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リーラーマウス小脳における抑制性神経回路の改変と
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北海道大学
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Altered distribution of inhibitory synaptic terminals in
reeler cerebellum with special reference to malposition of
GABAergic neurons

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Summary

In immunohistochemical reactions against glutamic acid decarboxylase (GAD), γ -aminobutyric acid (GABA) and glycine (Gly), neurons in the mouse cerebellum showed the following reactivities: 1) The dendrites and cell bodies of the Purkinje cells were only GAD-positive, but their axonal terminals were GABA- and GAD-positive. 2) In both stellate and basket cells the cell bodies and terminals were GABA- and GAD-positive but Gly-negative. 3) The Golgi cells were GABA-, GAD- and Gly-positive. 4) The granule cells were negative with all antibodies. Based on the populations of each type of neuron, identified by the properties mentioned above, the reeler cerebellum was divided into four regions, namely, 1) the molecular and Purkinje cell layers covering the surface of the cerebellum, where the stellate and basket cells were present as in normal mouse; 2) the granule cell layer, where the heterotopic Purkinje and stellate-type cells (including both stellate and basket cells) were present together with the granule and Golgi cells; 3) the region beneath the granule cell layer where Purkinje cells were present as clusters of several neurons, and in addition, the superficial zone of the central cell mass, where the stellate-type and Golgi cells were present among the Purkinje cells; and 4) the deep zone of the central cell mass, where the Golgi cells were exclusively present among the Purkinje cells. The heterologous synapses originating from inhibitory interneurons were formed on the Purkinje cells closely related to the distribution of these neurons.

Introduction

The reeler mutant mouse is an autosomal recessive mutant, suffering from reeling ataxic gait, tremor action, and dystonic posture (Falconer,1951,1952). In the central nervous system there are widespread abnormalities characterized by malposition of neurons (Hamburgh,1960; Sidman,1968; Caviness and Rakic,1978). In the reeler cerebellum the lobular formation is extremely disturbed, and the layered architecture of the cortex, consisting of the molecular, Purkinje cell, and granule cell layers, is present but poorly developed beneath the pia mater, whereas the majority of malpositioned Purkinje cells remain in the central area together with other neuronal elements, referred to as the "central cell mass" (Rakic,1976; Caviness and Rakic,1978; Goffinet et al.,1984; Goffinet,1990; Inoue et al.,1990; Maeda et al.,1990). The Purkinje cells in the cortex and in the central cell mass reveal greater diversity in dendritic extensions and external features as shown by Golgi silver-impregnation and IP₃-receptor immunohistochemistry (Rakic,1976; Mariani et al.,1977; Heckroth et al.,1989; Inoue et al.,1990). Thus the neuronal connectivity with the Purkinje cells is possibly altered as was reported previously; for example, heterologous synaptic contacts with mossy fibers on the Purkinje dendrites, or multiple innervations by climbing fibers in the central cell mass (Rakic,1976; Mariani et al.,1977; Sotelo and Privat,1978; Mariani,1982; Goffinet et al.,1984; Terashima et al.,1985; Sotelo,1990,1991).

The interneurons in the reeler cerebellar cortex have been investigated by Golgi silver impregnation and electron microscopy (Rakic,1976; Mariani et al.,1977; Caviness and Rakic,1978; Wilson et al.,1981; Terashima et al.,1985), but the types of these neurons and the terminals on the Purkinje cells could not be clearly identified. Neurons other than the granule cells in the cerebellar cortex are exclusively inhibitory and GABAergic (Eccles et al.,1967; Ito,1984; Seguela et al.,1984; Ottersen and coworkers,1984,1987,1988). By immunohistochemical examination, the cell bodies, dendritic shafts, and terminals of the Purkinje cells were demonstrated to be glutamic acid decarboxylase (GAD)-positive, as were those of the stellate, basket and Golgi cells (McLaughlin et al.,1974; Oertel et al.,1981), and it is easy to identify each of these cell types based on their location within the cerebellar cortex. In the reeler cerebellum, on the other hand, it is impossible to determine the neuronal types of GAD-positive cells in accordance with their position because in the granule cell layer and central mass the neurons are intermingled with one another as described above. Thus cell markers other than GABA are necessary for the reeler study. In the present study I chose glycine (Gly) as a cell marker, which is reported to act as an inhibitory transmitter in the spinal cord (Aprison and Daly,1978; Fagg and Foster,1983) and retina (Ehinger and Dowling, 1987), and exists in the Golgi cells which are inhibitory neurons in the cerebellum (Ottersen et al.,1987,1988). The functions of Gly, i.e. it is in fact

