0014-4754/96/060531-04$1.50 + 0.20 © Birkhäuser Verlag Basel, 1996

Altered axon terminals containing concentric lamellar bodies of cerebellar Pukinje cells in Mongolian gerbil

I. K. Takeuchi[a,*], Y. K. Takeuchi[b], Y. Murashima[c] and A. Seto-Ohshima[a]

[a]*Institute of Developmental Research, Aichi Human Service Center, Kasugai, Aichi 480-03 (Japan)*
[b]*Anatomical Laboratory, Gifu Medical College of Technology, Seki, Gifu 501-32 (Japan)*
[c]*Department of Neurophysiology, Tokyo Institute of Psychiatry, Tokyo 156 (Japan)*
Received 2 May 1995; received after revision 14 September 1995; accepted 26 September 1995

Abstract. Altered axon terminals containing concentric lamellar bodies were observed in cerebellar and vestibular nuclei of the Mongolian gerbil. The terminals increased in number from 30 days of age onward, and reached about tenfold at 360 days. The numbers were the same in two gerbil strains with different susceptibility to spontaneous motor seizures by various stimuli, but about threefold those in Slc:Wistar rat.
Key words. Concentric lamellar body; Purkinje cell; axon terminal; ubiquitin; Mongolian gerbil.

Purkinje axon terminals in cerebellar and vestibular nuclei have been shown to form concentric lamellar bodies (CLBs) under some pathological conditions[1–5]. These CLBs are positive to acid phosphatase cytochemistry[1,5,6] and ubiquitin (Ub) immunocytochemsitry[7], and are thought to digest terminal cytoplasmic organelles in axonal remodeling process occurring in response to abnormal conditions[1], but the precise pathogenesis of CLB production remains nuclear.

Using Ub immunocytochemistry, we have examined the presence of the CLBs in brains of various normal and mutant experimental animals. This report describes the appearance of the CLBs in altered axon terminals in the cerebellar and vestibular nuclei of Mongolian gerbil with time. Since the Mongolian gerbil exhibits spontaneous motor seizures in response to a variety of stimuli, and the susceptibility varies with the strain[8,9], we also examined whether susceptibility to the seizure is related to the occurrence of the CLBs.

Materials and methods

To examine the distribution of altered axon terminals containing CLBs in brain, Mongolian gerbils of MGS/Idr strain at 360 days of age were used. This strain has been shown to display spontaneous motor seizures in all animals[10]. The gerbils were deeply anesthetized by ethyl ether, and killed by intracardiac perfusion with 10% Formalin after blood vessels had been flushed out with physiological saline. After removal from the skull, the brains were bisected coronally, fixed with Bodian II solution for 24 h, and embedded in paraffin wax. Coronal sections 8 μm thick were mounted on albumin-coated slides, and immunostained with anti-Ub antibody according to the methods previously reported[7]. Briefly, deparaffinized slides were treated for 30 min with 0.3% hydrogen peroxide in methanol, rehydrated through a descending ethanol series to water, and heated in a 20% solution of zinc sulfate in water in a microwave oven for 10 min. Cooled slides were rinsed in distilled water and 0.01 M phosphate-buffered saline, (PBS) pH 7.2. Then, sections were first incubated with 0.5% casein in PBS for 30 min at room temperature, followed by incubation with a primary antibody (a mouse monoclonal antibody to Ub; Chemicon Int. Inc.) for 24 h at 4 °C at a dilution of 1:10,000 with 0.5% casein in PBS. Immunoreactive sites were visualized by treatment with Vectastain ABC kits and Tris-HCl bufier (pH 7.6) containing 0.05% 3,3′-diaminobenzidine (DAB) and 0.002% hydrogen peroxide. Control sections were processed as above, but the primary antibody was replaced with a non-immune mouse IgG diluted 1:200.

Some gerbils were killed by intracardiac perfusion with a mixture of 2% paraformaldehyde, 0.25% glutaraldehyde, 10% dimethyl sulfoxide and 8% sucrose in 30 mM PIPES buffer (pH 7.2) at room temperature. After overnight fixation with the same solution, 40 μm thick vibratome sections of cerebella were made, and those containing cerebellar nuclei were treated with 0.5% casein in PBS for 30 min at room temperature. They were then incubated at 4 °C overnight with the anti-Ub antibody at a dilution of 1:1,000 with 0.5% casein in PBS. These sections were treated with Vectastain ABC kits, fixed with 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 10 min at 4 °C, and incubated with 0.05% DAB in Tris-HCl buffer for 30 min at room temperature, followed by a mixture of 0.05% DAB and 0.002% hydrogen peroxide in Tris-HCl buffer for 5 min. Cerebellar nuclei were cut out under a binocular microscope, post-fixed with 1% osmic acid in 0.1 M cacodylate

* Corresponding author.

