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CEREBELLAR HYPOPLASIA IN THE HYPERBILIRUBINEMIC GUNN RAT: MORPHOLOGICAL ASPECTS

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ABSTRACT

Gunn rats, a mutant strain of rats, suffer from autosomal recessive hyperbilirubinemia. The homozygotes (j/j) develop jaundice soon after birth and often exhibit kernicterus and cerebellar hypoplasia that are due to bilirubin. Therefore, j/j Gunn rats have been used as an animal model of bilirubin encephalopathy, as well as of neonatal hyperbilirubinemia. In this review, we discuss morphological aspects of the cerebellar hypoplasia that is due to bilirubin and describe the relationship between plasma bilirubin levels and cerebellar hypoplasia, as well as the pathogenesis of cerebellar hypoplasia, including abnormal histogenesis of the cerebellar cortex, abnormalities associated with Purkinje cells and abnormal synaptogenesis in j/j Gunn rats.

Key Words: Gunn rats, Bilirubin, Cerebellar hypoplasia, Purkinje cells, Synaptogenesis

INTRODUCTION

In 1938, C.K. Gunn described a mutant strain of Wistar rats that suffered from hereditary hyperbilirubinemia[1]. The mutant rats have been called Gunn rats after their discoverer. The hyperbilirubinemia (jaundice) trait is transmitted by a single autosomal recessive gene[1–3]. Johnson et al.[2] proposed that the jaundice gene is represented by a small letter j and the normal gene by a capital letter J. The homozygotes (j/j) lack hepatic UDP-glucuronosyltransferase (UDPGT) activity towards bilirubin[4,5] and develop jaundice several hours after birth. The jaundice is due to high levels of unconjugated bilirubin in the blood and persists throughout the life of the animal. Recently, the genetic defect of bilirubin-UDPGT in j/j Gunn rats was proved to be a single-base frameshift mutation[6–8].

Bilirubin is a neurotoxic compound and 70% to 75% of j/j Gunn rats develop overt signs of central nervous system disease during the first 18 to 21 days after birth[9], and kernicterus and cerebellar hypoplasia (Fig. 1) are often observed in j/j animals. The cerebellar hypoplasia in j/j Gunn rats seems to be due to hyperbilirubinemia. This putative association is supported by the fact that administration of novobiocin during a limited period to neonatal and infant Wistar rats induces transient hyperbilirubinemia with resultant cerebellar hypoplasia[10,11], and by the fact that photoirradiation of neonatal and infant j/j Gunn rats, which decreases serum and plasma levels of bilirubin, protects cerebella from hypoplasia[12–15]. Heterozygous (J/j) Gunn rats show neither hyperbilirubinemia nor any abnormalities in various organ systems, including the central

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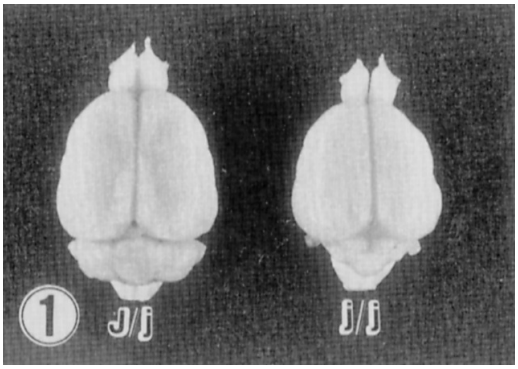

Fig. 1. Brains from heterozygous (J/j) and homozygous (j/j) 30-day-old Gunn rats. The j/j rat cerebellum is highly hypoplastic.

nervous system, even though bilirubin-UDPGT activity in J/j Gunn rats is about half of that in normal Wistar and Sprague-Dawley rats[16].

PLASMA BILIRUBIN LEVELS AND CEREBELLAR HYPOPLASIA

The severity of cerebellar hypoplasia in j/j Gunn rats varies greatly among animals with advancing age[17,18]. In our earlier study, in which cerebella from j/j and J/j rats were weighed on postnatal day 30, about 40% of the j/j cerebella examined were similar in weight to the J/j cerebella (217.5 ± 7.0 mg: mean ± S.D.), but about 18% were severely hypoplastic, weighing less than 110 mg, while the remaining 42% were moderately hypoplastic, ranging in weight from 111 to 190 mg. Total plasma bilirubin levels in j/j Gunn rats also varied with the individual at all stages examined (days 3, 7, 12, 15 and 18). In j/j Gunn rats, the cerebellar weight on day 30 was significantly and negatively correlated with the total plasma bilirubin levels on days 3 and 7 (correlation coefficients were −0.66 and −0.82, respectively), but these parameters were not significantly correlated on days 12, 15 and 18[19]. The results of this study show that the severity of cerebellar hypoplasia in a j/j Gunn rat can be predicted from the total plasma bilirubin level on day 3 or day 7.