inhibitory, are uncertain as yet, since it has been reported that Gly may be involved in the potentiation of the NMDA receptor channel in cultured neurons (Johnson and Ascher, 1987) or in *Xenopus* oocytes implanted with NMDA receptor channel m-RNA (Meguro et al., 1992; Kutsuwada et al., 1992; Ikeda et al., 1992). However, I could immunohistochemically distinguish the Golgi cells from stellate and basket cells using the novel monoclonal antibody against Gly as well as that against GABA, both of which had been established at our laboratory.

Thus in this way I elucidated the population of each type of inhibitory neuron in the reeler cerebellum and to demonstrate the distribution of the inhibitory terminals, which is possibly altered in the disturbed cytoarchitecture.

Materials and Methods

1) Monoclonal antibodies against the conjugated GABA or Gly

GABA and Gly were conjugated to bovine serum albumin (BSA) as described by Storm-Mathisen et al. (1983). In addition, ϵ -amino caproic acid (ACA), α -alanine (α -Ala), β -alanine (β -Ala), aspartic acid (Asp), GABA, glutamine (Gln), glutamic acid (Glu), Gly, histidine (His), hydroxyproline (Hpr), isoleucine (Ile), methionine (Met), proline (Pro), tyrosine (Tyr), and valine (Val) were conjugated to ovalbumin (Ova) in a similar manner. Balb/c mice were immunized by several intraperitoneal injections of 100 μ g of GABA or Gly conjugated with BSA and emulsified in equal amounts of complete Freund's adjuvant. Three days

after the last injection without adjuvant, spleen cells were prepared and fused with P3-U1 myeloma cells. Hybridoma cells producing antibodies which selectively bind to conjugated GABA or Gly were screened by using GABA-Ova- or Gly-Ova-coated immunoplates (Sumitomo, Japan). Cross-reactivity with other amino acids was checked in a similar manner by using immunoplates coated with various conjugated amino acids. Two novel hybridoma clones, HE-4 (anti-GABA) and RS 3-21 (anti-Gly), were chosen for use in this study. HE-4 reacted strongly with conjugates of both GABA and ACA, but showed no cross-reactivity with other conjugated amino acids (α -Ala, β -Ala, Asp, Gln, Glu, Gly, His, Ile, Met, Pro, Tyr, and Val) nor Ova (Fig.1a). RS 3-21 bound only to the Gly conjugate (Fig. 1e).

2) Animals

The reeler strain (B6C3Fe-a/a-rl) was obtained from Jackson Laboratories (Bar Harbor, Maine, USA), and maintained in our laboratory. Adult reeler mutant mice and their littermates (rl/+ or rl/-) were used in this study.

3) Immunohistochemistry

(1) Glutamic acid decarboxylase (GAD)

Mice were fixed by transcardial perfusion with a solution of 4% paraformaldehyde in 100 mM phosphate buffer adjusted to pH 7.4, after being anesthetized deeply with ether. Their brains were immersed overnight in the same fixative, and 50- μ m-thick sagittal sections were cut with a

microslicer (DTK-1000, Dosaka, Japan). Sections were incubated in antiserum against rat GAD diluted 1:4000 and stained by the ABC method (Vectastain kit, Vector Laboratories, Inc.). Their cross-reactivity with mouse GAD had been revealed in our previous study (Inoue et al., 1993).

