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A Morphometric Study on Postnatal Development of the External Granular Layer of Mice Cerebella, Focusing on Local Difference

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ABSTRACT

The external granular layer (EGL) of the cerebellum thickens, thins and disappears in its developmental process. We examined the thickness of the EGL, both intralobule differences and interlobule differences, in the whole midline sections of mouse cerebella for the entire postnatal period up to disappearance. The thinnest site in each lobule was located at the outer apex throughout the observation period, the thickest site was the portion facing the inner apex during early period, and that facing the convexity where the EGL curved in the later period. The observed interlobular differences of the EGL thickness were statistically divided into three groups, referred to as the early developing group (EDG), the late developing group (LDG), and the mixed-type group (MTG). The EDG consisted of the whole anterior lobe and a site in lobule VI facing the fissura prima. These sites thickened earlier, and showed a similar thickness on each observation day. The LDG was composed of all sites in the posterior lobe, with the exception of two sites where lobules VIII and IX confronted each other as well as the site included in the EDG. The sites in the LDG thickened later. They demonstrated a similar thickness during thickening, but varied during thinning. The MTG, consisting of two sites where lobules VIII and IX facied each other, showed features similar to the EDG in the mitotic zone and the LDG in the premigratry zone. These data may serve as the basis for studies on regional differentiation of the cerebellum.

Key words: *Cerebellum, External granular layer, Development, Mice*

The cerebellum appears as a thickening in the anterior part of the roof of the fourth ventricle, rhombic lip[11]. The rostral part of the rhombic lip gives rise to the external granular layer (EGL), where granule cells divide and proliferate within a given period of time. The EGL covers the surface of the cerebellum, consisting of two layers: the mitotic zone where the granule cells undergo mitosis, and the premigratory zone where the granule cells extend parallel fibers and stay before starting to migrate. By division and proliferation, the EGL increases in thickness. As the granule cells migrate into the internal granular layer, the EGL reduces in thickness and disappears.

Proliferation and migration of the granule cells have been investigated by observing mitotic figures and by autoradiography[8,11]. In addition, studies on the thickness of the EGL provide us with valuable information in interpreting the EGL development. In the pyramis of rats, Altman[4] observed the depth of cells in the mitotic zone and the premigratory zone. In the sagittal planes of mice, Fujita et al[7] described the thickness of the two layers as basic data to determine the nature of granule cells, and Fujita[8] reported on a further study in culmen and central. In the mid-sagittal plane, Mares and Lodin[9] attempted to elucidate the mechanism of the process of gyrification, by comparing the morphology and proliferation of the whole EGL at the bottom of fissures with those on the surface of the lobule. In these papers, however, either the width and location of the selected sites for observation in the mid-sagittal sections varied from author to author, or the selected sites were not precisely described. Among these various sites observed in previous studies, local differences were discernible. A detailed description of the whole EGL thickness with a focus on local differences has not yet been provided in previous reports.

In the present paper, the authors attempted to delineate the normal postnatal changes of the

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EGL thickness with systematic observations, in whole mid-sagittal sections until the EGL disappeared. We discuss our results in relation to the recent studies on genes expressed in cerebella.

MATERIALS AND METHODS

1) Animals

Jcl:ICR mice from Japan CLEA Co., Ltd. (Tokyo) (8–12 weeks old) were used for this study. The day when a vaginal plug was found was designated as gestation day 0 (GD 0). Only pups born on GD 19 were used. The day of birth was designated as postnatal day 0 (PD 0). Pups culled to 12 per dam at PD 0 consisted of six males and six females. Only females were used for this study. Each age group consisted of two or three litters.

2) Histological observation

a) Tissue preparation

As the EGL of the cerebella disappeared around PD 21, observation days were set for PD 1, 4, 7, 10, 13, 16, 19, or 22. Mid-sagittal sections of the cerebellar vermis were served for observation. On observation days, all mice in a litter were given a single intraperitoneal injection of 5-bromo-2'-deoxyuridine (BrdU) (Cell proliferation kit, RPN 20, Amersham) at a dose of 30 mg/kg body weight. One hour after BrdU injection, two mice from each litter were sacrificed after proper anesthesia. Cerebella were removed, fixed in Bouin's fixative for 2 hours at 4°C, and embedded in paraffin after dehydration in graded ethanol series. Sections were cut serially at 8 μm thickness in parasagittal planes. For the proper orientation, only those sections that satisfied the following two conditions were selected for this study: (1) the aqueduct of cerebrum appeared longitudinally through the mid-anteroposterior axes, (2) dendrites of the Purkinje cells could be traced for considerable distances in all lobules in the sagittal planes.

