Acta Neuropathol (1989) 78:131–136

Acta Neuropathologica

Distribution and morphology of brain stem plaques in Alzheimer's disease*

E. Iseki[1,2], M. Matsushita[1], K. Kosaka[2], H. Kondo[2], T. Ishii[3], and N. Amano[4]

[1] Department of Psychiatry and Neurology, Yokohama City University School of Medicine, 3-46 Urafune-cho, Minami-ku, Yokohama 232, Japan
Departments of [2] Neuropathology and [3] Molecular Biology, Psychiatric Research Institute of Tokyo, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156, Japan
[4] Division of Psychiatry and Neurology, Kanagawa Rehabilitation Center, Nanasawa 516, Atsugi-shi, Kanagawa-ken, Japan

Summary. The morphology, incidence and distribution of senile plaques in the brain stem were examined in 15 cases of Alzheimer's disease, using mainly the Methenamine-Bodian method. The plaques were found in all cases and were grouped into three types according to their morphology. They were not randomly scattered in the brain stem, but had a distribution common to all cases. There were numerous plaques in the periaqueductal gray, superior colliculus, fourth-ventricle floor and superior central nucleus. The plaques were also found less abundantly in the reticular formation, substantia nigra, pontine nucleus and inferior olivary nucleus. There was a tendency for certain plaque types to be associated with specific regions. In the familial cases, the plaques tended to occur even in the regions where they were rare in other cases. The capillaries with plaque-like degeneration were observed not infrequently in the brain stem. The distribution of plaques did not always coincide with that of neurofibrillary tangles.

Key words: Senile plaque – Alzheimer's disease – Brain stem – Distribution – Morphology

Senile plaques (SPs) and neurofibrillary tangles (NFTs) are the most striking features in the brains of patients with Alzheimer's disease (AD) and senile dementia of the Alzheimer's type (SDAT). SPs are composed of amyloid, degenerative neurites and reactive cells. They are classified into three types (classic, primitive and compact plaques) according to the arrangement and relative proportions of those components [25].

* Supported by local specialized studies subsidy granted by Yokohama City

Offprint requests to: E. Iseki (address see above)

In AD/SDAT, there have been a number of studies concerning the distribution and morphology of SPs in the cerebral cortex [2, 3, 5, 19]. However, studies regarding the subcortical SPs have been few. Recently topographical studies of SPs in the striatum and diencephalon [20] and in the cerebellum [16] have been reported. SPs were considered to be much less numerous in the brain stem than in the cerebral cortex, being found only in the tegmentum of the midbrain and pons [23], until Rudelli et al. [21] mapped the thioflavin S-positive SPs in the brain stem.

Here we examine the brain stem sections in 15 cases of AD, using mainly the Methenamine-Bodian (M-Bodian) method [11], and describe the morphology, incidence and distribution of SPs in the brain stem.

Materials and methods

We used 10% formalin-fixed brain stem tissues from 15 patients with AD of presenile onset. These cases are the only AD cases in which the brain stem could be sufficiently examined, having been autopsied from 1982 to 1986 at the Psychiatric Research Institute of Tokyo and the Department of Psychiatry and Neurology, Yokohama City University School of Medicine (Table 1). Three of these cases had a familial history of the disease. The diagnosis of AD was made clinically and was confirmed neuropathologically because of the numerous NFTs and SPs throughout the cerebral cortex. The paraffin-embedded sections (6–10 μm thick) were stained with PAS, Bodian, methenamine-Bodian and Congo red, and 2-μm-thick sections with modified periodic acid-methenamine silver (PAM) [28]. They were examined by light microscopy. Also, selected sections were examined by the anti-β-protein immunoperoxidase Avidin-biotin-peroxidase complex (ABC) method [9].