PATHOGENESIS OF CEREBELLAR HYPOPLASIA

1. Changes in cerebellar weight and growth of cerebellar lobules

Weights of cerebella from j/j Gunn rats are not significantly different from those from J/j Gunn rats until postnatal day 10. In j/j Gunn rats with plasma total bilirubin levels of 9−10 mg/dl on day 3 or day 7, the cerebellar weight shows little increase after day 10, being significantly lower than that of J/j Gunn rats on day 12 and thereafter (Fig. 2)[20]. In hypoplastic j/j cerebella, hemispheres are extremely thin and the vermis is attenuated (Fig. 1). In the vermis, anterior and dorsal lobules, such as the centralis, culmen, declive and tuber, are markedly reduced in size, while posterior lobules, such as the nodulus and ventral parts of the uvula, are less severely affected[20,21]. The growth of anterior and dorsal lobules is affected from day 12 onwards[20].

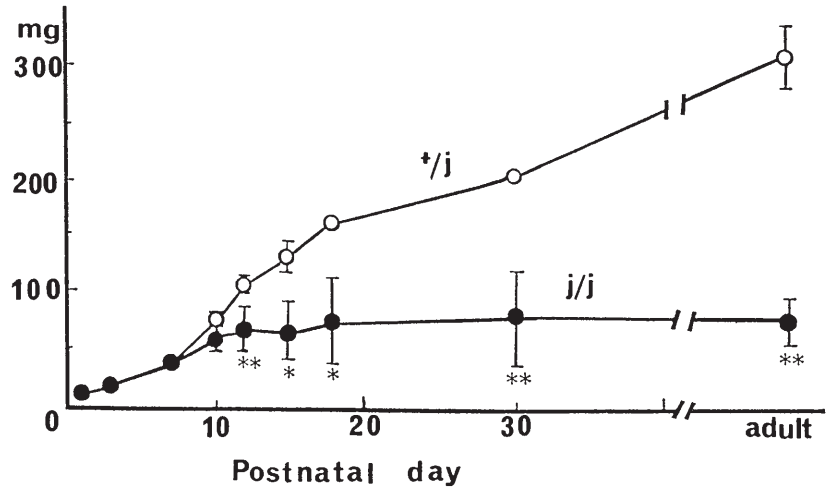

Fig. 2. Postnatal changes in cerebellar weights for J/j and j/j Gunn rats. Each point represents mean ± S.D. *$p<0.05$, **$p<0.01$. "+/j" in the Figure is equivalent to "J/j". (From Takagishi, 1989[20]. Reprinted with permission.)

2. Histogenesis of the cerebellar cortex

The following description is based upon observations of the cortex of the culmen in cerebella of J/j and j/j rats[20]. In J/j cerebella, the external granular layer is 6 to 8 cells deep on postnatal days 1–3. The upper part of the layer, namely, the proliferative zone[22], consists of roundish cells and contains mitotic cells. The lower part of the layer, namely, the premigratory zone[22], contains smaller round cells (when sectioned in the sagittal plane). The thickness of the layer increases up to day 10 until the layer 10 to 11 cells deep and it decreases thereafter. The external granular layer disappears by day 23 in J/j cerebella. The molecular layer has not yet formed by days 1–3 in many J/j cerebella. However, this layer becomes distinct on day 7 and steadily increases in thickness up to the adult stage. Cells in the molecular layer are frequently found after day 10 and are abundant at all levels of this layer on day 18 in J/j cerebella. Cells in the internal granular layer are scarce on days 1–3 but they become concentrated on day 7 and, thereafter, they are densely packed and form a cluster in J/j cerebella.

In j/j cerebella, the external granular layer is of the same thickness as that in J/j cerebella from days 1 to 10, but it is thinner than that in J/j cerebella on days 12 and 15. By day 18, the external granular layer disappears locally, although rows of 2 to 3 cells can be seen in places in this layer on day 18. On day 23, many individual cells from the external granular layer remain at the uppermost level of the molecular layer. The molecular layer is thinner in j/j cerebella than in J/j cerebella on day 10 and thereafter. In j/j cerebella, this layer increases slightly in thickness after day 10. Consequently, the thickness of the molecular layer in j/j cerebella is 25% to 20% of the thickness of that in J/j cerebella at the adult stage. Cells in this layer are scarce in j/j cerebella at all ages (Figs. 3–5). The cells seem to be produced in a reduced number in j/j cerebella. In the internal granular layer, the density of cells in j/j cerebella is the same as that in J/j cerebella until day 10, but it is lower than that in J/j cerebella on day 12. Furthermore, this layer is thinner in j/j cerebella than in J/j cerebella on day 12. The paucity of cells in this layer becomes conspicuous and this layer is very thin from day 15 onwards (Fig. 5).