Mid-sagittal sections were stained in hematoxylin and eosin (H-E) for routine histology and also by BrdU/anti-BrdU immunohistochemistry in order to detect BrdU. In order to confirm whether only cells in the mitotic zone have the ability to take up BrdU, immunostained sections and H-E sections were compared.

b) Detection of cells taking up BrdU

For immunohistochemical staining, sections were deparaffinized, rehydrated, incubated in 1N hydrogen chloride for 30 min at 37°C, and in methanol containing 3% hydrogen peroxide for 20 min at room temperature. All reagent was used and the protocol was carried out according to the description of the Amersham Kit.

c) General view of the structure

In order to observe the general view of structures in entire midsagittal sections, they were photographed on PD 1, 4, 7, 10, or 16 with a dissection microscope (Nikon SMZ-U) at the same magnification.

d) The morphology of the EGL

The morphology was observed with a microscope (Nikon OPTIPHOT UFX-IIA).

d-i) Thickness of the EGL within each lobule (intralobule differences)

In order to ascertain the thickest and thinnest sites within a lobule, the thickness of the EGL was observed in each lobule from PD 4 when most of the main lobules were formed, to PD 13 when the EGL could still be observed in most lobules (n = 4, respectively).

d-ii) Thickness of the EGL among lobules (interlobule differences)

The thickness of the EGL was observed among lobules of cerebella on PD 1, 4, 7, 10, 13, 16, 19 and 22 (n = 4, respectively). The thicknesses of the mitotic zone and the premigratory zone in the EGL were measured separately in terms of the number of cells serially aligned perpendicular to the surface of the fissure wall at the midpoint between the top and bottom of the fissure or, in a straight segment closest to that midpoint where the fissure was not straight (Fig. 1). To assure that one determination of the number of cells in a site would be sufficient to represent the thickness for the site, the measured value was compared with those at two loci, 20 μm apart from the measured point. For this study, 23 points were selected at random, equivalent to 5% of those observed in our main study. The result showed that a single determination for each locus would be sufficient to describe the thickness of the site. Since integral numbers were not sufficient to exhibit the thickness of the EGL due to its non-uniformity of cell arrangement, the cell number was expressed to

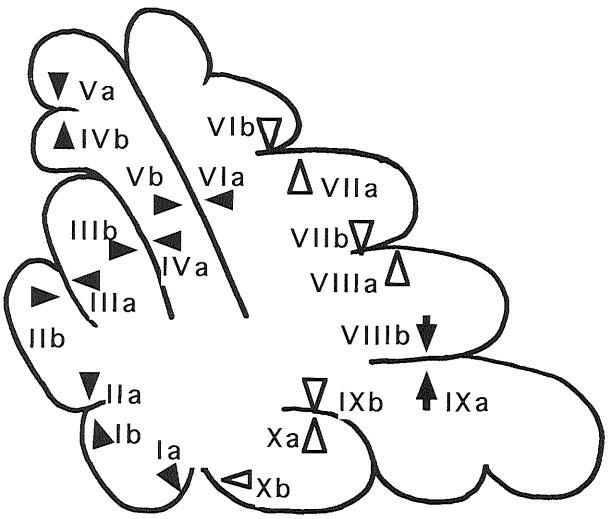

Fig. 1. Sites selected for observing interlobule difference.
The sites are selected in the middle of each fissure. These sites are divided into three groups: an early developing group (filled arrowheads), a late developing group (white arrowheads), and a mixed-type group (arrows) (see text for details).

the nearest 0.5 (Fig. 2). We avoided counting cells at successive adjacent sites to avoid bias by the previous result.

3) Statistical Analysis

Observed sites were grouped, based on data obtained from PD 4 to PD 19 by Hierarchical Cluster Analysis (Ward Method, Square Euclid Method) with the SPSS Base 10.0J[17]. Analyses of variance (ANOVAs) were examined, and Games-Howell method was used for post hoc tests[16] to compare group means on each developmental day.

RESULTS

1) Results of the intralobule observation

In order to clarify the intralobule difference in the thickness of the EGL in each lobule, we observed its thickness in sections from PD 4 to PD 19. The results revealed that the thinnest part of almost all lobules was located at the outer apex throughout the period of observation. The thickest part was shown to face the inner apex during the early days (to PD 4 in anterior lobe, to PD 13 in posterior lobe) and was located in the convexity where the EGL curved in the later days (Fig. 3). The most consistent thickness of a lobule appeared, throughout the period of observation, along the straight border between adjoining lobules. Such consistent thickness varied somewhat from lobule to lobule.

2) Results of the interlobule observation

Interlobule differences were determined by measuring, in every lobule, the thickness at the midpoint along the straight portion of each of the two streches of the most consistent thickness (Fig. 1). Sections from newborn mice, however, did not include as many sites as those from adult mice did for observation, because of the immaturity of the folial formation (Table 1).

