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Diffuse type of senile plaques in the cerebellum of Alzheimer-type dementia demonstrated by β protein immunostain

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Summary. We studied senile plaques (SP) in the cerebella of six autopsied subjects with Alzheimer-type dementia (ATD) and ten non-ATD autopsied subjects between the ages of 78 and 90. Neither SP nor amyloid angiopathy (AA) was observed in any of the non-ATD subjects. In the four of the six ATD subjects, diffuse plaques in the molecular layer were seen as ill-defined areas of fine fibrillar materials by β protein immunostaining with formic acid pretreatment, the modified Bielschowsky stain, and periodic acid-methenamine silver (PAM) stain. The plaques were not visible with Bodian, Congo red, or periodic acid-Schiff stains. Compact plaques in the Purkinje cell or in the granular cell layers were found in three of the six subjects. Their amyloid core was often surrounded by areolar amyloid deposits. AA was observed in three of the six subjects. The argyrophilia of the diffuse and compact plaques, demonstrated by the modified Bielschowsky and PAM stains, became undetectable when the sections were first treated with formic acid. Such treatment made the plaques immunoreactive with β protein antiserum. The findings suggested that cerebellar diffuse plaques and compact plaques consist mainly of an amyloid component, and are characteristic of ATD.

Key words: Alzheimer-type dementia – Senile plaques – β Protein – Formic acid treatment – Cerebellum

The abundant formation of senile plaques (SP) in the cerebral cortex is a characteristic pathological findings of Alzheimer-type dementia (ATD), but histopathological studies of cerebellar SP are rare [10, 11]. Research on β protein, one of the component of senile cerebral amyloid [3], has identified the nucleotide sequence and predicted amino acid sequence of a cDNA clone that encodes the precursor protein [4]. Antisera against synthetic β peptide recognize SP and amyloid angiopathy (AA) [1, 5, 9, 12, 19]. β Protein immunostain is useful for the detection of a variety of cerebral amyloid deposits [20].

Here, we have studied SP in the cerebellum, and divided them into two classes: cerebellar diffuse and compact plaques. The effects of formic acid pretreatment for silver stains were examined and discussed in relation to the amyloid component of cerebellar SP.

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Materials and methods

We examined brains obtained from autopsy of two kinds of subjects. The ATD group consisted of five subjects with senile dementia of the Alzheimer type (SDAT), who died at the ages of 75 to 83 years, and a 62-year-old patient with Alzheimer's disease (AD). The non-ATD group consisted of ten elderly subjects who died aged between 78 and 90 (mean, 83.4). None of our subjects had a family history of dementia. The cerebella from autopsied subjects were fixed in formalin and cut sagittally 2 cm lateral to the midline. The cerebellar hemisphere was embedded in paraffin. Deparaffinized serial sections, 6 μm-thick, were used.

A rabbit was immunized with a synthetic peptide consisting of residues 1–28 of β protein [4] to which keyhole limpet hemocyanin was coupled through the N-terminal cysteine [20]. The antiserum did not recognize Alzheimer's neurofibrillary tangles, Pick's bodies, or amyloid from either primary amyloidosis or familial amyloid polyneuropathy. The antiserum was absorbed first with human immunoglobulin G because of their partial cross-reactivity. Before the immunostaining, deparaffinized sections were treated with formic acid (99%) for 5 min at room temperature to enhance the immunostaining of β protein [5]. This step was omitted for some sections. Sections were then incubated with β protein antiserum (diluted 1:2,000), stained by the avidin-biotin peroxidase complex (ABC) method (Vector Lab., USA), and counterstained with hematoxylin. Negative controls included sections incubated with normal rabbit serum or β protein antiserum completely saturated with synthetic β peptide.