Results

The SPs were detected most clearly by the M-Bodian method. Though they varied in size, they were grouped into three types according to their morphology

Table 1. Fifteen cases of Alzheimer's disease

Case	Age at death (years)	Sex	Duration (years)	Brain weight (g)
1	72	f	12	1170
2	65	m	5	1250
3	66	m	10	1070
4	68	f	10	1050
5	63	m	7	1235
6	57	f	12	800
7	48	m	8	1250
8	74	f	15	940
9	72	f	11	780
10	65	f	4	1020
11	61	f	14	775
12	71	f	14	800
13 (F)	45	m	7	1300
14 (F)	58	m	12	1000
15 (F)	46	m	10	1250

F, familial case

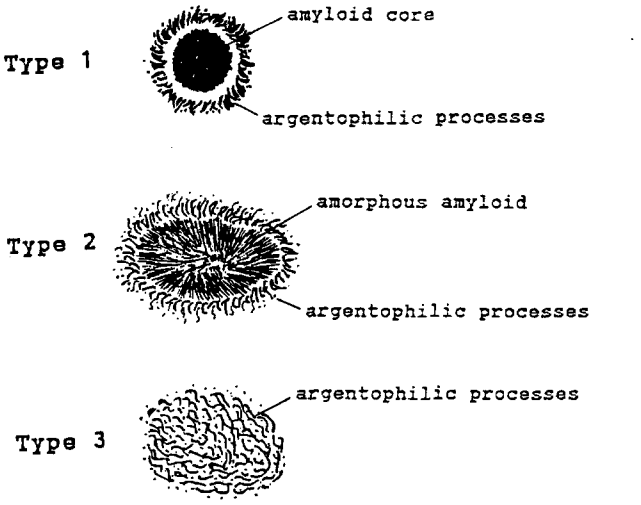

Fig. 1. Morphology of three senile plaque (SP) types in the brain stem

(Fig. 1). The M-Bodian staining generally colored the degenerative neurites greenish-black and the amyloid reddish-brown. Type 1 SPs had an amyloid core surrounded by a small amount of argentophilic processes (see Fig. 4). They resembled classic or compact SPs. They were smaller (5 – 50 μm in diameter) than those in the cerebral cortex. Type 2 SPs had amorphous, faintly stained amyloid, which did not form an amyloid core (see Fig. 5). This was also surrounded by argentophilic processes. Type 3 SPs were an ill-defined aggregation of the fine argentophilic processes (Fig. 2). These three types had some degree of intergradation with each other. Type 1 could be identified by all of the staining methods. With Congo red, the amyloid cores showed green birefringence with polarizing microscopy. Type 2 appeared to be an aggregation of the black fiber bundles (see Fig. 6) with the PAM and a lightly stained spot with PAS. It was mostly unidentified with Bodian and Congo red. Type 3 could not be identified by conventional staining methods other than M-Bodian. The anti-β-protein antibodies reacted with all types of SPs. The differentiation between types 2 and 3 was determined by the differences in the density of the immune products (Figs. 3, 7).

The SPs detected by M-Bodian were regionally counted at a magnification of $\times 40$ (field size 0.53 mm^2), and their incidence was graded as follows: (+++) $\geq$ 10/field, (++) 1 – 9/field, (+) 1/several fields, (±) rare, and (–) absent. The relative proportion of plaque types was expressed as (1, 2) when the incidences of types 1 and 2 were roughly even, and as (1 > 2) or (2 > 1) when they were much different. The results are shown in Table 2.

The SPs were found to a greater or lesser degree in the brain stem sections of all cases. It was noteworthy that they were not randomly scattered in the brain stem, but had a distribution common to all cases. Furthermore, there was a tendency for certain SP types to be associated with specific regions, although the SPs of different types were usually intermingled there.

The regional characteristics of SPs in the brain stem were as follows. They were numerous in all cases in the periaqueductal gray, superior colliculus, fourth-ventricle floor and superior central nucleus. In the periaqueductal gray, type 3 was predominant in the dorsal part (Fig. 2), while in the ventral part including the dorsal tegmental and dorsal raphe nuclei, type 2 was predominant. In the superior colliculus, type 3 was predominant in most cases, while type 1 stood out in two cases (Fig. 4). The SPs also appeared in all cases in the reticular formation and substantia nigra, although their incidence was less than in the regions mentioned above. In the compact zone of the substantia nigra, a few type 1 SPs similar to compact SP were noticed in 9 of the 15 cases. In the pontine nucleus, the SPs were present in 9 of the 15 cases. In the inferior olivary nucleus, large type 2 SPs (up to 200 μm in diameter) occurred in 13 of the 15 cases (Fig. 5). In the red nucleus, the SPs were few, and were very scarce in the vestibular nucleus and/or posterior funicular nucleus. In the white matter, the SPs rarely appeared. A schema outlining the distribution of SPs in the brain stem is shown in Fig. 8. The dots roughly indicate the regional proportion of SP incidence.