The morphology and function of the mitotic zone and the premigratory zone were first confirmed with H-E stained sections and immunostained sections (Fig. 4). The cells in the EGL were divided into two layers; one beneath the pial surface and the other (the inner layer) between the outer layer

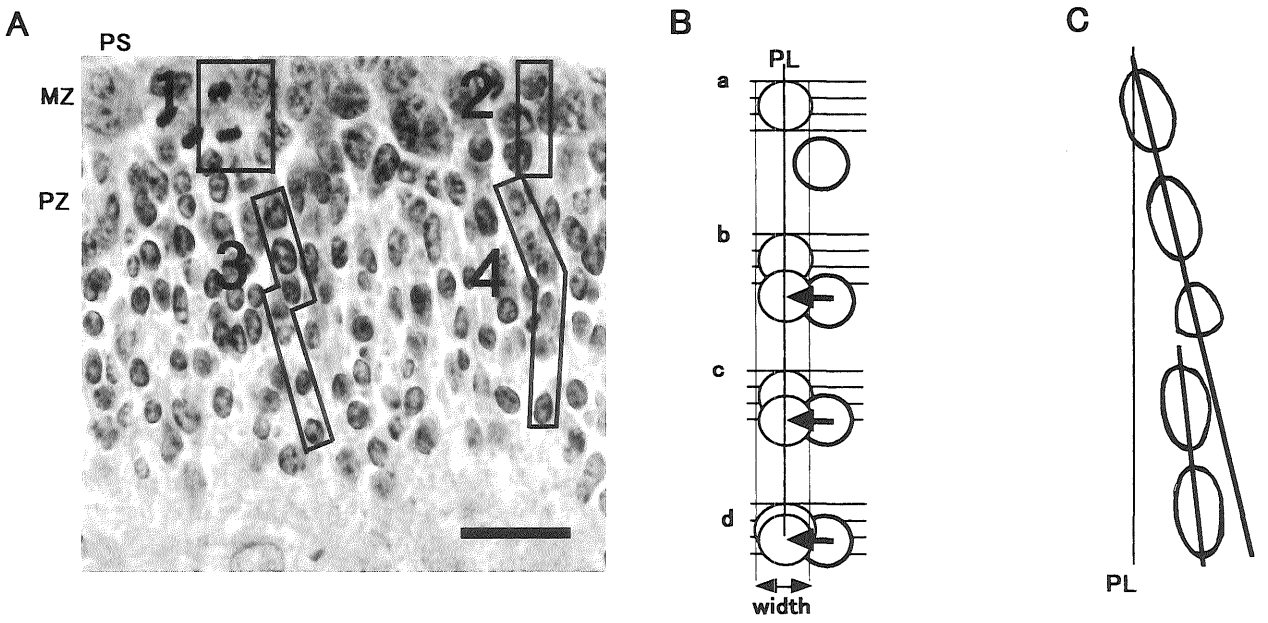

Fig. 2. Identification of the boundary between mitotic zone (MZ) and premigratory zone (PZ), and counting cells for measuring thickness of the EGL.

The EGL is divided into two layers morphologically: an outer layer beneath the pial surface and an inner one between the outer layer and the molecular layer (A). Cells in the outer layer lie close together in irregular arrangement. Those in the inner layer are oval and aligned perpendicular to the pial surface (perpendicular line, PL), with their long axes in the same direction. The outer layer was shown to be as the MZ, and the inner one the PZ.

For measuring the thickness of the EGL, nuclei in each layer were separately counted to express the number of cells. For a given thickness, all cells were counted if any part of each successive cell lay within the width of the cell beneath the pial surface in the direction of the PL (A). When these cells were not aligned along a PL, they were counted as follows. All cells were considered to be aligned in a PL. If two successive cells overlapped with each other by no more than one third of the diameter of the earlier encountered cell (B-a, b), they would be counted as separate cells. If the overlap was more than one third but less than two thirds (B-c), they were counted as one and a half. If the overlap exceeded two thirds (B-d), two cells were counted as one. In the PZ, cells are simply counted along their alignment, which often deviated from the PL. When a line of cells appears to stop in the middle of the PZ, but another line appears immediately below (C), thickness of the PZ is expressed by adding cell numbers of two segments. If one third to two thirds of the cell is visible, it is counted as half a cell (third cell in C). Examples of cells counted in this way: A-1 = 2.5, A-2 = 3, A-3 = 6, A-4 = 6. Abbreviation: PS, the pial surface. Scale bars: 10 μm