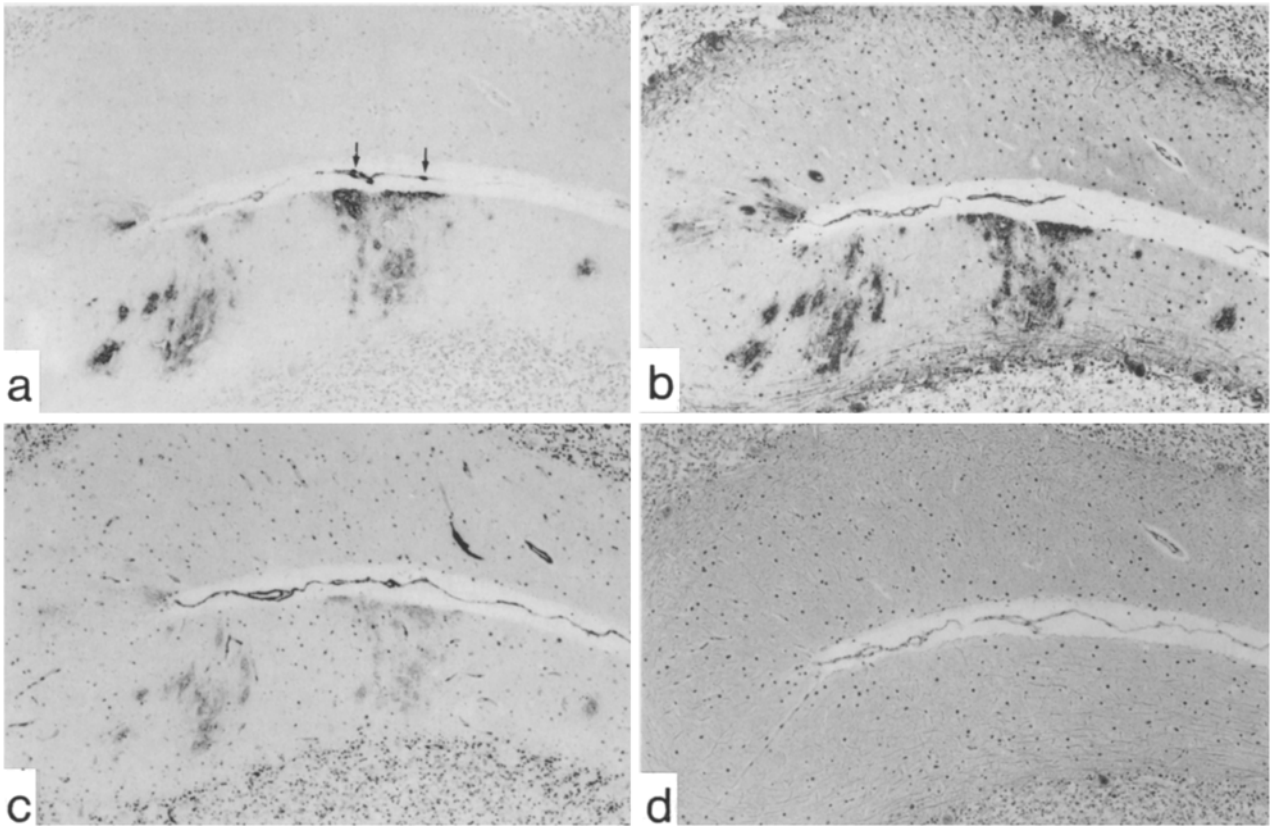

Fig. 1. Serial sections stained with β protein immunostain (**a**), modified Bielschowsky stain (**b**), periodic acid-methenamine silver (PAM) stain (**c**), and Bodian stain (**d**), showing diffuse plaques as polymorphous areas in the molecular layer. *Arrows* indicate amyloid angiopathy. Alzheimer's disease (AD), $\times 70$

Bodian staining was done with Albumosesilber (Merck, Art. 7447, FRG). Periodic acid-methenamine silver (PAM) staining (the same as Jones' method for kidney; [6]), and modified Bielschowsky staining [21] were also used. To study the effect of formic acid pretreatment on these three kinds of silver stains, sections were treated beforehand with formic acid (99%) for 5 min at room temperature. For the double staining, some sections were stained primarily with the usual Bodian stain, then treated with formic acid, and processed for the β protein immunostain.

For conventional histological examination, sections were stained with hematoxylin and eosin, Congo red, periodic acid-Schiff (PAS), or Klüver-Barrera stains.

Results

Distribution of SP in the non-ATD group

Neither SP nor AA was seen in any cerebellum from ten non-ATD subjects.