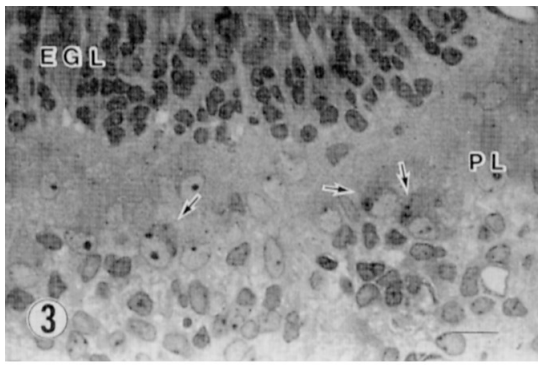

Fig. 3. A light micrograph of part of the culmen from a j/j rat cerebellum, 3 days after birth. Under the thick external granular layer (EGL), the Purkinje cell layer (PL) is present, and it is 2–3 cells deep. Several Purkinje cells contain osmiophilic granules (arrows). The molecular layer is not clearly defined. Bar=10 μm.

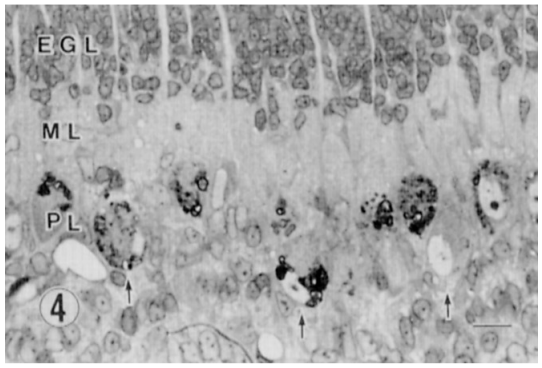

Fig. 4. Part of the culmen from a 7-day-old j/j rat. Purkinje cells contain a large number of osmiophilic granules and small vacuoles. The Purkinje cell layer (PL) is disorganized and some Purkinje cells are not located in the layer (arrows). EGL, external granular layer; ML, molecular layer. Bar=10 μm.

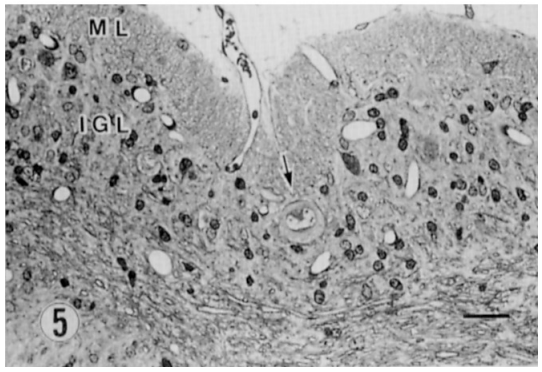

Fig. 5. Part of the culmen from a 6-month-old j/j rat. The molecular layer (ML) and the internal granular layer (IGL) have atrophied and contain small numbers of cells. Only one Purkinje cell with clear cytoplasm is present (arrow). The Purkinje cell does not contain any osmiophilic granules. Bar=20 μm.

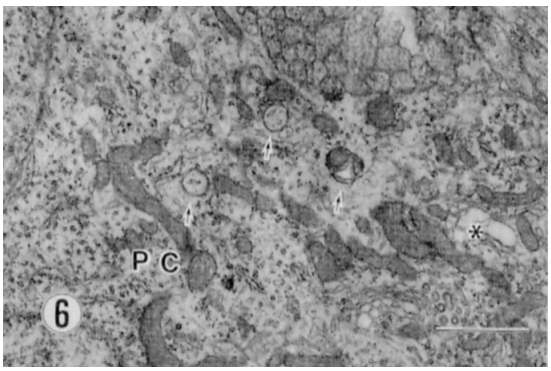

Fig. 6. A Purkinje cell (PC) from a 3-day-old j/j rat. Membranous whorls (arrows) are present and Golgi lamellae are dilated (*). Bar=1 μm.