(2)GABA and Gly

Mice were fixed by transcardial perfusion with a mixture of 2.5% paraformaldehyde and 1.5% glutaraldehyde in 100 mM phosphate buffer (pH 7.4). The brains were immersed for several days in the same solution, and sectioned sagittally at a thickness of 500 μ m with a microslicer. The sections were dehydrated in a graded series of ethanol, and embedded in Epon in the usual manner. Semi-thin 0.5 μ m sections were prepared with glass knives, and mounted on slide glasses. They were etched with sodium methoxide (Mayor et al., 1961), and immunostained with HE-4 culture supernatant diluted 1:500 for GABA or RS 3-21 culture supernatant diluted 1:100 for Gly. Immunohistochemical staining was performed by the ABC method (Histofine, Nichirei Corp., Japan).

(3)Absorption experiment with amino acid conjugates

Sections were stained with each diluted culture supernatant which was preincubated with one of the three kinds of conjugated amino acids, GABA-Ova, Glu-Ova, or Gly-Ova.

Results

1) The normal cerebellum

(1) GAD immunohistochemistry

In the cerebellar cortex, the stellate, basket, Purkinje, and Golgi cells were positively labeled (Figs.2a and 2b). In the molecular layer, terminals of the stellate cells on the Purkinje dendrites and the basket terminals enclosing the Purkinje cell bodies were also immunostained (Fig.2a). In the granule cell layer the ring-shaped terminals at the periphery of the protoplasmic island (glomeruli), corresponding to the varicose plexuses of the Golgi cell axons, were labeled (Fig.2b).

In the cerebellar nuclei, large immunonegative neurons were surrounded by GAD-positive terminals. Small neurons were also immunonegative (Fig.2c).

(2) GABA and Gly immunohistochemistry

The stellate and basket cells in the molecular layer were labeled with GABA antibody, but were negative with Gly antibody. In the Purkinje cell layer small cell bodies which immunoreacted with both GABA and Gly antibodies were present. Around the Purkinje cell bodies GABA-positive basket-like terminals, but no Gly-positive terminals, were detected (Fig.3a,3b). In the granule cell layer, Golgi cell bodies and ring-shaped structures, suggestive of parts of glomeruli, were stained with both antibodies (Figs.3a and 3b). Gly-positive fibrous processes were abundantly present in the molecular layer, probably representing dendrites of the Golgi cells (Fig.3b). In the cerebellar

nuclei, many GABA-positive and several Gly-positive terminals were observed around the large neurons which were negative with both antibodies. Small GABA-positive cells, most of which simultaneously showed Gly-immunoreactivity, were present among the large neurons (Figs.3c and 3d).

The immunohistochemical properties of the neurons and terminals in the normal cerebellum are summarized in Table I. The stellate and basket cells had very similar properties, so they are referred to as "stellate-type cells" in this reeler study. The neuronal types in the reeler cerebellum were identified on the basis of these criteria.

2) Immunohistochemistry of GABA, Gly, and GAD in the reeler cerebellum

In the molecular and Purkinje cell layer covering the entire pial area of the cerebellum, stellate-type cells, which were GABA-positive and Gly-negative, were present, and terminal boutons with the same immunoreactivity were found mainly on the dendritic shafts of the Purkinje cells or formed basket-like structures around the Purkinje cell bodies, as observed in the normal cerebellar cortex (Figs.4a-c). In the granule cell layer the Golgi cells and their ring-shaped terminals composing the cerebellar glomeruli could be identified since they were GABA- and Gly-positive. However, near the malpositioned Purkinje cells within the granule cell layer stellate-type cells were present, and the GABA- and GAD-positive terminals were aligned on the dendritic shafts of the Purkinje cells or

formed baskets around the soma as well (Figs.4a-c).

Within the small clusters of the Purkinje cells located between the granule cell layer and white matter, stellate-type cells and Golgi cells were intermingled (Figs.5a,5b,6a,6b). In these clusters many GAD-positive punctate terminals were aligned on the Purkinje dendrites extending towards the pia mater (Fig.6c), and basket-like terminals enclosed the Purkinje cell bodies (Figs.5a,6a,6c). In addition, Gly-positive terminals were sometimes detected around the Purkinje cell bodies (Figs.5b and 6b).