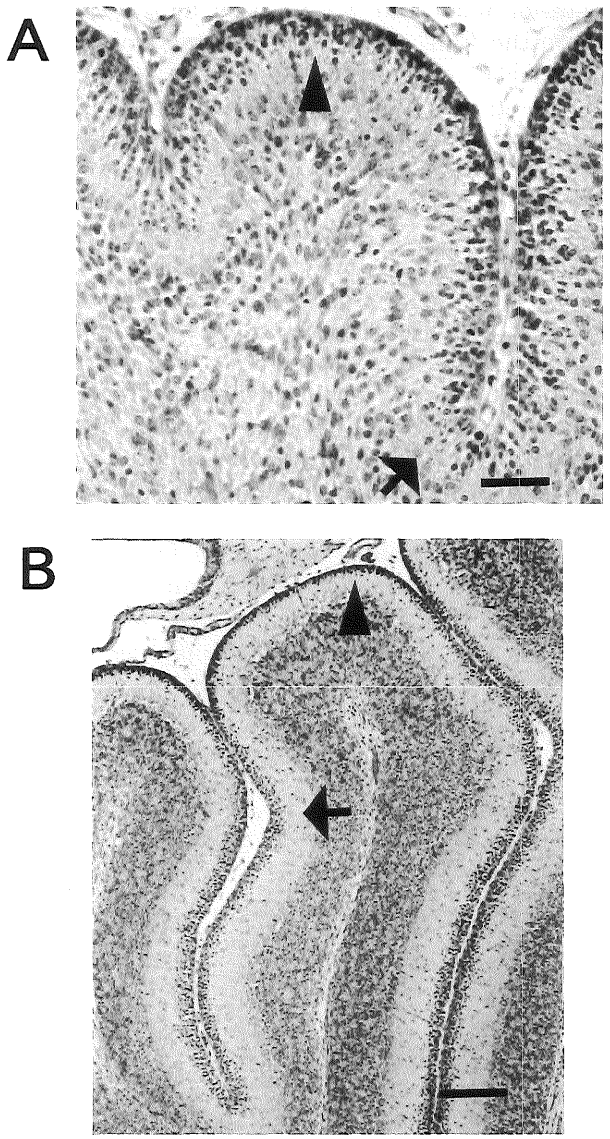

Fig. 3. Intralobule difference of the EGL on PD 1 (A) and PD 10 (B).
The thinnest part of the lobule is located at the outer apex (arrowheads). The thickest part is shown to face the inner apex on PD1, and is in the convexity where the EGL curves (arrows) on PD 10. Scale bars: A 30 μm; B 100 μm.

and the molecular layer. The cells in the outer layer were bigger, round, close to each other, in irregular arrangement. Those in the inner layer were smaller, oval and vertically aligned with their long axes in the same direction. The BrdU labeled cells were observed mainly in the outer layer, recognized as the mitotic zone. Some labeled cells were also found in outer parts of the inner layer, presumably ones migrating into the inner layer after labeled in the mitotic zone. The inner layer was recognized as the premigratory zone.

We observed the interlobule differences in the thickness of the EGL in selected sites of the EGL from PD 1 to PD 22 when the EGL disappeared. All data in 20 sites from PD 4 to PD 16 were statistically grouped, according to the indicated

Table 1. Observable sites in each development age

Observation Site	PD1	PD4	PD7	PD10	PD13	PD16	PD19	PD22
I a	–	+	+	+	+	+	+	+
I b	–	–	–	±	±	±	±	+
II a	–	–	–	±	±	±	±	+
II b	–	+	+	+	+	+	+	+
III a	–	+	+	+	+	+	+	+
III b	+	+	+	+	+	+	+	+
IV a	+	+	+	+	+	+	+	+
IV b	–	–	–	+	+	+	+	+
V a	–	–	–	+	+	+	+	+
V b	+	+	+	+	+	+	+	+
VI a	+	+	+	+	+	+	+	+
VI b	–	–	+	+	+	+	+	+
VII a	–	–	+	+	+	+	+	+
VII b	–	+	+	+	+	+	+	+
VIII a	–	+	+	+	+	+	+	+
VIII b	+	+	+	+	+	+	+	+
IX a	+	+	+	+	+	+	+	+
IX b	–	+	+	+	+	+	+	+
X a	–	+	+	+	+	+	+	+
X b	–	+	+	+	+	+	+	+

a, b for observation sites shown in Fig. 1.

developmental features. They were divided into three groups based on their developmental features: an early developing group, a late developing group, and a mixed-type group (Fig. 5).

The early developing group was examined in all observation sites in the anterior lobe, and one site in lobule VI facing the first fissure. The late developing group was composed of all sites in the posterior lobe, with the exclusion of the site included in the early developing group, and two more sites where lobules VIII and IX confronted each other. The mixed-type group was composed of these two sites.

