random directions of the stem dendrites or some dendrites extending toward the opposite direction.

A partial recovery from the derangement of Purkinje cells was observed on P16. At this age Purkinje cells in the control were perfectly aligned and their dendritic arbors densely filled the molecular layer (ML) (Fig. 2E). Purkinje cells in the irradiated cerebellum also formed well-branched dendritic arbors and many of them were normally located in the Purkinje cell layer. However, some Purkinje cells remained in a 2-cell-thick layer or scattered in the IGL with randomly directed stem dendrites (Fig. 2F).

Bergmann Cells and Their Fibers in the Irradiated Cerebellum

Figure 3 shows the localization of Bergmann cells and their fibers. Bergmann cell bodies were localized beneath the Purkinje cell layer and their fibers extended into the EGL of the control cerebellum on P4 (Fig. 3A). In the irradiated rat of the same age, Bergmann cell bodies were localized below randomly scattered Purkinje cells and some were close to the pial surface of the cerebellum (Fig. 3B). Bergmann fibers appeared crumpled and were generally shorter and abnormally oriented. By P9 the EGL in irradiated rats recovered, and Bergmann cells were no longer found in the EGL. Bergmann cell bodies were observed beneath Purkinje cells, extending their fibers into the EGL perpendicular to the pial surface (Fig. 3D); the arrangement of Bergmann cells and their fibers compared with that of the control cerebellum (Fig. 3C).

Foliar Abnormality in Adult Rats

To test the critical stage in induction of the abnormal foliation of the cerebellum, a litter of pups were exposed to 2.5 Gy X-irradiation on P4 as well as on GD-21. Two pups were killed 6 h after exposure and destruction of the
Fig. 5. Immunoreactivity of NCAM (A, B), fibronectin (C, D) and tenascin (E, F) in cerebella prenatally exposed to X-irradiation (right column) and sham-exposed controls (left column). PCL: Purkinje cell layer, IGL: internal granular layer. Strong NCAM immunostain around Purkinje cells and weak stain in the IGL were seen in the control of P6 (A). Strong NCAM immunostain was seen in 2- to 4-cell-thick Purkinje cell layer and around heterotopic Purkinje cells of the irradiated cerebellum of P6 (B). Immunoreactivity of fibronectin (C, D) and tenascin (E, F) were strong around Purkinje cells and weak in the EGL and IGL in both control and irradiated cerebella of P4. Scale bars A, B = 40 μm, and C, D, E, F = 50 μm.
Fig. 6. Immunoreactivity of Reelin in cerebellar prenatally exposed to X-irradiation (right column) and sham-exposed controls (left column). A, B: 12 h after exposure, C, D: P1, E, F: P4, G, H: P9. EGL: external granular layer, ML: molecular layer, IGL: internal granular layer. In controls on P0 through P9, cell bodies in the inner half of the EGL and outer part of the IGL were strongly Reelin immunoreactive (A, C, E, G). Reelin was present in the EGL and IGL 12 h after X-irradiation (B). Reelin decreased on P1 (D) through P4 (F). In addition to the EGL and IGL, the ML was also diffusely immunoreactive in the control on P9 (G). Reelin became visible both in the EGL and IGL of the irradiated rat on P9 (H). Immunofluorescence stain with antibody CR-50. Scale bars = 30 μm.

EGL was confirmed histologically. Eight pups were reared to adulthood. We selected P4 because this is the stage when Purkinje cells are in alignment as mentioned above. When X-radiation was performed in the prenatal period, GD-21, the pattern of cerebellar foliation was remarkably abnormal: The number of lobules was larger and the direction of the fissures was almost vertical, whereas the direction of most fissures was horizontal in the control (Fig. 4A, B). In contrast, no apparent foliar abnormalities were found in the cerebellum exposed on P4 (Fig. 4C). These results suggest that the Purkinje cell alignment in the early neonatal days (P0 to P4) is important for normal foliation of the cerebellum.