The central cell mass in the cerebellar hemisphere, which consisted mainly of the heterotopic Purkinje cells, occupied the area from the white matter to the cerebellar nuclei. In the superficial zone of the cell mass beneath the white matter many stellate-type cells were present (Figs.5c,5d,6d,6e). GAD-positive punctate boutons were aligned on the Purkinje dendrites, which extended across the white matter up to the granule cell layer (Figs.5e and 6f), and GABA- and GAD-positive baskets probably originating from the neighboring stellate-type cells closely enclosed the Purkinje cell bodies (Figs.5c,5e,6d,6f). In the deep zone near the cerebellar nuclei, on the other hand, only a few GABA-positive interneurons were present and most of them were Gly-positive Golgi cells as ascertained in the adjacent sections (Figs.5f,5g,6d,6e). The GABA- and GAD-positive punctate terminals surrounded the Purkinje cell bodies (Figs.5f,5g,6h,6j), whereas they were rarely aligned on their dendrites which extended in a disorderly manner in the

compact cluster of cell bodies (Figs.5h and 6j). In addition, Gly-positive terminals were observed around the Purkinje cell bodies (Figs.5g,6e,6i).

In the cerebellar vermis the central cell mass was present from the white matter to the ventricular wall. Except for the deep zone near the ventricular surface, the stellate-type cells and Golgi cells were scattered in the compact cluster of the heterotopic Purkinje cells (Figs. 4d and 4e). Numerous GAD-positive punctate terminals were present in the dendritic area of the Purkinje cells, and basket-like terminals enclosed their cell bodies (Figs.4d and 4f). In addition, Gly-positive terminals were sometimes present closely surrounding the Purkinje cell bodies (Fig.4e).

In the cerebellar nuclei, small GABA-positive neurons, most of which simultaneously displayed Gly-immunoreactivity, were present. Large neurons were negative for all antibodies, and were surrounded by numerous GABA-positive and a few Gly-positive boutons. This feature was identical to that observed in the normal mouse (Figs.5i-k,6h-j).

Discussion

1) Identification of neuronal types in the cerebellum by means of GAD-, GABA- and Gly-immunohistochemistry

Recently the distribution and features of Purkinje cells in the reeler cerebellum have been examined immunohistochemically with antibodies against available markers of the Purkinje cells, such as cGMP-dependent

protein kinase (Heckroth et al.,1989), P6 (Goffinet et al.,1984), and IP₃ receptor/P₄₀₀ protein (Inoue et al.,1990). However, the precise distribution of interneurons and their synaptic connectivity with the Purkinje cells have not been reported, since there have been no reliable methods of marking each type of interneuron and its synaptic boutons. In the present study, inhibitory neurons in the normal cerebellum were divided into two groups by means of immunostaining for GAD, GABA, and Gly, as follows. One group consisted of stellate and basket cells, referred to as stellate-type cells in this study, which were GABA (GAD)-positive and Gly-negative, and the others of Golgi cells which were GABA (GAD)-positive and Gly-positive. Neurons of the latter group included not only the Golgi cells in the granule cell layer but also some small neurons in the Purkinje layer. It could not be definitely determined whether these small cells were another type of Golgi cell or some other class of neuron (for example, Lugaro cells. (Ottersen et al.,1988)). In the cerebellar nuclei GABA- and GAD-positive terminal boutons surrounding the large neurons were considered to have originated mainly from the Purkinje cells, and in part from small inhibitory interneurons within their own cerebellar nuclei, since the immunopositive terminals of IP₃ receptor (Maeda et al.,1989,1990; Inoue et al.,1990), which is specific for all elements of the Purkinje cells, were fewer in number than the GABA- and GAD-positive terminals.