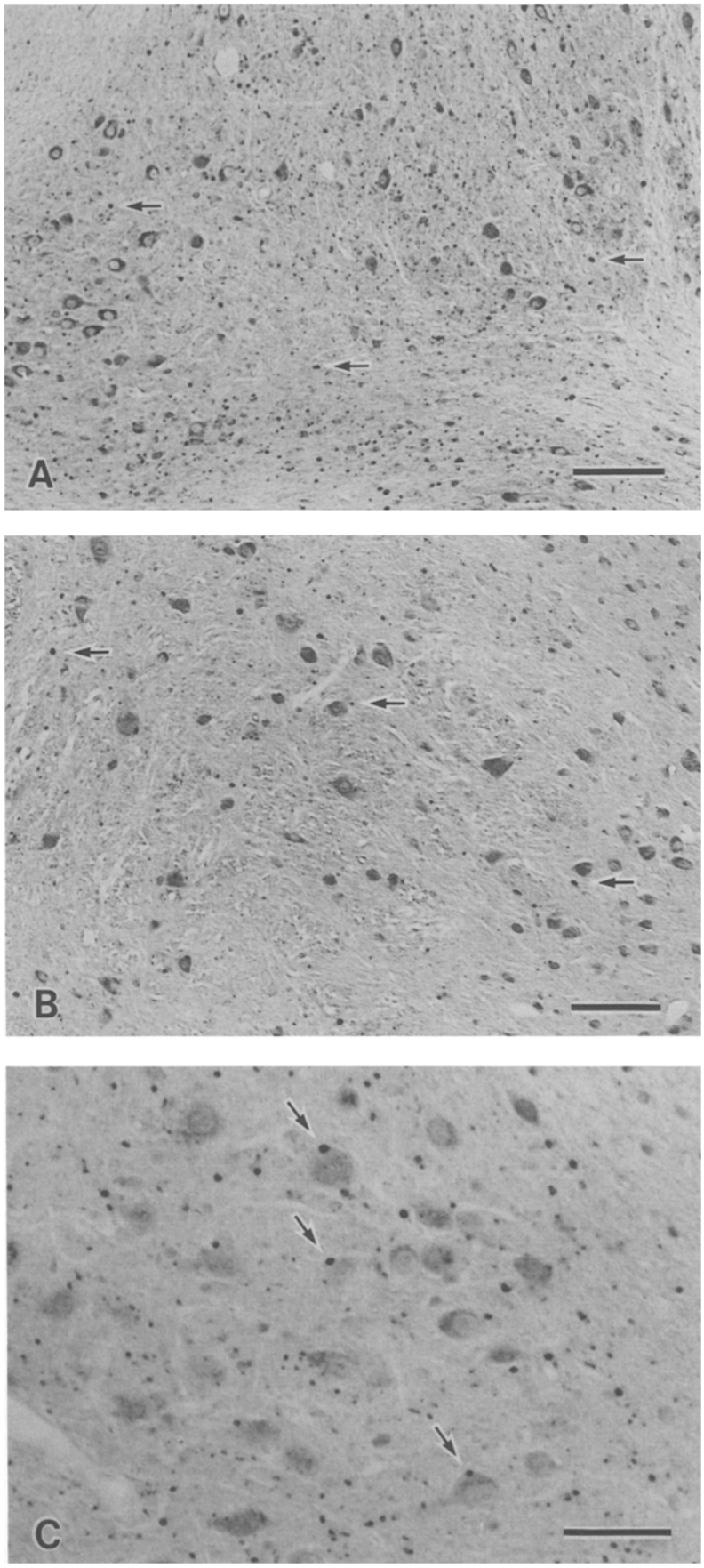

Figure 1. Photomicrographs showing many dot-like immunodeposits (arrows) after ubiquitin immunohistochemistry in the neuropil of lateral cerebellar nucleus (*A*) and spinal vestibular nucleus (*B*) of 360-day-old MGS/Idr gerbil. Some immunodeposits are attached to the surface of neurons (*C*). Bar in *A* and *B* = 100 µm, in *C* = 50 µm.