In the three familial cases (cases 13 – 15), the SPs tended to occur even in regions such as the red nucleus,

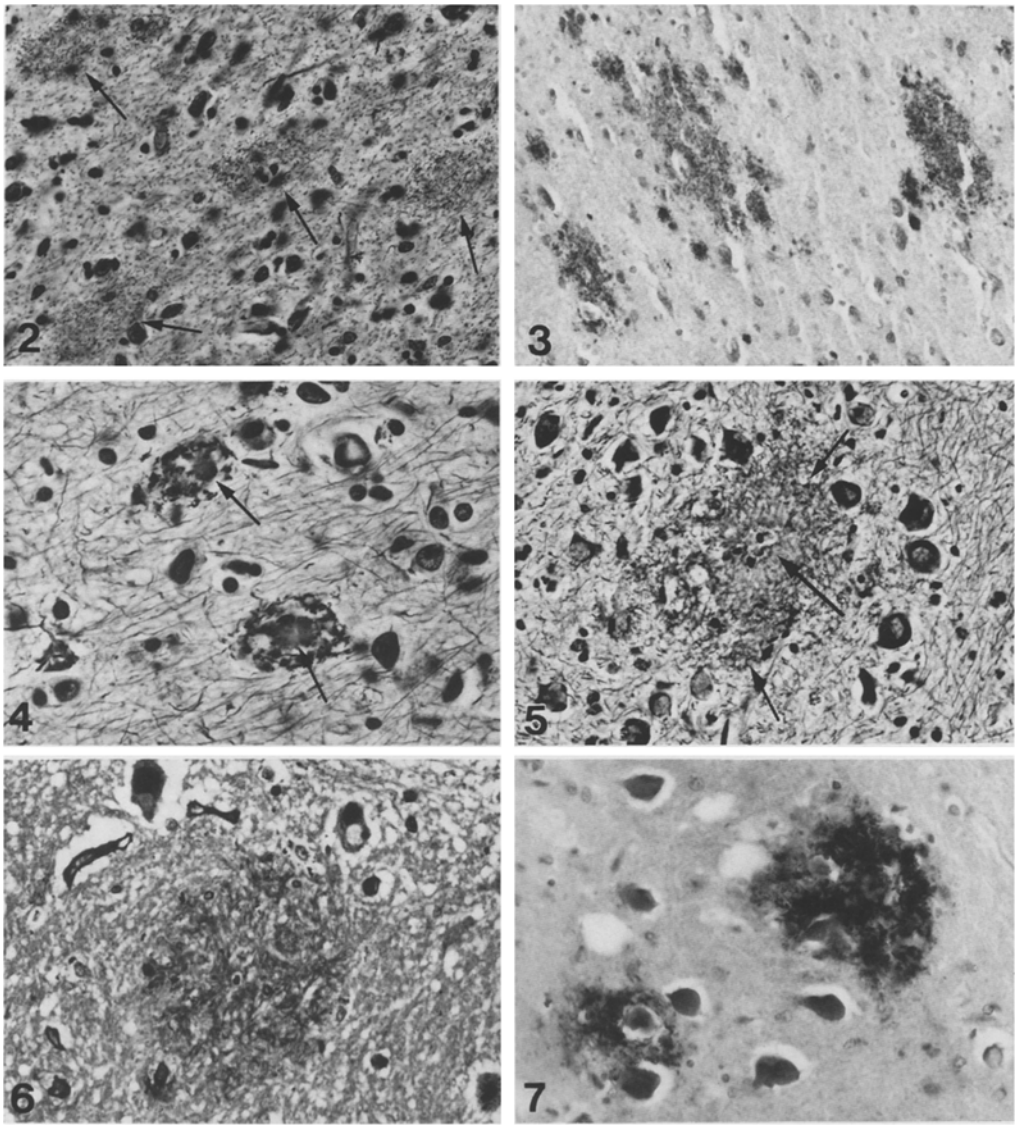

Fig. 2. Periaqueductal gray. Four type 3 SPs (*arrows*). Methenamine-Bodian (M-Bodian) stain, ×230