Levels of NCAM, Fibronectin and Tenascin

Figure 5 shows that neither the levels of NCAM, fibronectin, or tenascin were affected by irradiation. Strong expression of NCAM was observed around Purkinje cells even in the heterotopic location on P6. Comparable levels between control and irradiated groups were constantly observed throughout the period from P0 to P7 (data not shown).

Immunohistochemistry of Reelin

Figure 6 shows the levels of Reelin. In the control cerebellum on P0 through P9, Reelin was present on the
cell body, but not in the nucleus, especially in the inner half of the EGL (premigratory zone) and in the outer part of the IGL (Fig. 6A, C, E, G). Twenty-four hours after exposure (P0), the level of Reelin was still comparable to that in the control (Fig. 6A, B), however, it markedly decreased in the irradiated rat on P1 until P4 (Fig. 6C, D). Decreased levels of Reelin were observed throughout the cerebellar cortex (Fig. 6D, F, H), and Reelin tended to recover on P9, although the intensity of the immunostaining was still lower than that in the control.

Northern Blot Analysis of Reelin mRNA

Quantitative analysis of Reelin expression was also performed. Northern blot analysis was employed for this purpose because we found that the available anti-Reelin antibody, CR-50, failed to visualize Reelin protein in Western blot analysis (unpublished data). Figure 7A shows reelin mRNA as a band at 12 kb. Figure 7B shows the densitometric analysis of the band for reelin after correction for the amount of mRNA for the housekeeping gene, GAPDH. The abundance of reelin mRNA was almost constant throughout the observation period from P0 to P9 in the control. X-radiation caused a rapid decrease in reelin mRNA expression. The decrease was already evident on P0 when Reelin protein still remained comparable with the control. The expression decreased further, being almost one third of the level of the control group on P4. Although the expression tended to recover after P5, the levels were significantly lower than those of controls up to P9 (the end of the observation period).

DISCUSSION

By consecutive observation using immunohistochemistry, the present study demonstrated sequential changes
Fig. 7. Northern blot analysis of reelin mRNA from P0 through P9. (A) Visualized by autoradiography. A GAPDH (glyceraldehyde-3-phosphate dehydrogenase) probe was used for the loading control. (B) Visualized by densitometry. Expression of reelin mRNA was decreased in the X-irradiated cerebellum from P0 through P9. Data were presented as mean ± SE of 9 × replicate values for reelin mRNA expression. Analysis by two-way ANOVA. The difference between control and irradiated groups was statistically highly significant (p < 0.0001).

in cellular events in the cerebellum of rats after prenatal X-irradiation. X-radiation caused extensive cell death in the EGL as an acute effect, followed by recovery of the EGL. Although Purkinje cells were resistant to X-ray-induced mortality, they exhibited disturbed alignment with abnormal dendritic arborization. These were partially corrected during the following development, but the incomplete recovery resulted in disorganized Purkinje cell layer and heterotopic Purkinje cells in the IGL with abnormally oriented dendritic arbors. Bergmann cells with abnormal fibers also showed heterotopic locations. These abnormalities occurred before recovery of the EGL. When the EGL recovered, all Bergmann cells were located beneath the Purkinje cell layer and extended their fibers to the pial surface, being comparable to the control. These results suggest that recovered granule cells push Bergmann cells into the proper area, enabling extension of their fibers. As a result of recovery of the EGL, no
heterotopic granule cells in the ML were found in adult rats (data not shown).

Abnormal foliation induced by X-irradiation seems to correlate with abnormal cellular events described above. On GD-21, migration of immature Purkinje cells from the ventricular zone to the cortical area of cerebellum was almost complete, however, immature Purkinje cells were still in a multilayer (17, 18). X-irradiation at this stage induces abnormal foliation. In contrast, when rats were exposed on P4 to the same dose, almost normal foliation developed even though the majority of granule cells in the EGL were destroyed. Therefore, the critical stage for cerebellar foliation is in the early postnatal period and the alignment of Purkinje cells seems important for a normal pattern of the foliation.

A heterotopic localization of Bergmann cells at the early neonatal days may also be involved in abnormal foliation. When Purkinje cells were in a heterotopic location, Bergmann cells followed the Purkinje cell movement and their fibers were abnormally arranged at this stage. This abnormal arrangement of Bergmann fibers may affect the direction of migrating granule cells and change the direction of fissuration of the cerebellum.