3. Abnormalities associated with Purkinje cells

In j/j Gunn rats, Purkinje cells have been reported to be exclusively affected in the development of cerebellar hypoplasia[9,20,21,23], even though, on postnatal days 12 and 15, more degenerating cells in the internal granualr layer are found in j/j cerebella than in J/j cerebella (Takagishi and Yamamura, in preparation). The severity of Purkinje cell abnormalities varies among the cerebellar lobules; in anterior and dorsal lobules, such as the centralis, culmen, declive and tuber, the Purkinje cells are much more severely affected than those in posterior lobules, such as the nodulus and ventral part of the uvula[21]. Furthermore, the time at which lesions are first identified in Purkinje cells depends on the details of mating, in particular, on the genotype of the mother. In animals from j/j × j/j matings, osmiophilic granules are first found in the cytoplasm of Purkinje cells at 30 hours, and in the cytoplasm of Purkinje cells of offspring from J/j(♀) × j/j(♂) matings they are found 72 hours after birth[9]. Here we shall briefly describe the development of Purkinje cell abnormalities in the culmen of the cerebellum from j/j and J/j Gunn rats that are the product of J/j(♀) × j/j(♂) matings[20,23].

On days 1–3, several rows of Purkinje cells are seen in the Purkinje cell layer in j/j (Fig. 3) and in J/j cerebella. Osmiophilic granules are present in the cytoplasm of a few Purkinje cells in j/j cerebella on day 3 (Fig. 3). These granules are observed as loosely arranged whorls of membranes under the electron microscope (Fig. 6)[9,20] and they are the first distinct morphological signs of Purkinje cell abnormalities in hyperbilirubinemic rats[9,11]. In addition to membranous whorls, some minor cytoplasmic alterations, such as vacuolated Golgi vesicles or cisternae (Fig. 6), membranes stacked in parallel arrays and dilated endoplasmic reticulum, are occasionaly observed in j/j cerebella on day 3[20].

On day 7, Purkinje cells are arranged in a monolayer in J/j cerebella. In j/j cerebella, 85% of the somata of Purkinje cells appear abnormal, containing osmiophilic granules and small vacuoles (Fig. 4)[23]. Such abnormal cells occasionally lie along and/or are buried in the internal granular layer, and consequently the layer of Purkinje cells is disorganized (Fig. 4). Under the electron microscope, the whorled membranes, varying in size, can be seen to become more compact. Mitochondria are often enlarged and contain a vacuole that includes dense granules, which, from results of an α-amylase digestion test, appear to contain glycogen (Fig. 7)[24].

On day 10, Purkinje cells are at a very advanced stage of degeneration in j/j cerebella. Most cells are filled with osmiophilic granules. Some cells are vacuolated. These severely damaged

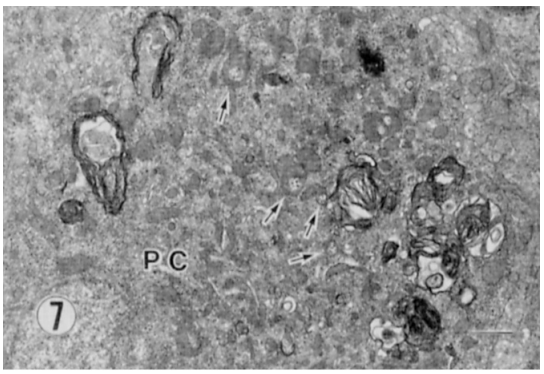

Fig. 7. A Purkinje cell (PC) from a 7-day-old j/j rat. Many membranous whorls of various sizes are present in the cytoplasm. Mitochondria are enlarged and contain electron-dense granules (arrows). Bar=1 μm.

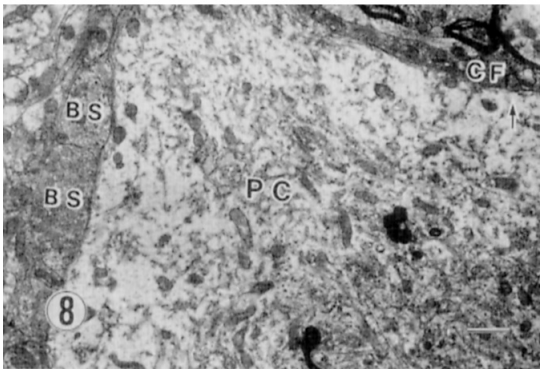

Fig. 8. A Purkinje cell (PC) from a 6-month-old j/j rat. The cell organelles are scarce at the periphery of the soma. The synapse of a climbing fiber (CF) with a perisomatic process (arrow) can still be found. Basket cell synapses (BS) are frequently seen on the soma. Bar=1 μm.

cells are surrounded by thin processes of astrocytes. About 50% of Purkinje cells in the culmen of j/j cerebella disappear from days 7 to 12[23]. On day 12, many of the remaining Purkinje cells in j/j cerebella are at an advanced stage of degeneration, as seen also on day 10.

On days 18–23, a great number of Purkinje cells are absent in j/j cerebella. Most of the remaining Purkinje cells are severely damaged.