In the reeler cerebellum these properties of neurons

were considered to be retained on the basis of evidence from previous studies as follows: 1) marker molecules of the Purkinje cells existed even in the heterotopic Purkinje cells (Caddy et al.,1982; Goffinet et al.,1984; Wuenschell and Tobin,1988; Inoue et al.,1990; Maeda et al.,1990), 2) malpositioned Purkinje cells produced GABA, since expression of GAD mRNA was demonstrated (Wuenschell and Tobin,1988), 3) Golgi cells in the central cell mass as well as those in the granule cell layer expressed proenkephalin mRNA (Wuenschell and Tobin,1988), and 4) grafted neurons also contained a similar transmitter even in the host tissue (Cassel et al.,1993). Therefore, it was reasonable to apply the criteria of the normal cell types to those in the reeler cerebellum. In the reeler cerebellum many Gly-positive boutons were observed around the Purkinje cell bodies in the central mass. These Gly-positive boutons might correspond to the heterologous synapses originating from the Golgi cells based on the following reasons: 1) In the normal cerebellum Gly immunoreactivity was mainly demonstrated in the Golgi cells, together with GABA-immunoreactivity. 2) In the reeler cerebellum many GABA-positive boutons were detected around the same Purkinje cell bodies as shown in the adjacent sections. Thus, these Gly-positive terminals probably contained GABA and originated from the Golgi cells which are inhibitory interneurons.

2) Heterogeneity of the population of interneurons in the reeler cerebellum

It is generally accepted that the granule cells are generated from the external germinal layer and descend from the pial side, whereas the Purkinje and Golgi cells originate from the internal germinal layer located on the ventricular side (Altman,1982). The stellate and basket cells, on the other hand, have been generally considered to originate from the external germinal layer (Altman,1982); however, recently some researchers have claimed that some of the small neurons in the molecular layer originate from the internal germinal layer, as do Purkinje and Golgi cells (Hallonet et al.,1990; Otero et al.,1993). The pial region of the cerebellum was covered with a layered structure consisting of a thin molecular layer, incompletely aligned Purkinje cells and a discontinuous granule cell layer, resulting from the normal migration of neurons which, however, were fewer in number than normal. In the molecular layer, stellate and basket cells but no Golgi cells were found, and the terminals from these cells maintained the same connections with the dendrites and cell bodies of the Purkinje cells as in the normal molecular layer, although the GABA-positive terminals were more numerous than in the normal cerebellum.

Beneath the molecular and Purkinje cell layer, in contrast, the population of each type of neuron varied with the depth of the cerebellum, and this region was divided into three areas, as follows: 1) the granule cell layer, where heterotopic Purkinje cells and stellate-type cells were found together with the granule and Golgi cells; 2)

small clusters of Purkinje cells which existed between the granule cell layer and white matter, and in addition, the superficial zone of the central cell mass, where the Purkinje dendrites extended widely and among which stellate-type and Golgi cells were present and; 3) the deep zone of the central mass enclosing the cerebellar nuclei or facing the fourth ventricle, where the Purkinje cells and Golgi cells were exclusively present. Thus there was a reverse gradient in cell population between the stellate-type and Golgi cells, and this supports the hypothesis that most of the stellate-type and granule cells originated from the external germinal layer and the Purkinje and Golgi cells from the internal germinal layer. According to a developmental analysis using ^3H -thymidine (Altman, 1982), the different types of neurons in the normal cerebellum are produced in strict chronological order in four nonoverlapping periods; i.e., the Purkinje cells are generated between the 13th embryonic day (E13) and E16, the Golgi cells on E19, the basket cells from the 6th postnatal day (P6) to P7, and the stellate cells from P8 to P11, and the granule cells are produced during the entire period until P21. The time schedule is preserved in the reeler cerebellum (Goffinet, 1983, 1984, 1990). In the normal cerebellum the stellate and basket cells descend in the molecular layer among the dendritic trees of the Purkinje cells and radial processes of the Bergmann glial cells, which migrated much earlier than neurons of the external germinal layer and are already aligned in the Purkinje cell