Fig. 3. Periaqueductal gray. Three type 3 SPs. Anti-β-protein method with Mayer's hematoxylin counterstain, ×180

Fig. 4. Superior colliculus. Two type 1 SPs having an amyloid core (*arrows*). M-Bodian stain, ×390

Fig. 5. Inferior olivary nucleus. A type 2 SP having amorphous amyloid (*long arrow*) surrounded by argentophilic processes (*short arrows*). M-Bodian stain, ×250

Fig. 6. Inferior olivary nucleus. A type 2 SP. Periodic acid-methenamine silver stain, ×160

Fig. 7. Inferior olivary nucleus. Two type 2 SPs. Anti-β-protein method with Mayer's hematoxylin counterstain, ×230

vestibular nucleus and posterior funicular nucleus where they were rare in the other cases.

In the brain stem, arteries or arterioles showing congophilic angiopathy were rare, but a number of SPs appeared to be somewhat correlated with the capillaries, regardless of SP types. The β-protein-positive capillaries with plaque-like degeneration frequently occurred.

NFTs were also found in the brain stem in all cases. They occurred preferentially in the nuclei such as the periaqueductal gray, superior central nucleus, locus ceruleus, reticular formation, substantia nigra, etc. Their distribution overlapped that of the SPs. But NFTs were very rare in the pontine nucleus and inferior olivary nucleus where the SPs were frequently found.

Table 2. Regional incidence of plaques in the brain stem

		Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Midbrain	PG	+++ 3>2>1	+++ 2, 3>1	++~+++ 2>3>1	++~+++ 3>2	++ 2>1, 3	++~+++ 3>2	++~+++ 2, 3>1
	SC	++~+++ 3>1, 2	++~+++ 1>2, 3	++ 3>2	++ 3>2	++~+++ 1>2, 3	++~+++ 3	++~+++ 3>2
	RF	++ 3>1, 2	++ 1, 2, 3	+~++ 2, 3	+~++ 2>3	+~++ 1, 2>3	++ 3>2	+~++ 2, 3
	RN	± 2	± 2, 3	± 2	± 2	−	±~+ 2, 3	±~+ 2
	SN	++ 3>1, 2	++~+++ 3>1, 2	+~++ 2, 3>1	++ 2, 3>1	+~++ 2>1	++ 3>2	++~+++ 2>3>1
Pons	FF	++~+++ 2>1, 3	+~++ 2, 3	+~++ 2, 3	++ 2, 3	+~++ 2>3	++~+++ 2, 3	++~+++ 2>3
	SCN	++~+++ 2>1, 3		++ 2>3		++ 1, 2>3	++~+++ 2>3	++~+++ 2>3>1
	RF	++ 2>1, 3	+ 1, 2, 3	+ 2, 3	+~++ 2, 3	+~++ 1, 2>3	++ 2, 3	+~++ 2>3>1
	PN	+~++ 2>1, 3	+ 2>1, 3	−	−	−	+~++ 3>2	++ 2, 3>1
Medulla	FF	+~++ 2>3	+~++ 2>3	+ 2	± 3	+ 2, 3	+~++ 2, 3	++ 2>3
	VN, PFN	−	−	−	−	−	−~± 2	± 2
	RF	+~++ 2>1, 3	+ 2>1, 3	+ 2	+ 2>1	± 2	+~++ 2>3	++ 2>3>1
	ION	+ 2>3	+ 2>3	+ 2	+ 2	± 2	+~++ 2>3	± 2