Developmental variations in the thickness of the EGL are described below. The EGL gained or sustained its thickness until PD 7, and lost its thickness almost constantly afterwards. In the early developing group, the EGL gained its thickness from PD 1 to PD 4, preserved it until PD 7, lost it constantly and disappeared on PD 13 in the mitotic zone, and on PD 16 in the premigratory zone (Fig. 6). The early developing group, including the sites formed late, showed almost the same thickness of the EGL throughout development. All the observation sites in the late developing group were not formed yet on PD 1. The EGL thickened from PD 4 to PD 7, thinned from PD 7 to PD 13 or PD 16 in the mitotic zone and to PD 16 or PD 19 in the premigratory zone. The late developing group demonstrated almost the same thickness of the EGL during thickening. However, the thickness varied from PD 7 when it started thinning. The later the observational sites were formed, the later the thickness became thinner. The mixed-type group indicated a similar developmental pattern to the early developing group in the mitotic zone,

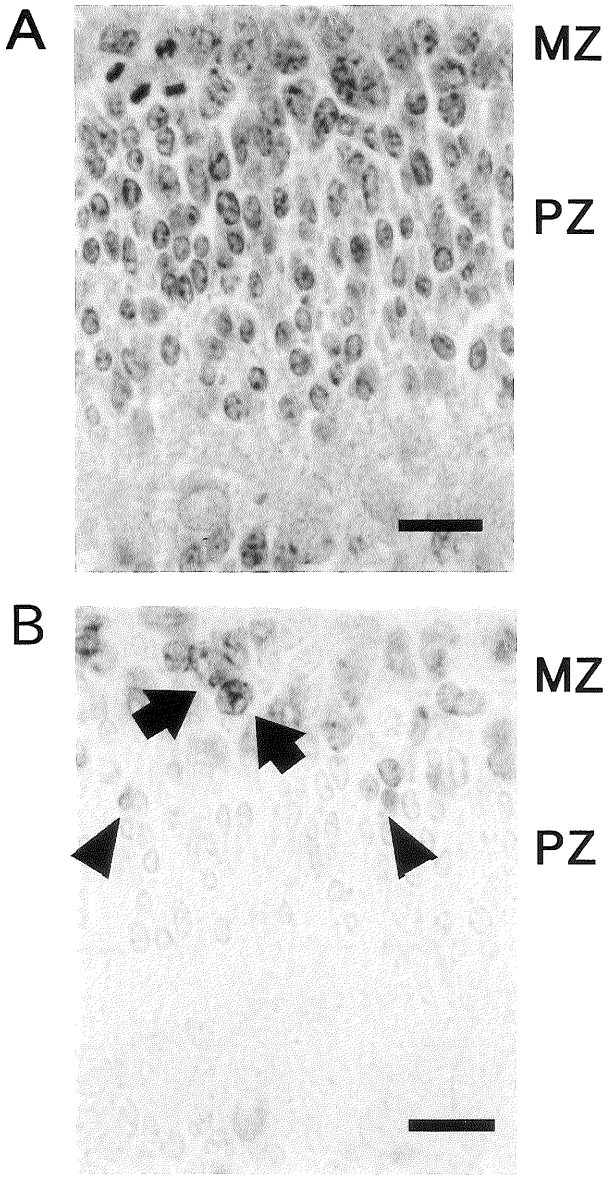

Fig. 4. Mitotic zone (MZ) and premigratory zone (PZ) of the EGL in PD 4 serial sections.
A: H-E stained section exhibiting morphology of MZ and PZ. B: immunostained section 1 h after injection, with most of BrdU-labeled cells (arrows) in MZ. Labeled cells in PZ (arrowheads) are considered to have started to migrate. Scale bars: 10 μm.