layer; the stellate and basket cells never cross over this layer to invade the granule cell layer. In the reeler, on the other hand, the Purkinje and Golgi cells, which develop and migrate from the ventricular site earlier, remain numerous in the central region of the cerebellum. The rest of the neurons, however, migrate as usual to form markedly incomplete alignment of the Purkinje and the Golgi cell populations in the granule cell layer. In addition, the Bergmann cells are located in an unusual site in the molecular and Purkinje cell layer (Rakic, 1976; Terashima et al., 1985), and some of them extend their processes into the granule cell layer. After the Purkinje cells become aligned, the stellate-type and granule cells descend from the external germinal layer and traverse the incomplete Purkinje cell layer to reach the granule cell layer and even the superficial zone of the central cell mass, to where the dendrites of the heterotopic Purkinje cells extend. Thus the compact alignment of the Purkinje and Bergmann cells in the Purkinje cell layer and the extension of the Purkinje dendrites and radial processes of the Bergmann cells into the molecular layer might play an important role in the descent of the stellate and basket cells and their restricted presence in the molecular layer. In the deep zone of the central cell mass near the cerebellar nuclei or 4th ventricle, on the other hand, stellate-type cells were scarce. The compressed dendrites of the Purkinje cells in the packed cluster of cell bodies might obstruct their further descent into the deep zone.

3) Synaptic rearrangement in the reeler cerebellum

The reeler cerebellum was divided into four regions on the basis of the populations of Purkinje cells (Goffinet et al., 1984; Heckroth et al., 1989; Inoue et al., 1990) and inhibitory interneurons, the stellate-type and Golgi cells, described above. The distribution of inhibitory terminals on the Purkinje cells differed among these four regions, as summarized in Table II. These regional differences were closely related to the diversity of the population of inhibitory neurons. In the molecular and Purkinje cell layers, terminals of the stellate cells were present on the Purkinje dendrites, and basket-like terminals were formed around the Purkinje cell bodies, as was observed in normal mice. However, the density of the stellate terminals was higher than that in the normal molecular layer. The probable reasons for the higher density are as follows: 1) the neuronal population became relatively dense because of the reduction of the molecular layer volume, and 2) reduced synaptic contact with the parallel fibers might give rise to an increase in formation of other synapses as reported for other mutant mice (Inoue et al., 1993). In areas where both the stellate-type and Golgi cells were present, a few heterologous synapses originating from Golgi cells were detected around the Purkinje cell bodies as well as those originating from the stellate-type cells on the dendrites or somata. In the deep zone of the central cell mass, on the other hand, where the Golgi cells were exclusively present

in the compact cluster of Purkinje cells, many Gly-positive synapses were formed heterologously around the Purkinje cell bodies. The GABA- and GAD-positive terminals occurring in the deep zone possibly originated from both the recurrent axons of the heterotopic Purkinje cells and the Golgi cells, since the IP₃ receptor protein-positive terminals found in this region were much fewer (Inoue et al.,1990) and the stellate-type cells were scarce. In previous electron microscopic studies (Mariani et al.,1977; Sotelo and Privat,1978; Wilson et al.,1981, Sotelo,1990,1991), it was noted that the stellate axon terminals were in contact with the Purkinje cell bodies and the Golgi cells formed synapses on the dendritic spines of the Purkinje cells. This heterologous synapse formation could not be detected in this immunohistochemical study.

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Figure legends

Fig.1 Specificity of two novel monoclonal anti-bodies.

Enzyme-linked immuno-solvent assay of the reactivities of HE-4 and RS 3-21: HE-4 reacted strongly with conjugates of both GABA and ACA, but showed no cross-reactivity with other conjugates (a). RS 3-21 bound only the Gly conjugates (e).

Inhibition experiment with three conjugated amino acids, GABA(+GABA), Glu(+Glu), and Gly(+Gly): Immunoreactivity of HE-4 was inhibited by GABA-Ova (b), and RS 3-21 by Gly-Ova (h), but the other conjugates had no effect (c,d,f,g).

Fig.2 Normal cerebellum stained with anti-GAD antiserum. a and b, the cerebellar cortex of the normal mouse; c, the cerebellar nucleus (Nucl. medialis).