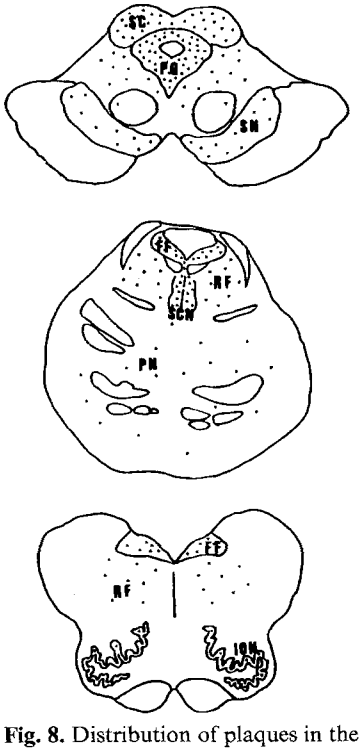

Fig. 8. Distribution of plaques in the brain stem

Discussion

The accepted techniques for the demonstration of SPs are the silver-impregnation methods such as the Bodian and the modified Bielschowsky method [6], and the amyloid-staining methods such as the PAS, Congo red and thioflavin T. The Bodian method has been most commonly used because it stains consistently, but it does not always demonstrate all SPs present [29].

The PAM method, which had so far been used for the visceral organs, was recently reapplied to the central nervous system [12]. It is similar to the modified Bielschowsky method in SP-demonstrating efficiency. It preferentially stains the amyloid rather than the degenerative neurites.

On the other hand, the M-Bodian method we devised is prepared by adding methenamine to the protein-silver in the standard Bodian [11]. It separately stains both amyloid and degenerative neurites, so that the various types of SPs can be easily and efficiently distinguished. This method should be useful for further studies of SPs.

Case 8	Case 9	Case 10	Case 11	Case 12	Case 13 (F)	Case 14 (F)	Case 15 (F)
++~+++ 3>2	++ 2>3	++ 2>3	++ 2>3	+~++ 3>2		++~+++ 3	+++ 2, 3
++ 3>2	+~++ 2, 3	++ 3>2	++ 2, 3	+~++ 3		++ 3	+++ 3>2
+~++ 3>2	+ 1, 2, 3	+ 2, 3	+~++ 2>3	+ 3		+ 3	++~+++ 2, 3
–	–	–		–	± 3	++ 3>2	++ 2, 3
+~++ 2>3	±~+ 1, 3	+ 2>3	+ 2>3	+ 2, 3>1	++~+++ 2, 3>1	++~+++ 3>2	+++ 3>2>1
++~+++ 3>2	+~++ 3>2	+~++ 2>3	+~++ 2>3		++~+++ 2>3	++~+++ 2, 3	++~+++ 3>2
					+++ 2>3		++ 2, 3
+~++ 2>3	± 3	± 3	+~++ 2>3		++ 2, 3	++ 2>3	+~++ 2, 3
+ 2>3	± 2	–	–		++ 3>2>1	+~++ 2, 3	+~++ 3>2
+ 3	± 3	± 3	+ 2, 3	+~++ 2, 3	++~+++ 3>2	++ 2, 3	++ 3>2
–	–	–	–	–	± 2	±~+ 3>2	±~+ 2>3
+~++ 2>3	–	–	+ 2	+ 2>1, 3	++ 2>3	+ 3>2	+ 2>3
–	±~+ 2	–	± 2	+~++ 2>3	+ 2	+~++ 2>3	+~++ 2>3

Abbreviations: F, familial case; PG, periaqueductal gray; SC, superior colliculus; RF, reticular formation; RN, red nucleus; SN, substantia nigra; FF, fourth-ventricle floor; SCN, superior central nucleus; PN, pontine nucleus; VN, vestibular nucleus; PFN, posterior funicular nuclei; ION, inferior olivary nucleus

Also the anti-β-protein immunohistochemical method has been applied recently to demonstrate SPs, because the antibodies to a polypeptide purified from cerebrovascular amyloid (β-protein) react not only with cerebrovascular amyloid but also with SPs amyloid [26].