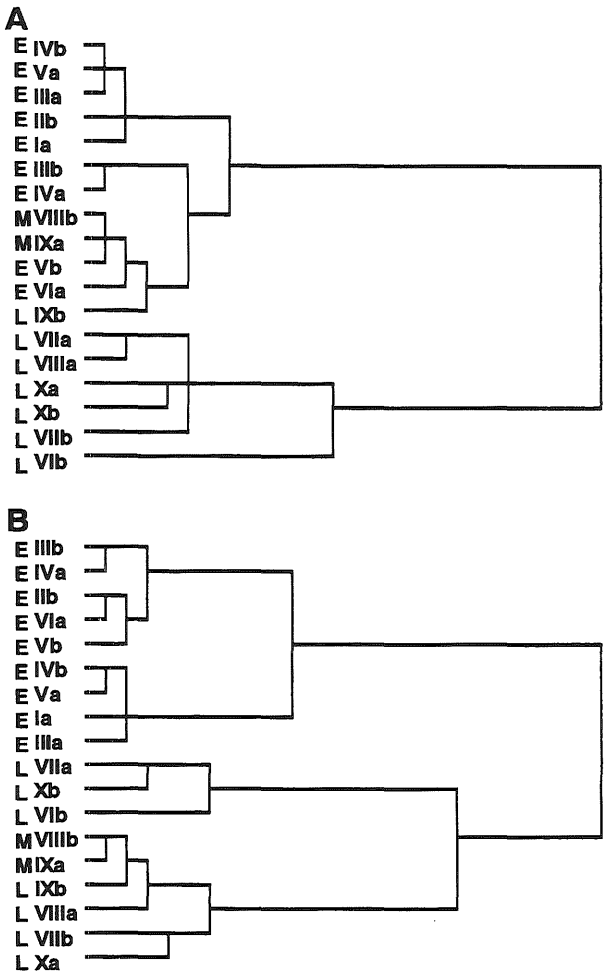

Fig. 5. Statistical grouping of the observed sites using the data from PD 4 to PD 19 by Hierarchical Cluster Analysis (Ward Method, the squared Euclidean distance).
A: the mitotic zone (MZ), B: the premigratory zone (PZ). All observed sites are divided into two groups in both MZ and PZ. The sites composing each group are almost the same in MZ and PZ, except for VIIIb and IXa. These two sites are referred to as M. The remainder of the sites, divided into two groups, are referred to as E and L, according to their developmental characteristics (described later). Abbreviations: E, early developing group; L, late developing group; M, mixed-type group.

and to the late developing group in the premigratory zone.

We further compared all data in three groups on each observation day with ANOVAs (Table 2). The result of the mitotic zone provided a positive proof that the developmental features of the mixed-type group showed similarity to the early developing group; i.e. absence of significant differences of the EGL thickness between the early developing group and the mixed-type group indicated the similarity of the two groups. The thickness of the mixed-type group and that of the late developing group were often significantly different, showing the distinct developmental features of two groups in the mitotic zone. In the premigratory zone, the statistics did not lend support for the grouping. Significant differences were demonstrated on some days between the early developing group and the mixed-type group but they were also confirmed between the late developing group and the mixed-type group.

DISCUSSION

We observed postnatal variations in the EGL thickness until the EGL disappeared, with systematic procedures in whole midsagittal sections of the mice cerebella.

The results revealed the following intralobule differences in the EGL thickness: 1) the thinnest parts were located at the outer apex, 2) the thick-

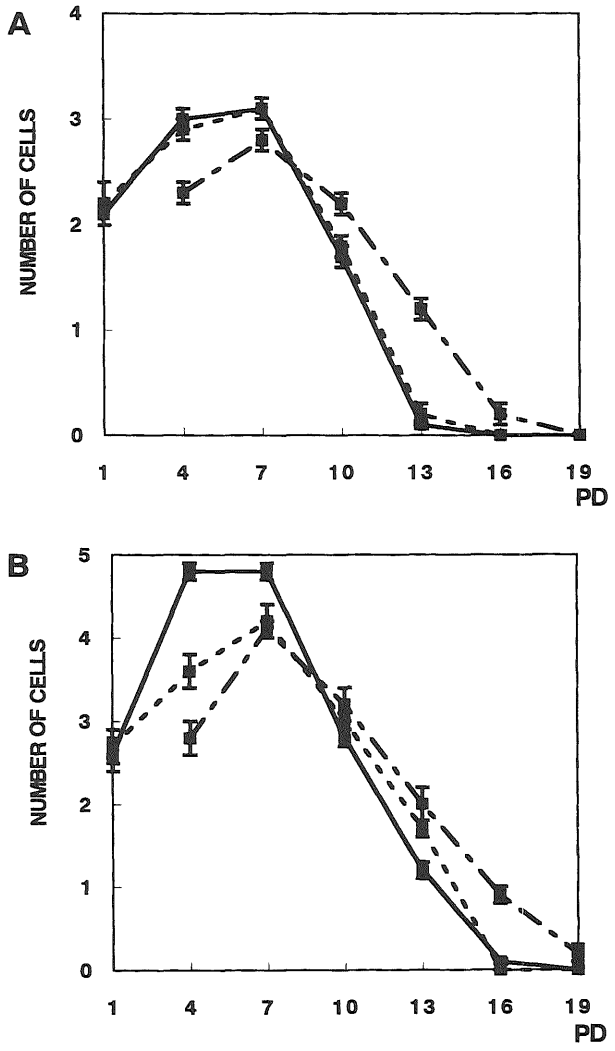

Fig. 6. Means of EGL thickness in three groups on each developmental day.
A: thickness of the mitotic zone; B: thickness of the premigratory zone. Solid lines, early developing group; chain lines, late developing group; dotted lines, mixed-type group. Error bars indicate SEM.

est parts were shown to face the inner apex during the early days and were located in the convexity where the EGL curved in the later days. The developmental features of the EGL thickness in the whole midline sections beyond lobules were divided into three groups, the early developing group, the late developing group, and the mixed-type group.

The characteristic gain and loss of the EGL thickness described above was reflected in the change of area proportion in the sections (Fig. 7). Until PD 7, when the size of the anterior lobe increased more than that of the posterior lobe, the EGL in the early developing anterior lobe was mostly thicker in the posterior lobe.

From PD 7 to PD 16 when the dorsal part of the posterior lobe expanded its area the most, the EGL in the dorsal region remained thicker than in other parts (data not shown).

Table 2. Comparison of the cell numbers in the three groups in a verebellum with ANOVAs.