On day 30, severely damaged Purkinje cells are no longer seen and the remaining Purkinje cells appear normal or only mildly affected, containing small numbers of osmiophilic granules at the periphery of the soma.

At the adult stage, only a few Purkinje cells are present in j/j cerebella (Fig. 5). They contain no osmiophilic granules, but their cytoplasm appears clear, especially at the periphery of the soma. Under the electron microscope, the organelles are seen to be scarce at the periphery of the soma and free ribosomes especially are reduced in number. Small, spine-like processes are still occasionally found on the soma (Fig. 8).

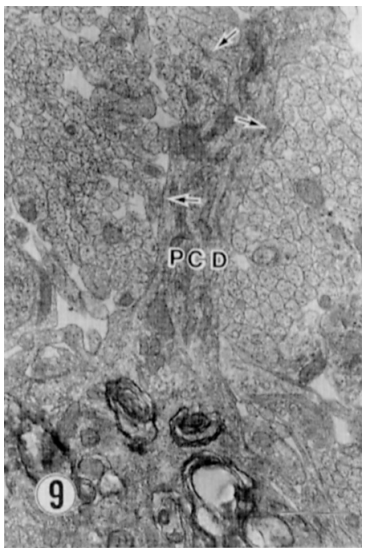

Fig. 9. A Purkinje cell dendrite (PCD) from a 7-day-old j/j rat. Parallel fibers make synaptic contacts with the smooth surface of the dendrite or the dendritic processes (arrows). Bar=1 μm.

4. Synaptogenesis in the development of cerebellar hypoplasia

The following description is based upon observations of the cortex of the culmen in J/j and j/j rat cerebella[23].

Formation of synapses between parallel fibers and Purkinje cells

On postnatal day 7, parallel fibers are seen to be in synaptic contact with proximal dendritic shafts or their processes in j/j cerebella (Fig. 9). The complete and mature synapse between parallel fibers and Purkinje cells is a synapse between parallel fiber boutons and spines which arise from dendritic branches of Purkinje cells. However, in j/j cerebella, synapses between parallel fibers and Purkinje dendritic shafts are present from day 7 through the adult stage, whereas these synaptic junctions are found during only the first four weeks in J/j cerebella. On day 12, parallel fiber-spine synapses are apparent but they are less frequent in j/j cerebella than in J/j cerebella. Spines arise not only from the dendritic branches of Purkinje cells but also from the trunks.

On days 18—30, the concentration of parallel fiber-spine synapses is very low in j/j cerebella. These synapses are found on the spines that emerge from rather thick dendrites as well as on branchlet spines (Fig. 10). In j/j cerebella, parallel fiber boutons are often enlarged. The enlargement becomes very conspicuous with age. In j/j cerebella, parallel-fiber synaptic boutons often face astrocytic processes in the absence of their postsynaptic targets (Fig. 11). These synaptic boutons are variable in size and have the same features as those that make contact with spines. Dense materials are usually present in the space between the presynaptic membrane of the parallel fiber and the cell membrane of the astrocytic processes. These presynaptic elements are found from day 18 to the adult stage.

In cerebella from adult j/j rats, parallel fiber-spine synapses are frequently seen only in the molecular layer above the remaining Purkinje cells. Synaptic junctions between parallel fibers

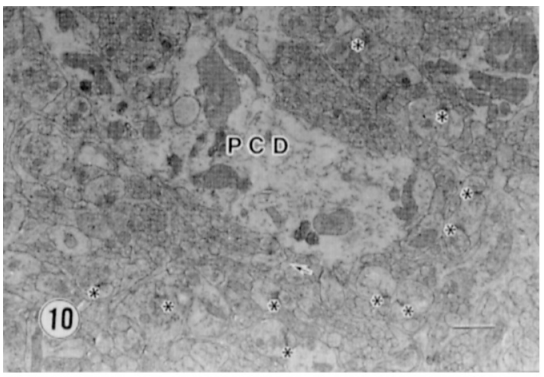

Fig. 10. The molecular layer from an 18-day-old j/j rat. Synapses between parallel fibers and dendritic spines of Purkinje cells (*) are present. A spine (arrow) arising from a rather thick dendrite (PCD) also makes synaptic contact with the parallel fiber. Bar=1 μm.