To study the molecular mechanism of the abnormal patterning of Purkinje cells by X-irradiation, we first examined the expressions of NCAM, fibronectin, and tenascin. As reported previously in the mouse cerebellum (4–6), strong immunostaining of NCAM was observed around Purkinje and granule cells. In irradiated rats, NCAM expression was also detected with similar intensity to that in the control. Similarly, strong immunostaining of fibronectin and tenascin was visible in the Purkinje cell layer both in control and irradiated cerebellum. Although these molecules have been reported as critical factors for the cellular arrangement of the brain, results of the present study suggest that these molecules are not involved in the derangement of Purkinje cells in the irradiated cerebellum.

It should be noted that strong expression of NCAM was observed around Purkinje cells even showing derangement, indicating that Purkinje cells are able to function normally and are resistant to X-rays. This suggests that the derangement of immature Purkinje cells is not due to the direct effect of X-irradiation on Purkinje cells but due to changes in the surrounding milieu after exposure.

We focused on Reelin as a candidate to mediate the abnormal patterning of Purkinje cells induced by X-irradiation. Reelin is a secretory extracellular protein and has already been suggested to mediate neuronal adhesion and migration at critical stages of development (13, 19–23). In reeler mutant mice, in which Reelin is absent, Purkinje cells do not migrate but remain as clusters at deep cerebellar areas (23–27). When normal external granule cells were explanted, reeler Purkinje cells started to migrate around the explanted granule cells (23). These results strongly suggested that Reelin plays a pivotal role in the migration of Purkinje cells. However, in such mutant mice, Reelin is absent throughout the life, and the lack of this molecule affects not only Purkinje cell migration but also the entire structure of cerebellum and cerebrum. Therefore, it is not possible to specify the critical stage for Reelin to affect the arrangement of Purkinje cells and to discuss the relationship between the Purkinje cell arrangement and foliation. In this study, using X-irradiation, we were able to induce the lack of Reelin at the specific time during cerebellar development. Immunohistochemistry of Reelin in this study clearly demonstrated that X-irradiation on GD-21 caused the reduction of Reelin 48 h after exposure and the decrease continued up to P9, the end of the observation period. During the time when the lack of Reelin occurred in the irradiated pups the alignment of Purkinje cells was completed in the control. Our results thus render evidence that Reelin is important for the arrangement of Purkinje cells at the critical stage.

The decreased reelin expression was also demonstrated quantitatively at the level of mRNA. The decrease in reelin mRNA was already evident 24 h after exposure, the level was lowest around P4 and started to recover afterward. The decreased reelin expression seemed due to cell loss of the EGL. However, there was a lag between the recovery of the EGL and that of Reelin expression because histological examination revealed that recovery of the EGL had already started on P4. A report on Reelin secretion by postmitotic granule cells, but not by proliferating granule cells (8), may explain this lag.

In summary, our results demonstrate that X-irradiation on GD-21 causes cell death in the EGL resulting in a decreased level of Reelin in the early neonatal period. The lack of Reelin at this stage may contribute to the permanent derangement of Purkinje cells, and possibly also to abnormal foliation of the cerebellum. Since the mechanism of cerebellar foliation is not yet known, results of the present study provide an insight into the development of folia.

ACKNOWLEDGMENTS

We wish to thank Dr. Tetsuichiro Saitoh, National Institute of Genetics, Japan, for providing Reelin cDNA and to Dr. David Walsh for reviewing the manuscript. We also thank to Sizu Hayasaka, Hossain M.D. Aolad, Yasuhiko Kanou, Sugiko Futaki, and Ayesha Siddiq for their experimental contributions to this study.

REFERENCES


17. Altman J, Bayer SA. Prenatal development of the cerebellar system in the rat II. Cytogenesis and histogenesis of the inferior olive, pontine gray and the precerebellar reticular nuclei. J Comp Neurol 1978;179:49–76


Received August 31, 1999
Revision received December 13, 1999
Accepted December 14, 1999