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Subtypes and differential laminar distributions of βA4 deposits in Alzheimer's disease: relationship with the intellectual status of 26 cases*

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Summary. βA4 immunoreactivity was studied in temporal neocortex, area 22, of 26 cases with graded intellectual status. Sampling was performed in psychometrically assessed women over 75 years, either intellectually normal or affected by senile dementia of Alzheimer type of various degrees of severity. βA4 antibodies labelled various types of βA4 deposits in 22/26 cases: (1) small, stellate deposits; (2) diffuse deposits, (3) primitive, (4) classic and (5) compact, or burn-out, plaques. The densities of the stellate deposits, primitive and classic plaques were always positively linked with the severity of the intellectual status, whereas those of the diffuse deposits were not. This was due to a single case with normal mental status and numerous βA4 deposits. Densities of stellate and diffuse deposits were higher in layers I, III and IV, whereas densities of primitive, classic, and neuritic plaques observed with Bodian's technique were higher in layers II and III. Topographical distribution of each subtype did not vary as a function of the severity of the intellectual status. These data suggest that deposits of βA4 protein appear a necessary but not a sufficient condition for inducing neuritic plaque formation, in the neocortex as in other brain areas. βA4 proteins could accumulate either as diffuse deposits, which do not cause an intellectual deficit, or as dense deposits, associated with argyrophilic neurites, i.e., classic neuritic plaques, highly correlated to the intellectual impairment. This evolution could depend on factors which are laminarily distributed in the neocortex.

Key words: Senile dementia of Alzheimer type – βA4 immunocytochemistry – Neurofibrillary degeneration – Morphometry

Various subtypes of lesions can be distinguished using βA4 immunocytochemistry in aging and Alzheimer's disease (AD) (Table 1). The stellate and the diffuse βA4 deposits are not stained by conventional methods [20, 28, 36]. Diffuse deposits [41], also called pre-amyloid [36] or pre-plaques [20], contain only rare bundles of amyloid fibrils at the ultrastructural level [42]. Primitive, classic and compact plaques are made of amyloid fibrils stained by Congo red and thioflavin S; contrarily to diffuse deposits, they are frequently associated with abnormal neurites [7, 39], reactive microglia [21, 22], reactive astrocytes [3, 38] and are immunoreactive to antibodies raised against various sequences of the amyloid precursor protein, other than the βA4 sequence [1, 22]. These diffuse deposits are supposed to occur early, before the appearance of amyloid fibrils [26, 32, 36]. Whether the diffuse deposits evolve directly into other types of βA4 deposits and neuritic plaques is currently uncertain.

To address this question, the relationship between the intellectual status and the various subtypes of βA4 deposits, their density and their laminar distribution in the neocortex, was studied in a series of 26 cases from the Charles Foix Prospective Study. This study involved women over 75 years of age, either intellectually normal or affected by senile dementia of Alzheimer type (SDAT) of various degrees of severity. Intellectual status was assessed by the test score of Blessed et al. [4]. βA4 antibodies and Bodian's method were used to stain contiguous sections of the temporal neocortex. Results suggested that βA4 deposits was a necessary but not a sufficient condition for the formation of neuritic plaques. βA4 accumulation may evolve into the diffuse form, not related to the intellectual status, and into dense deposits usually associated with argyrophilic neurites, the density of which is related to the severity of the intellectual impairment. The evolution into diffuse or dense deposits seem to depend on laminarily distributed factors.

Patients and methods

Patients

Twenty-six brains were collected from the Charles Foix Prospective Study. This prospective study involved 136 woman over 75 years of age living in the same long-term care unit. Clinical criteria were chosen so as to obtain all possible degrees of intellectual deterioration and to discard all known causes of dementia other than SDAT and every possible disorders interfering with psychometric

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Table 1. Classification of βA4 deposits in five morphological subtypes

Subtypes of βA4 deposits [references]	Diameter (μm)	Central core	Morphological characteristics	Density of labelling	Congo red	Degenerating neurites
Stellate deposits [9, 20, 28, 40]	2– 20	–	Small granules with fibrillary process (Fig. 1a)	Strong	–	–
Diffuse deposits, (or preamyloid or preplaques) [9, 20, 28, 36, 38, 40]	10–200	–	Weakly demarcated, irregularly shaped (Fig. 1b)	Weak	–	–
Primitive plaques [20, 37]	20– 60	–	Round shaped well demarcated (Fig. 1c)	Moderate	+	+
Classic plaques [20, 28, 37]	20– 60	+	With a βA4 crown (Fig. 1d)	Strong	+	+
Compact plaques (or burned out) [20, 28, 37]	5– 15	+	Without βA4 crown (Fig. 1e)	Strong	+	–