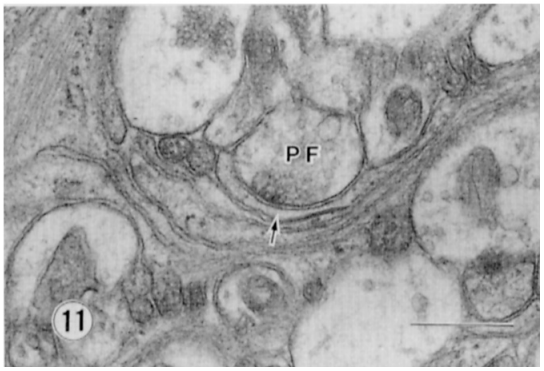

Fig. 11. The molecular layer from a 30-day-old j/j rat. A parallel-fiber synaptic bouton (PF) without a postsynaptic target faces an astrocytic process (arrow). Bar=0.5 μm.

and Purkinje dendritic shafts are still present. Parallel-fiber synaptic boutons without postsynaptic targets are frequently seen. Their features are the same as those observed on days 18–30.

The synaptic junctions between parallel fibers and dendritic shafts of Purkinje cells were previously considered to be transient[25,26] and to evolve into mature spine synapses[27]. However, results of an investigation using a freeze-fracture technique suggest that these shaft junctions are not simply a transient intermediate in the assembly of spine synaptic junctions, but are actually representatives of a class of synaptic junctions that have the capacity to dissociate in the remodeling process that accompanies normal development[28]. It is difficult to explain why the shaft junctions persist in the j/j cerebellum. They might have lost the capacity to dissociate as a result of the Purkinje cell abnormalities. Alternatively, the shaft junctions might compensate for a deficiency of mature spine synapses[23].

With respect to the development and fate of the presynaptic terminals in the absence of their postsynaptic targets, two hypotheses have been proposed[29,30]. One is that parallel fibers may be able autonomously to produce transient 'boutons en passant' with normal presynaptic organelles

in the absence of their postsynaptic targets. The other hypothesis is that presynaptic fibers in the cerebellum, once they have been stabilized on one occasion by the establishment of functional synapses, may be able to survive for long periods even if they lose their postsynaptic targets, while those that have never made synaptic contacts may be unable to survive independently, disappearing rapidly through the process of degeneration.

In j/j cerebella, there was no marked difference in the concentration of parallel-fiber synaptic boutons that were devoid of their postsynaptic targets between rats on postnatal day 30 and those of 5–10 months of age[23]. This observation suggests that, in j/j cerebella, the parallel-fiber synaptic boutons, which had been stabilized via synaptic contacts with the Purkinje spines at the earlier stages, lost their postsynaptic targets as a result of the progressive degeneration of Purkinje cells by day 30, and these boutons were able to survive to the adult stage without their postsynaptic targets, as in the nervous mutant mouse[30]. This speculation is supported by the observation that parallel fibers in contact with atrophied spines of Purkinje cells were found from days 18 to 23[23].

Formation of synapses between climbing fibers and Purkinje cells

During the normal development of the rat cerebellum, synapses between climbing fibers and Purkinje cells are first formed on the soma and/or perisomatic processes of Purkinje cells from postnatal day 5 and then they are translocated to the spines that arise from dendritic trunks from day 10 onwards[26].

In J/j cerebella, synaptic junctions between climbing fibers and perisomatic processes of Purkinje cells are very often observed on day 7. They diminish in number thereafter and are never found after day 18. Synapses between climbing fibers and spines that arise from dendritic trunks of Purkinje cells begin to be formed and increase in number from day 12.

In j/j cerebella, synaptic junctions between climbing fibers and the soma or perisomatic processes of Purkinje cells are seen on day 7 and they increase in number until day 12. These synapses are still frequently encountered on days 18–30 and , although few in number, they are even found at the adult stage (Fig. 8). This profile indicates a delay in or an arrest of the translocation of the synaptic sites of climbing fibers from the perisomatic processes to the spines of the dendritic trunks in j/j cerebella. Synapses between climbing fibers and spines that arise from Purkinje dendritic trunks are rarely seen on days 18–30 but are frequently observed at the adult stage (Fig. 12).

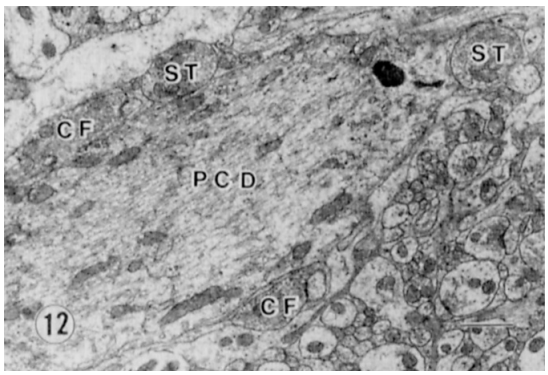

Fig. 12. A dendrite of a Purkinje cell (PCD) from a 16-month-old j/j rat. Climbing fibers (CF) and stellate cells (ST) are in synaptic contact with dendritic spines and the dendrite, respectively. Bar=1 μm.