Up to now, only brief descriptions of brain stem SP have been given, except for those in atypical AD [4, 14, 27]. The distribution pattern of brain stem SP in our study was similar to that of thioflavin S-positive SP reported by Rudelli et al. [21]. They reported that the SPs of amyloid type with little or no neuritic "halo" were predominant in the brain stem. In our study, the proportion of type 1 to whole SPs was rather low. The SPs described by Rudelli et al. [21] are likely to include type 1 and small-sized type 2 SPs. Type 2 and possibly some of type 3 SPs correspond to primitive SP in the earlier grouping. Yamamoto and Hirano [29] reported that the ill-defined fibrillary structures, resembling our type 3, were observed in the periaqueductal gray by the modified Bielschowsky method, but they were undetectable by the Bodian method or by thioflavin S. Taking into account the result of the anti-β-protein staining, type 3 SPs stained by the M-Bodian method may contain not only degenerative neurites, but also amyloid in small amounts.

In the brain stem, the distribution of SPs was more restricted than in the cerebral cortex. Furthermore, there was a tendency for certain SP types to occur predominantly in a particular region, as seen in the inferior olivary nucleus. These findings are important considering the mechanism of SP formation, even though they are partially attributable to the regional differences in anatomical architecture.

It has long been discussed whether amyloid angiopathy is essential for SP formation [15, 18, 22]. In our study, as in previous studies [13, 24], there were few vessels showing congophilic angiopathy. The pres-

ence of capillaries with plaque-like degeneration, however, indicates that amyloid angiopathy plays an important part in SP formation in the brain stem.

On the other hand, the distribution of NFTs in the brain stem [7, 8] does not always coincide, though overlapped considerably, with that of SPs. The fact that SPs and NFTs can occur separately suggests that their pathogenesis should also be considered separately [25].

Recently the attention has been directed to the relation between SPs and some neurotransmitters. It was shown with the immunohistochemical method that neurites containing acetylcholine [1], catecholamines [10], somatostatin [17], etc., were present within SPs. More detailed examination of SPs in the brain stem should help to understand what role a neurotransmitter system, including its projection pathways, plays in SP formation, in view of their restricted distribution.

Acknowledgement. We thank Mrs. K. Suzuki (Yokohama City University) for technical assistance.