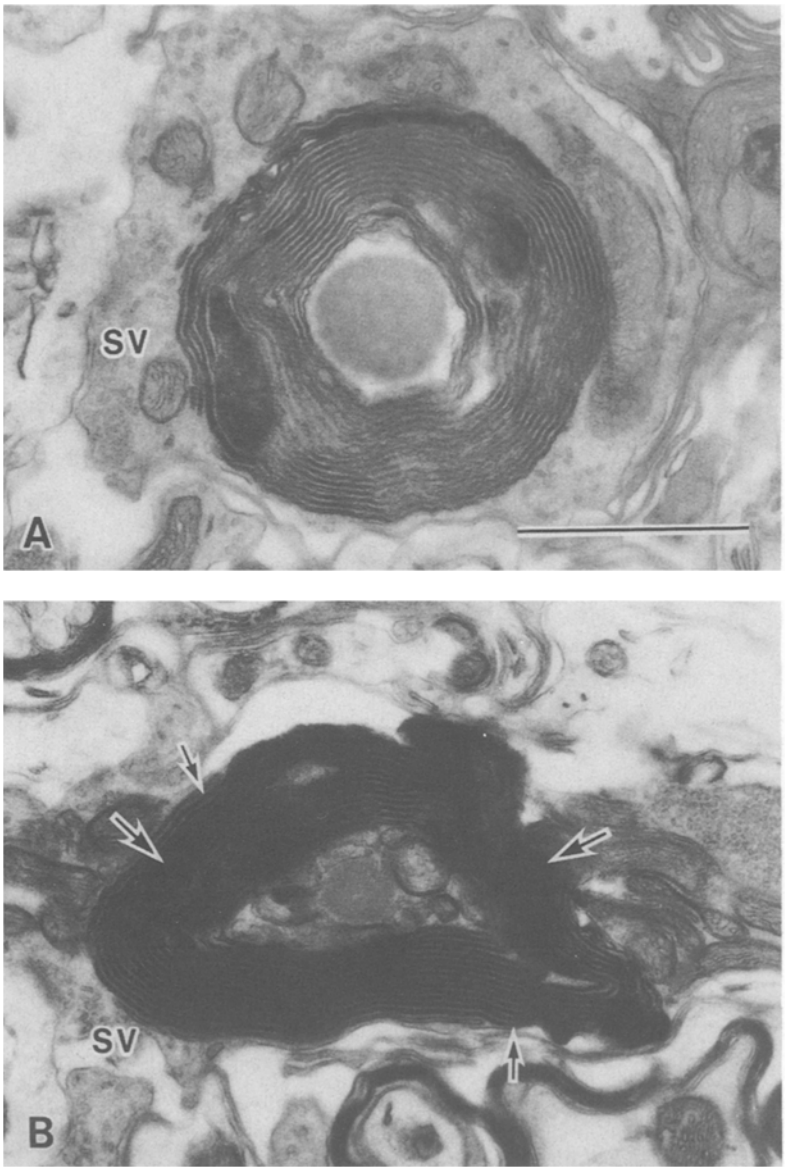

Figure 2. Electromicrographs of concentric lamellar bodies in axon terminals in lateral cerebellar nuclei of 360-day-old MGS/Idr gerbil after immunostaining with non-immune mouse IgG (*A*) and anti-ubiquitin antibody (*B*). Numerous synaptic vesicles (SV) are observed in the axoplasm near the synaptic contents. Both lamellar structures (small arrows) and degenerative amorphous materials (large arrows) are immunoreactive. Bar = 1 μm.

buffer, and embedded in Quetol 812. Thin sections were observed using a JEOL JEM 100B electron microscope after staining with lead nitrate. Staining specificity was assessed by replacing the primary antibody with the non-immune mouse IgG[7].

To examine time-related differences in the appearance of the altered axon terminals, paraffin sections of cerebella and underlying medulla were made from the MGS/Idr gerbils at 20, 30, 40, 60, 90, 180 and 360 days of age, and immunostained with anti-Ub antibody. Four gerbils were sacrificed at each day. Dot-like Ub immunodeposits in the neuropil were counted in 0.25 mm^2 areas of the right-hand lateral cerebellar nuclei.

To examine whether the gerbils' susceptibility to the seizure is related to the occurrence of Ub immunodeposits, four 360-day-old gerbils maintained by brother-sister mating for F65 as a seizure-resistant strain were sacrificed, and dot-like Ub immunodeposits were also counted in the right-hand cerebellar nuclei. For the study of strain differences in their occurrence, four 360-day-old Slc:Wistar rats were sacrificed and examined with the same methods. Statistical analyses were performed using the Mann-Whitney U test.