Distribution of SP in the ATD group

In the sections stained with β protein antiserum, the most prominent feature was an ill-defined polymorphous area of fine fibrillar materials in the molecular layers of the cerebellar hemisphere (Fig. 1a). This change exactly corresponded to the argyrophilic structure in the neighboring sections, which were stained with modified Bielschowsky and PAM stains (Fig. 1b, c). The change was not demonstrable by Bodian (Fig. 1d), Congo red, or PAS stains. The fibrillar materials were arranged vertically, parallel to Bergmann's fibers, or horizontally, like parallel fibers (Fig. 2); therefore, they seemed to be granular when an oblique section was observed (Figs. 1a and 3). This change, named "cerebellar diffuse plaques", was seen in four of the six subjects (Table 1). Most of the diffuse plaques had no apparent relationship to the capillaries, which were shown by the PAM stain (Fig. 2), although some had capillaries in their centers. An amyloid core was rarely seen in the diffuse plaques. The double stain of Bodian and β protein immunostain seemed not to be associated with neuritic abnormalities in the diffuse plaques (Fig. 3).

The second type of β protein-positive structure was compact plaques in the Purkinje cell or in the granular cell layers, seen in three of the six subjects (Table 1). The β protein immunostain showed wreath-like areolar deposits of amyloid surrounding the central core or conglomerate of amyloid (Fig. 4a–c).

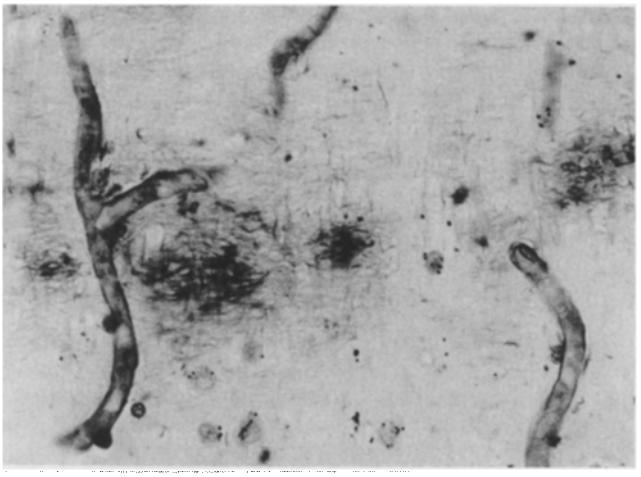

Fig. 2. Diffuse plaques of fibrillar materials arranged vertically or horizontally, with no apparent relationship to the capillaries. Senile dementia of the Alzheimer type (SDAT), PAM stain, ×330

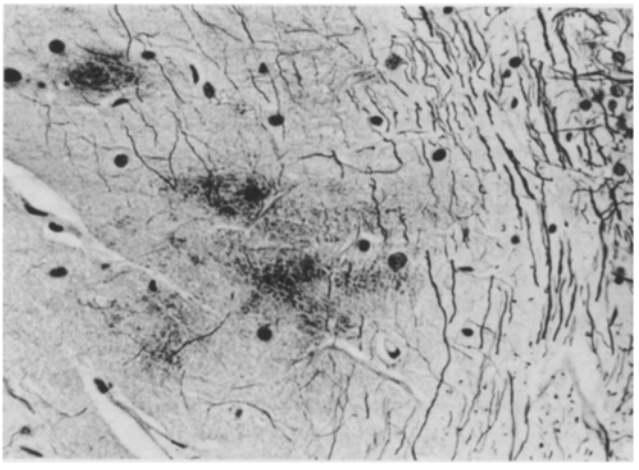

Fig. 3. Diffuse plaques in the molecular layer, not associated with neuritic abnormalities. AD, double stain of Bodian stain and β protein immunostain, ×280

The compact plaques were also seen by the modified Bielschowsky and PAM stains. However, they were shown as a spherical core or conglomerate of amyloid alone by the Congo red or PAS stains (Fig. 4d).

In the white matter, SP, consisting of meshy deposits of amyloid, were observed in two of the brain specimens (Fig. 5a). Only one brain had SP in the dentate nucleus (Fig. 5b). AA, clearly demonstrated with the β protein immunostain, was seen in four of the six subjects (Fig. 1a). One brain had neither SP nor AA. The characteristics of these two kinds of cerebellar SP are summarized in Table 2.