With respect to the persistence of synaptic junctions between climbing fibers and the soma or perisomatic processes of Purkinje cells, it has been suggested that the inadequate development of Purkinje cell dendrites deprives climbing fibers of a dendritic surface that would otherwise be available for formation of synapses and prevents the translocation of the fibers to the dendrites[31,32].

Formation of synapses between basket or stellate cells and Purkinje cells

In j/j cerebella, synapses between basket cell axon terminals and the somata of Purkinje cells are found from postnatal day 12, but they are not encountered frequently until day 30. In the normally developing rat cerebellum, most of the temporary synapses between climbing fibers and the somata or perisomatic processes of the Purkinje cells disappear before basket cell axon terminals begin to establish synapses on the somata of Purkinje cells[26]. However, in j/j cerebella, because of the delay in or arrest of the translocation of the synaptic sites of climbing fibers from the perisomatic processes to the spines of the dendritic trunks, most of the surface of the soma of each Purkinje cell, the main site for synapses with basket cell axons, is occupied by the perisomatic processes that form synapses with the climbing fibers. At the adult stage, more basket cell axons terminate on the somata of Purkinje cells in j/j cerebella (Fig. 8).

Synapses between stellate cell axon terminals and Purkinje cell dendrites are also rarely found from days 12 to 30 in j/j cerebella. Their absence may be due to the underdevelopment of Purkinje cell dendrites and, in addition, to the paucity of stellate cells. However, the synapses between stellate cells and Purkinje cells are occasionally concentrated on the dendritic trunks of Purkinje cells in the cerebella of adult j/j rats (Fig. 12).

REFERENCES

1) Gunn, C.K.: Hereditary acholuric jaundice in a new mutant strain of rats. *J. Hered.*, 29, 137–139 (1938).
2) Johnson, L., Sarmiento, F., Blanc, W.A. and Day, R.: Kernicterus in rats with an inherited deficiency of glucuronyl transferase. *Am. J. Dis. Child.*, 97, 591–608 (1959).
3) Tanase, H., Sudo, S. and Suzuki, Y.: Maintenance and propagation of jaundiced rat. *Exp. Anim.*, 17, 23–25 (1968).
4) Schmid, R., Axelrod, J., Hammaker, L. and Swarm, R.L.: Congenital jaundice in rats, due to a defect in glucuronide formation. *J. Clin. Invest.*, 37, 1123–1130 (1958).
5) Weatherill, P.J., Kennedy, S.M.E. and Burchell, B.: Immunochemical comparison of UDP-glucuronyltransferase from Gunn- and Wistar-rat livers. *Biochem. J.*, 191, 155–163 (1980).
6) Iyanagi, T., Watanabe, T. and Uchiyama, Y.: The 3-methylcholanthrene-inducible UDP-glucuronosyltransferase deficiency in the hyperbilirubinemic rat (Gunn rat) is caused by a −1 frameshift mutation. *J. Biol. Chem.*, 264, 21302–21307 (1989).
7) Sato, H., Aono, S., Kashiwamata, S. and Koiwai, O.: Genetic defect of bilirubin UDP-glucuronosyltransferase in the hyperbilirubinemic Gunn rat. *Biochem. Biophys. Res. Commun.*, 177, 1161–1164 (1991).
8) Roy-Chowdhury, J., Huang, T., Kesari, K., Lederstein, M., Arias, I.M. and Roy-Chowdhury, N.: Molecular basis for the lack of bilirubin-specific and 3-methylcholanthrene-inducible UDP-glucuronosyltransferase activities in Gunn rats: the two isoforms are encoded by distinct mRNA species that share an identical single base deletion. *J. Biol. Chem.*, 266, 18294–18298 (1991).
9) Schutta, H.S. and Johnson, L.: Bilirubin encephalopathy in the Gunn rat: a fine structure study of the cerebellar cortex. *J. Neuropathol. Exp. Neurol.*, 26, 377–396 (1967).
10) Yamamura, H., Yoshioka, F., Oyama, K. and Sawano, J.: Cerebellar hypoplasia induced by experimental hyperbilirubinemia in rats. In *Proceedings of the 10th International Congress of Anatomists*, Tokyo, edited by Yamada, E., p.171 (1975), Science Council of Japan, Tokyo.
11) Takagishi, Y. and Yamamura, H.: The critical period of Purkinje cell degeneration and cerebellar hypoplasia due to bilirubin. *Acta Neuropathol.*, 75, 41–45 (1987).