Mitotic Zone

	PD4	PD7	PD10	PD13	PD16
ANOVAs	$p < .001$	$p < .005$	$p < .001$	$p < .001$	$p < .005$
E-L	$p < .001$	$p < .005$	$p < .001$	$p < .001$	N.S.
E-M	N.S.	N.S.	N.S.	N.S.	equal
L-M	$p < .005$	$p < .005$	N.S.	$p < .001$	N.S.

Premigratory Zone

	PD4	PD7	PD10	PD13	PD16	PD19
ANOVAs	$p < .001$	$p < .001$	N.S.	$p < .001$	$p < .001$	$p < .001$
E-L	$p < .001$	$p < .001$	N.S.	$p < .001$	$p < .001$	$p < .005$
E-M	$p < .001$	N.S.	N.S.	$p < .005$	$p < .005$	equal
L-M	$p < .005$	N.S.	N.S.	N.S.	$p < .001$	$p < .005$

Games-Howell for multiple comparison test.
Abbreviations: E. the early developing group; L, the late developing group; M, the mixed-type group.

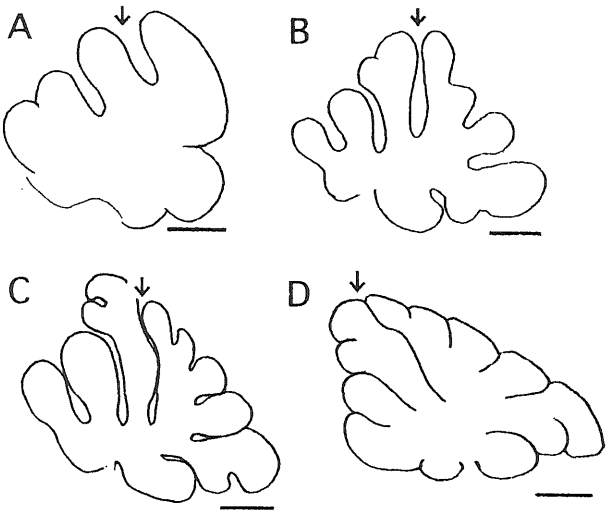

Fig. 7. Proportion of areas in anterior lobe and posterior lobe in midline sections.
From PD 1 to PD 7, the anterior lobe expanded more than the posterior lobe. After PD 7, the dorsal part of the posterior lobe increased in area. A, PD 1; B, PD 7; C, PD 10; D; PD 16. Arrows: fissura prima. Scale bars: A, 2.5 μm; B, 3.5 μm; C, 6.7 μm; D, 10 μm.

Altman[3] noted that the ventral lobes (lobules I and X) and the areas facing the fissura prima matured early, and the last maturing vermian lobes were lobules IV, V, VI and VII. His studies on lobules VI and VII are consistent with ours, but those of lobules IV and V are not. Mares[9] demonstrated that the average proliferation rate was higher in the EGL localized in fissures than at the superficial convexities of gyri. This supports our result that the thickest parts of the EGL faced the deep gyri in young animals.

In previous papers, the areas selected for observation of the EGL in sagittal planes were neither

consistent nor strictly localized. Our results suggest the necessity of carefully selecting the observation sites and provide a basic criterion for selecting sites. It also implies that, when the influence of chemicals or physical factors produce an alteration of the EGL thickness, the comparison should be made among carefully selected sites. Strictly corresponding sites would be an essential experimental condition for this kind of study.

Recently, molecular studies on cerebellar development have been refined, including those on lineage and area specificity. For example, mutant mice *rcm* and mice deficient in *Zic1*, whose phenotypes are characterized as rostral malformations in the midline cerebella, maintain a common boundary between malformation areas and intact lobe as the fissura prima[5,6].

The *meander tail* mutant mouse also exhibits the rostral malformation, but it involves a portion of the posterior lobe beyond the fissura prima[14,15]. Normal anterior expression of *Otx-2*, *Wint-7*, *En-1* is also observed beyond the fissura prima some time in development[12]. The expression in the cerebellar granular layer of a tyrosine phosphatase (RPTPρ) transcript and acidic fibroblast growth factor (FGF-1) mRNA appears beyond the fissura prima. However, the rostrocaudal boundary in lobule VI is close to, but not coincident with, the extent of the disorganized mea cerebella and the rostral extent of *Otx-2* expression[10].

The expression of genes and other factors in the anterior lobe beyond the fissura prima might be the cause of the demonstrated boundary between the early developing group and the late developing one.

The dorsal posterior lobe exhibiting the developmental delay in the present study might be coincident with the reduced area of *En-2* mutant[13], and with areas in which folial abnormality was found in *Otx-1* mutant[1,2].