Results and discussion

In the Ub-immunostained paraffin sections of the brains of 360-day-old MGS/Idr gerbils, numerous dot-like im-

Table 1. Number of dot-like immunodeposits in neuropil of lateral cerebellar nucleus (mean ± SD, per 0.25 mm^2).

Age (days)	Seizure-		Slc:Wistar rat
	sensitive gerbil	resistant gerbil	
20	5 ± 3	-	-
30	76 ± 8	-	-
40	99 ± 13	-	-
60	139 ± 28	-	-
90	147 ± 19	-	31 ± 10*
180	335 ± 25	-	-
360	809 ± 34**	824 ± 56**	271 ± 111

*Cited from Takeuchi et al.[7].
**$p < 0.05$ compared with Slc:Wistar rat.

munodeposits were found in the neuropil of lateral (fig. 1A), interposed and medial cerebellar nuclei. Although somewhat fewer than in the cerebellar nuclei, a number of the same immunodeposits were also found in the neuropil of superior, lateral, medial and spinal vestibular nuclei (fig. 1B). At higher magnification, some immunodeposits were observed to be attached to the surface of the neuronal cell bodies (fig. 1C).

Electronmicroscopic Ub immunocytochemistry revealed a number of immunostained CLBs in the altered axon terminals in the cerebellar nuclei of the 360-day-old gerbils. These CLBs measured about 1–5 μm in diameter, and consisted of compactly and concentrically arranged lamellar structures, and central cores containing degenerative cellular organelles. The lamellar structures had partially degenerated into osmiophilic amorphous materials (fig. 2A). The Ub immunodeposits were distributed in the lamellar structures and the amorphous materials (fig. 2B).

Table 1 shows changes in mean numbers of dot-like Ub immunodeposits in the neuropil of lateral cerebellar nuclei in the gerbils with time. The immunodeposits abruptly increased at 30 days of age and thereafter gradually increased, reaching about twofold at 90 days, fourfold at 180 days, and tenfold at 360 days. When compared at 360 days of age, the mean number of the Ub immunodeposits was not significantly different between seizure-sensitive and seizure-resistant gerbil strains, but they were three times as numerous as in the Slc:Wistar rats.

In the previous study[7], we observed an average of about 30 Ub immunodeposits per 0.25 mm^2 in the neuropil of lateral cerebellar nuclei of 90-day-old Slc:Wistar rats (table 1). In the present study, however, the mean number of Ub immunodeposits increased about ninefold in the 360-day-old rats. Thus, Purkinje axon terminals containing CLBs greatly increase in number with time both in gerbils and in rats. However, these altered axon terminals appeared much more numerous in gerbils than rats, and their numbers in gerbils did not correlate with susceptibility to seizure. In the studies hitherto published, numerous appearances of CLB-containing Purkinje axon terminals have been reported in animals under certain pathological conditions, such as in rats subjected to a prolonged hypoxia[2,3], in rats after an inferior olive lesion[4], and in groggy mutant rats displaying movement disorder[5]. Mongolian gerbils may be the first case showing the spontaneous appearance of a large number of these altered axon terminals with age, and offer some new clues to the study of the pathogenesis of CLB production in Purkinje axon terminals, especially in relation to aging of Purkinje cells.

1 Sotelo, C., and Palay, S. L., Lab. Invest. *25* (1971) 653.
2 Yu, M. C., Bakay, L., and Lee, J. C., Acta neuropathol. *22* (1972) 222.
3 Yu, M. C., Bakay, L., and Lee, J. C., Expl Neurol. *40* (1973) 114.
4 Rossi, F., Cantino, C., and Strata, P., Neuroscience *22* (1987) 99.
5 Takeuchi, I. K., Aoki, E., and Takeuchi, Y. K., Acta neuropathol. *87* (1994) 628.
6 Takeuchi, I. K., Aoki, E., and Takeuchi, Y. K., J. Electron Microsc. *44* (1995) 140.
7 Takeuchi, I. K., Takeuchi, Y. K., and Aoki, E., Acta histrochem. cytochem. *27* (1994) 275.
8 Jobe, P. C., Mishra, P. K., Ludvig, N., and Dailey, J. W., Crit. Rev. Neurobiol. *6* (1991) 183.
9 Loskota, W. J., Lomax, P., and Rich, S. T., Epilepsia *15* (1974) 109.
10 Seto-Ohshima, A., Ito, M., Kudo, T., and Mizutani, A., Acta neurol. scand. *85* (1992) 311.