ML, molecular layer; PL, Purkinje cell layer; GL, granule cell layer; Nu, cerebellar nucleus; S, stellate or basket cells; Go, Golgi cell; small arrows, terminal boutons of stellate cells; large arrowheads, basket-like terminals around the Purkinje cell bodies; arrowhead, ring-shaped terminals at the cerebellar glomeruli; asterisks, large neurons within the cerebellar nucleus.

Fig.3 Normal cerebellum stained with GABA (a,c) and Gly (b,d) antibodies. a and b, serial sections of the cerebellar cortex; c and d, serial sections of the cerebellar nucleus (Nucl. medialis). ML, molecular layer; PL, Purkinje cell layer; GL, granule cell layer; S, stellate or basket cells; arrows, small neurons located in the Purkinje cell layer

which were stained with both GABA and Gly antibodies; small arrows, Gly-positive fibrous processes in the molecular layer; crossed arrows, Golgi cells located in the granule cell layer; large arrowheads, basket-shaped terminals around the Purkinje cell bodies; small arrowheads, ring-shaped structures which were stained with both antibodies; asterisks, large neurons within the cerebellar nucleus; circle, a small neuron containing only GABA.

Fig.4 Sagittal sections of the vermis of reeler cerebellum stained with GABA antibody (a,d,g), Gly antibody (b,e,h), and GAD antibody (c,f,i). These regions are shown in the diagram (j).

a-c, the pial zone composed of the molecular layer, Purkinje cell layer, and granular layers; d-f, the voluminous central mass beneath the white matter; g-i, the ventricular zone.

Abbreviations in Figs. 4, 5, and 6: ML, molecular layer; PL, Purkinje cell layer; GL, granule cell layer; WM, white matter; CM, central cell mass; Nu, cerebellar nucleus; arrows, stellate-type cells (GABA-positive, Gly-negative); crossed arrows, Golgi cells (GABA-positive, Gly-positive); large arrowheads, basket-shaped structures enclosing the Purkinje cell bodies; small arrow, Purkinje dendrites; arrowheads, unusual Gly-positive terminal boutons around the Purkinje cell bodies.

Fig. 5 Paramedial sections where the medial nucleus was observed to be stained with GABA (a,c,f,i), Gly (b,d,g,j), and GAD (e,h,k) antibodies. The areas are shown in the diagram (l).

a and b, the small cluster of Purkinje cells located between the granule cell layer and the white matter; c-e, the superficial zone of the central cell mass; f-h, the deep zone of the central cell mass close to the medial cerebellar nucleus; i-k, the medial cerebellar nucleus; asterisks, large neurons within the cerebellar nucleus.

Fig.6 Sagittal sections of the reeler hemisphere stained with GABA (a,d,h), Gly (b,e,i), and GAD (c,f,j) antibodies. The areas are shown in the diagram (g).

a-c, a small Purkinje cell mass located under the granule cell layer; d-f, the central cell mass underlining the white matter; h-j, interpositus nucleus.

Table I Immunohistochemistry of the neurons in the normal cerebellum

	anti-GAD	anti-GABA	anti-Gly
stellate cell			
soma	+	+	-
terminal	+	+	-
basket cell			
soma	+	+	-
terminal	+	+	-
Purkinje cell			
soma	+	-	-
dendrite	+	-	-
terminal	+	+	-
granule cell			
soma	-	-	-
parallel fiber	-	-	-
Golgi cell			-
soma	+	+	+
terminal	+	+	+
cerebellar nucleus			
large neuron	-	-	-
small neuron			
soma	-	+	+
terminal	+	+	+
unknown type in the	+	+	+
molecular layer			

+: positive, -: negative

Table II Distribution of inhibitory terminals on the
Purkinje cells in four regions of the reeler cerebellum

	stellate cell	basket cell	Golgi cell
molecular and Purkinje cell layer	++	+	-
granule cell layer	+	+	-
central cell mass			
surface zone	+	+	+
deep zone	-	-	++