The mixed-type group, located in the dorsal region of lobule VIII and the ventral area of lobule IX, demonstrated several features shown by the early developing group which consisted mainly of the anterior lobe. This observation might be related to a partial distribution of spinocerebellar projections to the anterior region and to lobules VIII and IX of the posterior region. An especially interesting observation is that the *En-2* mutant exhibited an abnormal folial association of lobule VIII with lobule IX[12,13,18].

At present, the observed intra- and interlobule differences in thickness of the midline EGL cannot yet be attributed to variations in gene expression. Detailed morphological description is still essential in order to elucidate the basic mechanisms underlying the cerebellar morphogenesis. Although our observation on the thickness of the EGL is limited to the mid-sagittal plane of the cerebellum, it covers the EGL of whole midline sections extending throughout the entire postnatal period when the EGL could be observed, and may serve as a basis for studies on regional differentiation of the cerebellum.

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REFERENCES

1. **Acampora, D., Mazan, S., Avantaggiato, V., Barone, P., Tuorto, F., Lallemand, Y., Brulet, P. and Simeone, A.** 1996. Epilepsy and brain abnormalities in mice lacking the *Otx1* gene. Nat. Genet. **14:** 218–222.
2. **Acampora, D., Avantaggiato, V., Tuorto, F., Barone, P., Perera, M., Choo, D., Wu, D., Corte, G. and Simeone, A.** 1999. Differential transcriptional control as the major molecular event in generating *Otx1-/-* and *Otx2-/-* divergent phenotypes. Development. **126:** 1417–1426.
3. **Altman, J.** 1969. Autoradiographic and histological studies of postnatal neurogenesis. J. Comp. Neurol. **136:** 269–294.
4. **Altman, J.** 1972. Postnatal development of the cerebellar cortex in the rat. J. Comp. Neurol. **145:** 353–398.
5. **Aruga, J., Minowa, O., Yaginuma, H., Kuno, J., Nagai, T., Noda T. and Mikoshiba, K.** 1998. Mouse *Zic1* is involved in cerebellar development. J. Neurosci. **18:** 284–293.
6. **Eisenman, L.M. and Brothers, R.** 1998. Rostral cerebellar malformation (*rcm/rcm*): A murine mutant to study regionalization of the cerebellum. J. Comp. Neurol. **394:** 106–117.
7. **Fujita, S., Shimada, M. and Nakamura, T.** 1966. H3-thymidine autoradiographic studies on the cell proliferation and differentiation in the external and the internal granular layers of the mouse cerebellum. J. Comp. Neurol. **128:** 191–208.
8. **Fujita, S.** 1967. Quantitative analysis of cell proliferation and differentiation in the cortex of the postnatal mouse cerebellum. J. Cell. Biol. **32:** 277–287.
9. **Mares, V. and Lodin, Z.** 1970. The cellular kinetics of the developing mouse cerebellum. II. The function of the external granular layer in the process of gyrification. Brain Res. **23:** 343–352.
10. **McAndrew, P.E., Frostholm, A., Evans, J.E., Zdilar, D., Goldowitz, D., Chiu, I.-M., Burghes, A.H.M. and Rotter, A.** 1998. Novel receptor protein tyrosine phosphatase and acidic fibroblast growth factor transcripts deliniate a rostrocaudal boundary in the granule cell layer of the murine cerebellar cortex. J. Comp. Neurol. **391:** 444–455.

11. **Miale, I.L. and Sidman, R.L.** 1961. An autoradiographic analysis of histogenesis in the mouse cerebellum. Exp. Neurol. **4:** 277–296.
12. **Millen, K.J., Hui, C. and Joyner, A.L.** 1995. A role for *En-2* and other murine homologues of *Drosophila* segment polarity genes in regulating positional information in the developing cerebellum. Development **121:** 3935–3945.
13. **Millen, K.J., Wurst, W., Herrup, K. and Joyner, A.L.** 1994. Abnormal embryonic cerebellar development and patterning of postnatal foliation in two mouse *Engrailed-2* mutants. Development **120:** 695–706.
14. **Napieralski, J.A. and Eisenman, L.M.** 1993. Developmental analysis of the external granular layer in the *meander tail* mutant mouse: Do cerebellar microneurons have independent progenitors? Dev. Dynamics **197:** 244–254.
15. **Napieralski, J.A. and Eisenman, L.M.** 1996. Further evidence for a unique developmental compartment in the cerebellum of the meander tail mutant mouse as revealed by the quantitative analysis of Purkinje cells. J. Comp. Neurol. **364:** 718–728.
16. SPSS Base 10.0J User's Guide, SPSS inc., 1999, pp. 235–240.
17. SPSS Base 10.0J User's Guide, SPSS inc., 1999, pp. 299–306.
18. **Vogel, M.W., Ji, Z., Millen, K. and Joyner, A.L.** 1996. The *Engrailed-2* homeobox gene and patterning of spinocerebellar mossy fiber afferents. Dev. Brain Res. **96:** 210–218.