Effect of formic acid pretreatment

The argyrophilia of cerebellar diffuse and compact plaques, demonstrated by the modified Bielschowsky and PAM stains, completely disappeared after formic acid pretreatment (Fig. 6a, c). However, the axons in the modified Bielschowsky preparations remained

Table 1. Amyloid deposits in the cerebellum of subjects with Alzheimer-type dementia (ATD)

Case	Age	Class	Diffuse plaques	Compact plaques	Amyloid angiopathy
1	62	AD	+	−	−
2	75	SDAT	+	+	−
3	81	SDAT	+	+	+
4	81	SDAT	+	−	+
5	82	SDAT	−	+	+
6	83	SDAT	−	−	−
Total			4/6	3/6	3/6

AD: Alzheimer's disease; SDAT: senile dementia of the Alzheimer type

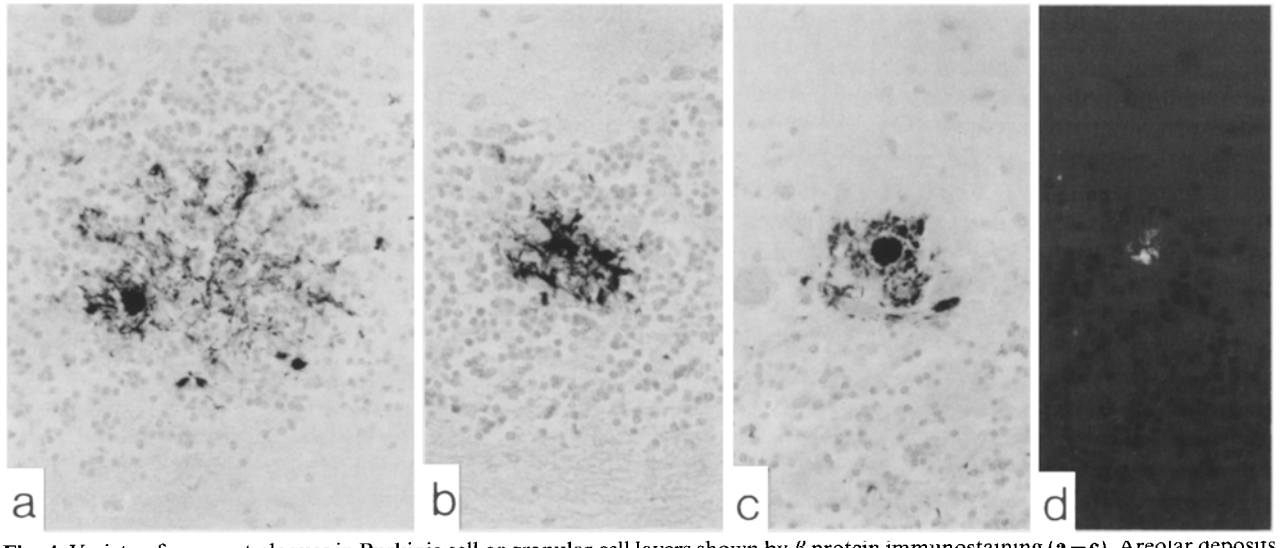

Fig. 4. Variety of compact plaques in Purkinje cell or granular cell layers shown by β protein immunostaining (**a**–**c**). Areolar deposits surrounding an amyloid core (**a, c**) were almost undetectable by Congo red stain (**d**). SDAT, ×280

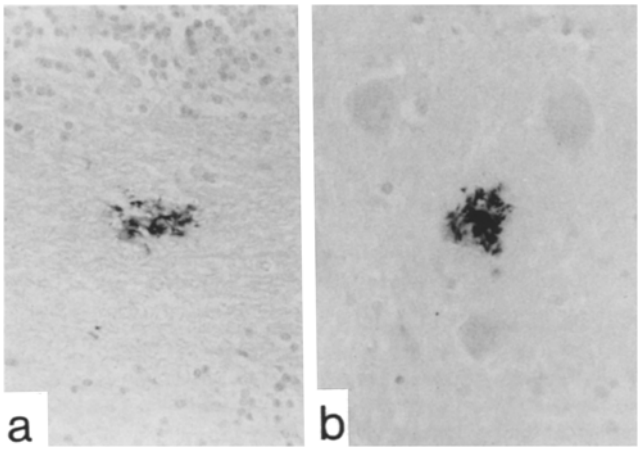

Fig. 5. Senile plaques in the white matter (**a**) and in the dentate nucleus (**b**). SDAT, β protein immunostain, $\times 280$