References

1. Armstrong DM, Bruce G, Hersh LB, Terry RD (1986) Choline acetyltransferase immunoreactivity in neuritic plaques of Alzheimer brain. Neurosci Lett 71:229–234
2. Braunmühl AV (1957) Alterserkrankungen des Zentralnervensystems, senile Involution, senile Demenz, Alzheimersche Krankheit. In: Lubarsch O, Henke F, Rossle R (eds) Handbuch der speziellen pathologischen Anatomie und Histologie, vol 13. Springer, Berlin, pp 337–539
3. Dayan AD (1970) Quantitative histological studies on the aged human brain. II. Senile plaques and neurofibrillary tangles in senile dementia. Acta Neuropathol (Berl) 16:95–102
4. Fukatsu R, Ikeda T, Ueno T, Tanabe M, Honma H, Kimura N, Takahata N (1980) An unusual case of presenile dementia with numerous argentophilic plaques and severe amyloid vascular changes. Adv Neurol Sci 24:271–282
5. Gibson PH (1983) Form and distribution of senile plaques seen in silver-impregnated sections in the brains of intellectually normal elderly people and people with Alzheimer-type dementia. Neuropathol Appl Neurobiol 9:379–389
6. Hirano A, Zimmerman HM (1962) Silver impregnation of nerve cells and fibers in celloidin sections. Arch Neurol 6:114–122
7. Hirano A, Zimmerman HM (1962) Alzheimer's neurofibrillary changes: a topographic study. Arch Neurol 7: 227–242
8. Ishii T (1966) Distribution of Alzheimer's neurofibrillary changes in the brain stem and hypothalamus of senile dementia. Acta Neuropathol (Berl) 6:181–187
9. Ishii T, Haga S, Kitaguchi N (1987) Immunohistochemistry and molecular biology of Glenner amyloid protein (GP). Neuropathology 8:173
10. Kitt CA, Strube RG, Cork LC, Mobley WC, Walker LC, Joh TH, Price DL (1985) Catecholaminergic neurites in senile plaques in prefrontal cortex of aged nonhuman primates. Neuroscience 16:691–699
11. Kondo H, Haga C, Kito T, Kosaka K, Matsushita M (1987) The Methenamine-Bodian staining for the demonstration of senile plaques. Byori-to-Rinsho 5:1365–1369
12. Makifuchi T, Watabe K, Ikuta F (1986) The study of senile plaques by the PAM staining: the detection of amyloid. Neuropathology 7:196
13. Mandybur TI (1975) The incidence of cerebral amyloid angiopathy in Alzheimer's disease. Neurology 25:120–126
14. Matsuoka T, Miyoshi K, Saka K, Kawagoe T, Nishikiori T, Suzuki S, Hirabayashi M, Shisozuka T, Suda K, Aoki A, Shimokawa K, Shiraki H (1967) A case of encephalopathy with plaque-like bodies, neurofibrillary change, angiopathy and amyotrophic lateral sclerosis-like lesions. Adv Neurol Sci 11:801–811
15. Miyakawa T, Shimoji A, Kuramoto R, Higuchi Y (1982) The relationship between senile plaques and cerebral blood vessels in Alzheimer's disease and senile dementia. Morphological mechanism of senile plaques production. Virchows Arch [B] 40:121–129
16. Morioka E (1985) Senile amyloid changes in the cerebellum: with special reference to senile plaques and amyloid angiopathy. Neuropathology 6:313–323
17. Morrison JH, Rogers J, Scherr S, Benoit R, Bloom FE (1985) Somatostatin immunoreactivity in neuritic plaques of Alzheimer's patient. Nature 314:90–92
18. Mountjoy CQ, Tomlinson BE, Gibson PH (1982) Amyloid and senile plaques and cerebral blood vessels: a semiquantitative investigation of a possible relationship. J Neurol Sci 57:89–103
19. Rogers J, Morrison JH (1985) Quantitative morphology and regional and laminar distributions of senile plaques in Alzheimer's disease. J Neurosci 5:2801–2808
20. Rudelli RD, Ambler MW, Wisniewski HM (1984) Morphology and distribution of Alzheimer neuritic (senile) and amyloid plaques in striatum and diencephalon. Acta Neuropathol (Berl) 64:273–281
21. Rudelli RD, Ambler MW, Wisniewski HM (1984) Pattern of distribution of Alzheimer plaques in brain stem. J Neuropathol Exp Neurol 43:306
22. Scholz W (1938) Studien zur Pathologie der Hirngefäße. II. Die drusige Entartung der Hirnarterien und Capillaren. Z Neurol 162:694–715
23. Tomlinson BE, Corsellis JAN (1984) Ageing and the dementias. In: Adams JH, Corsellis JAN, Duchen LW (eds) Greenfield's neuropathology, 4th edn. Edward Arnold, London, pp 951–1025
24. Tomonaga M (1981) Cerebral amyloid angiopathy in the elderly. J Am Geriatr Soc 29:151–157
25. Wisniewski HM, Terry RD (1973) Reexamination of the pathogenesis of the senile plaque. Prog Neuropathol 11:1–26
26. Wong CW, Quaranta V, Glenner GG (1985) Neuritic plaques and cerebrovascular amyloid in Alzheimer's disease are antigenically related. Proc Natl Acad Sci USA 82: 8729–8732
27. Worster-Drought C, Greenfield JG, McMenemey WH (1944) A form of familial presenile dementia with spastic paralysis. Brain 67:38–43
28. Yajima G (1976) The periodic acid-methenamine silver staining for renal glomeruli. Rinsho-Kensa 20:14–19
29. Yamamoto T, Hirano A (1986) A comparative study of modified Bielschowsky, Bodian and thioflavin S stains on Alzheimer's neurofibrillary tangles. Neuropathol Appl Neurobiol 12:3–9

Received September 26, 1988/Revised December 17, 1988/ Revised, accepted February 13, 1989