12) Sisson, T.R.C., Goldberg, S. and Slaven, B.: The effect of visible light on the Gunn rat: convulsive threshold, bilirubin concentration, and brain color. *Pediatr. Res.*, 8, 647–651 (1974).
13) Sato, H., Katoh-Semba, R. and Kashiwamata, S.: Effect of irradiation on the erythrocyte membranes of homozygous Gunn rat sucklings. *Biol. Neonate*, 32, 158–165 (1977).
14) Keino, H., Sato, H., Semba, R., Aono, S., Aoki, E. and Kashiwamata, S.: Mode of prevention by phototherapy of cerebellar hypoplasia in a new Sprague-Dawley strain of jaundiced Gunn rats. *Pediatr. Neurosci.*, 12, 145–150 (1985–86).
15) Keino, H. and Kashiwamata, S.: Critical period of bilirubin-induced cerebellar hypoplasia in a new Sprague-Dawley strain of jaundiced Gunn rats. *Neurosci. Res.*, 6, 209–215 (1989).
16) Strebel, L. and Odell, G.B.: Bilirubin uridine diphospho-glucuronyltransferase in rat liver microsomes: genetic variation and maturation. *Pediatr. Res.*, 5, 548–559 (1971).
17) Katoh-Semba, R., Aoki, E. and Kashiwamata, S.: Cerebellar hypoplasia in Gunn rats: effects of bilirubin on postnatal changes in cerebellar constituents. *Biomed. Res.*, 3, 319–329 (1982).
18) O'Callaghan, J.P. and Miller, D.B.: Cerebellar hypoplasia in the Gunn rat is associated with quantitative changes in neurotypic and gliotypic proteins. *J. Pharmacol. Exp. Ther.*, 234, 522–533 (1985).
19) Takagishi, Y. and Yamamura, H.: Severity of cerebellar hypoplasia is predictable from total plasma bilirubin level at 3 to 7 days of age in jaundiced Gunn rats. *Lab. Anim. Sci.*, 39, 158–160 (1989).
20) Takagishi, Y.: Development of cerebellar hypoplasia in jaundiced Gunn rats: a morphological study. *Cong. Anom.*, 29, 275–294 (1989).
21) Sawasaki, Y., Yamada, N. and Nakajima, H.: Developmental features of cerebellar hypoplasia and brain bilirubin levels in a mutant (Gunn) rat with hereditary hyperbilirubinaemia. *J. Neurochem.*, 27, 577–583 (1976).
22) Altman, J.: Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular layer. *J. Comp. Neurol.*, 145, 353–398 (1972).
23) Takagishi, Y. and Yamamura, H.: Purkinje cell abnormalities and synaptogenesis in genetically jaundiced rats (Gunn rats). *Brain Res.*, 492, 116–128 (1989).
24) Schutta, H.S., Johnson, L. and Neville, H.E.: Mitochondrial abnormalities in bilirubin encephalophathy. *J. Neuropathol. Exp. Neurol.*, 29, 296–305 (1970).
25) Mugnaini, E.: Ultrastructural studies on the cerebellar histogenesis. II. Maturation of nerve cell populations and establishment of synaptic connections in the cerebellar cortex of the chick. In *Neurobiology of Cerebellar Evolution and Development*, edited by Llinás, R., pp.749–782 (1969), American Medical Association, Chicago.
26) Altman, J.: Postnatal development of the cerebellar cortex in the rat. II. Phases in the maturation of Purkinje cells and of the molecular layer. *J. Comp. Neurol.*, 145, 399–464 (1972).
27) Foelix, R.F. and Oppenheim, R.: The development of synapses in the cerebellar cortex of the chick embryo. *J. Neurocytol.*, 3, 277–294 (1974).
28) Landis, D.M.D.: Initial junctions between developing parallel fibers and Purkinje cells are different from mature synaptic junctions. *J. Comp. Neurol.*, 260, 513–525 (1987).
29) Sotelo, C.: Electrical and chemical communication in the central nervous system. In *International Cell Biology*, edited by Brinkley, B.R. and Porter K.R., pp.83–92 (1977), The Rockefeller University Press, New York.
30) Sotelo, C. and Triller, A.: Fate of presynaptic afferents to Purkinje cells in the adult nervous mutant mouse: a model to study presynaptic stabilization. *Brain Res.*, 175, 11–36 (1979).
31) Landis, D.M.D. and Landis, S.C.: Several mutations in mice that affect the cerebellum. *Adv. Neurol.*, 21, 85–105 (1978).
32) Landis, D.M.D. and Sidman, R.L.: Electron microscopic analysis of postnatal histogenesis in the cerebellar cortex of staggerer mutant mice. *J. Comp. Neurol.*, 179, 831–864 (1978).