Table 2. Characteristics of the two major classes of senile plaques in the cerebellum

	Diffuse plaques	Compact plaques
Incidence		
ATD	4/6 Cases	3/6 Cases
non-ATD	0/10 Cases	0/10 Cases
Location	Molecular layer	Purkinje cell and granular cell layers
Shape	Polymorphous	Nearly spherical
Size	Variable	20~100 μm
Amyloid core	Rare	Frequent
Stainings		
β protein	(+)	(++) Core and crown
Bielschowsky	(+)	(++) Core and crown
PAM	(+)	(++) Core and crown
Bodian	(−)	(±)
Congo red	(−)	(+) Core alone
PAS	(−)	(+) Core alone

argyrophilic after pretreatment. The Congophilia of the amyloid core disappeared with pretreatment. In contrast, the β protein immunostaining was enhanced by the pretreatment, and cerebellar diffuse plaques became immunoreactive with β protein antiserum only after the pretreatment (Fig. 6b, d).

Discussion

Using β protein immunostaining and silver impregnation methods, we recently divided cerebral SP into four classes: diffuse plaques, consisting of fine fibrillar amyloid; primitive plaques, which had conglomerate of amyloid and swollen neurites; classic plaques, with an amyloid "core" and a "crown" made of swollen neurites and fibrillar amyloid, and compact (burned-out) plaques, with an amyloid core alone [20]. Here, we showed that the SP appearing in the cerebellum were of two major classes: diffuse and compact plaques. They differed from each other in many points (Table 2), suggesting that the morphology of the SP depended mostly on the location. The structure of the cerebellar diffuse plaques resembled that of the cerebral diffuse plaques except that the accumulated fine fibrillar amyloid was arranged vertically or horizontally in the cerebellum, but was arranged in various directions in the cerebrum. The cerebellar compact plaques usually consisted of a core and a crown, resembling the cerebral classic plaques, although there were no swollen neurites in the crowns of the cerebellar compact plaques.

It is generally accepted that SP rarely affect the cerebellum in ATD [14], although in a few special conditions, such as primary idiopathic cerebrovascular amyloidosis in children [13], there are abundant amyloid-rich plaques in the cerebellum. Pro et al. [11] examined the cerebella of 24 subjects with ATD, and showed amyloid plaques (similar to our compact plaques) in seven of the ten subjects with AD, but in none of the 11 subjects with SDAT (age at onset recorded in only 21 subjects). Because five of these seven subjects with AD had a family history of the disease, the authors suggested that amyloid plaques are a characteristic of familial AD. Morioka [10], however, found cerebellar SP, most of which were compact plaques, in all five subjects with non-familial AD, in six of the 13 subjects with SDAT, and in one of the nine none-SDAT subjects. The incidence of our compact plaques was similar. These studies failed to demonstrate another class of cerebellar SP, diffuse plaques, because Congo red, PAS, and Holmes silver stains were used [11], or else Congo red, PAS, and Bodian stains [10]. Here, we showed that the cerebellum was frequently affected with SP (5/6, 83%) in ATD, when examination involved sensitive methods: modified Bielschowsky stain, PAM stain, and β protein immunostain. The PAM stain is as sensitive as the Bielschowsky stain for the detection of cerebral SP [7].

Diffuse plaques were noted by earlier observers. Uyematsu [16] demonstrated a "diffuse form without nuclear-like central mass" in the molecular layer of the cerebellum using the Levaditi silver impregnation method. Tsujiyama [15] found diffuse plaques in two of the three cerebella from subjects with SDAT, and reported that the diffuse plaques might correspond to the "Filzwerk" described by Creutzfeldt and Metz [2]. These findings, however, drew little attention, because diffuse plaques were not readily visible by the Bodian stain, one of the most widely-used silver impregnation methods.

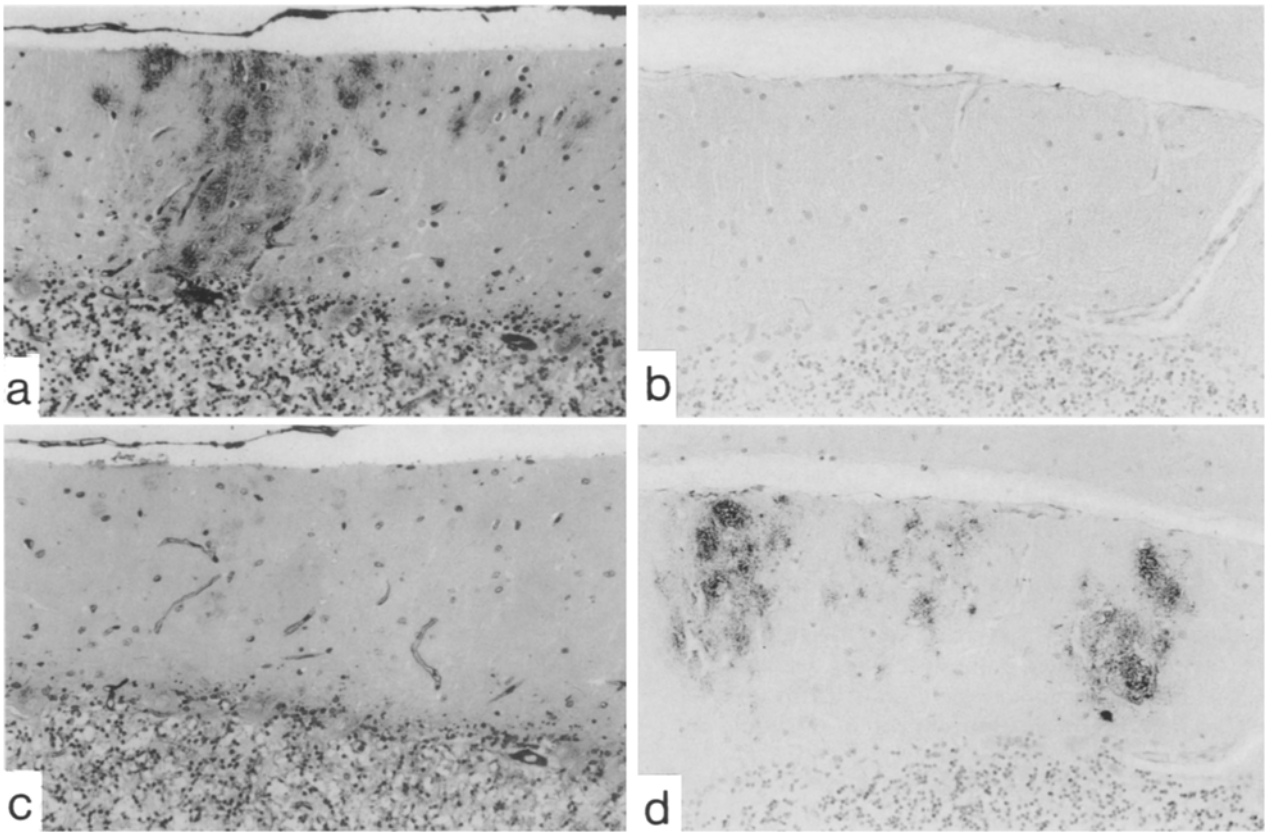

Fig. 6. Paired sections stained with PAM stain (**a, c**) and β protein immunostain (**b, d**); without (**a, b**) and with (**c, d**) formic acid pretreatment. The diffuse plaques lost their argyrophilia by pretreatment (**a, c**) and simultaneously developed immunoreactivity to β protein antiserum (**b, d**). AD, $\times 140$

Masters et al. [8] found that formic acid solubilized the plaque core amyloid. Kitamoto et al. [5] reported that formic acid pretreatment resulted in enhanced β protein immunoreactivity of the cerebral amyloid in spite of the loss of Congophilia. Formic acid pretreatment may destroy the β-pleated sheet structure of amyloid, uncovering the buried epitopes of the β protein, and thus enhance the β protein immunoreactivity of the amyloid. These structural changes in amyloid chains may also be responsible for the loss of argyrophilia of the cerebellar diffuse and compact plaques. The findings show that the amyloid component of the SP have argyrophilia in the sections stained by the modified Bielschowsky and the PAM methods, and that the cerebellar diffuse and compact plaques consist mainly of amyloid components, not neuritic components.

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