assessment. Seventy-seven patients were excluded because of cerebrovascular disease, Parkinson's disease, alcoholism, chronic psychiatric syndromes, blindness, deafness, or other disabling disease [12]. The included patients were considered intellectually normal or affected by SDAT. Their intellectual status was evaluated by the Blessed test score (BTS) [4]. The lowest values of BTS indicate the most severe cognitive impairment. Thirty-seven patients died. Eigth patients are still under study. Macroscopic and microscopic examinations excluded two cases with small infarcts and one case with cirrhosis. Twenty-six patients were investigated in this study. Age at death varied from 71 to 99 years (mean: 88 years). Postmortem delays ranged from 2 to 57 h (mean: 25 h) (Table 2).

Sampling

One hemisphere (randomly left or right) was formalin-fixed; the other was frozen at −80° C for biochemical analyses. The topography of the samples was recognized before sectioning and the samples were labelled at the surface of the brain. The hemisphere was then sectioned coronally. In all cases, sections from the hippocampus, the nucleus basalis of Meynert, the cerebellum and the frontal, temporal, parietal and occipital neocortices were examined. The present investigation was performed on formalin-fixed samples of the first temporal gyrus (area 22, just under the precentral gyrus). Contiguous 7-μm-thick paraffin sections were obtained.

Staining

Immunocytochemistry was performed with a rabbit antiserum raised against a synthetic peptide corresponding to the 42 residues [23] of the full length sequence of the βA4 protein (a gift of K. Beyreuther and C. Masters). Epitopes recognized by this βA4 antibody are located between residues 10 and 28 of the βA4 protein sequence [9]. This antibody has been shown to be specific in a series of studies [9, 32]. Sections were pretreated with a 80% formic acid solution and incubated for 16 h with 1/400 βA4 antibodies. Immunolabelling was revealed using a streptavidin-biotin peroxidase method and diaminobenzidine as chromogen [17]. Bodian's protargol (Roques, France) method, associated with luxol fast blue [14], was used to reveal the neuritic component of the senile plaques and the neurofibrillary tangles in contiguous sections of the same samples.

Classification of the lesions

The βA4 deposits were classified into five subtypes (Table 1, Fig. 1) according to morphological criteria adapted from Davies et al. [9], Ikeda et al. [20], Ogomori et al. [28], Tagliavini et al. [36], Yamaguchi et al. [40, 41], Wisniewski et al. [37, 38]: stellate deposits (Fig. 1B), diffuse deposits (Fig. 1A), primitive plaque (Fig. 1C), classic plaque (Fig. 1D), compact plaque (Fig. 1E). Neurofibrillary tangles (NFT) were not labelled by the βA4 antibody.

Plaques were called neuritic when they were made of abnormal argyrophilic processes, sometimes surrounding a pale-blue amyloid core, as seen with Bodian technique associated with luxol fast blue.

Morphometry

The density of lesions and their location in the thickness of the neocortex were evaluated for each case and both staining methods. Columns of contiguous fields (graticule of 143 μm × 215 μm, magnification of ×400) were assessed by one observer from the pial surface to the white matter perpendicularly to the gyrus axis. Columns were made of 9 to 14 fields. βA4 deposits, neuritic senile plaques (SP) and NFT were counted in ten adjacent columns. The classification of βA4 deposits into five morphological subtypes was assessed by examining at least two columns exhibiting at least 15 βA4 deposits. In case 8, six columns had to be screened. Results obtained after Bodian's method on 12 cases of this series have been previously published [13, 14, 24]. Counts of these 12 cases have been reassessed in order to use results obtained by only one observer. As far as the total or the diffuse βA4 deposits were concerned, the results fitted a Gaussian distribution only when 25 cases out of 26 were considered. They did not fit the Gaussian distribution when a non-demented patient (case 3) with a very high density of βA4 lesions was included. This case was considered to be singular and has been published as such [11]. Correlations were studied by Pearson's r coefficient. Coefficients were calculated with and without this particular case, as mentioned in the text.

The number of fields making up a column varied from case to case because the thickness of the neocortex was variable. It was necessary to standardize the data to compare the results. The densities of the five subtypes of βA4 deposits and of neuritic SP were expressed on a ten-level scale from the pial surface (level 1) to the white matter (level 10). The layer probability at each level of this scale had been previously estimated [13].

Results

Intellectual status (BTS) and density of βA4 deposits, neuritic SP and NFT

Four non-demented or mildly affected patients had no βA4 deposit in the temporal neocortex. βA4 deposits were very few in case 8. They were numerous in the other 21 cases (Table 2). Density of βA4 deposits was correlated neither with age at death ($r = 0.01$) nor with post-

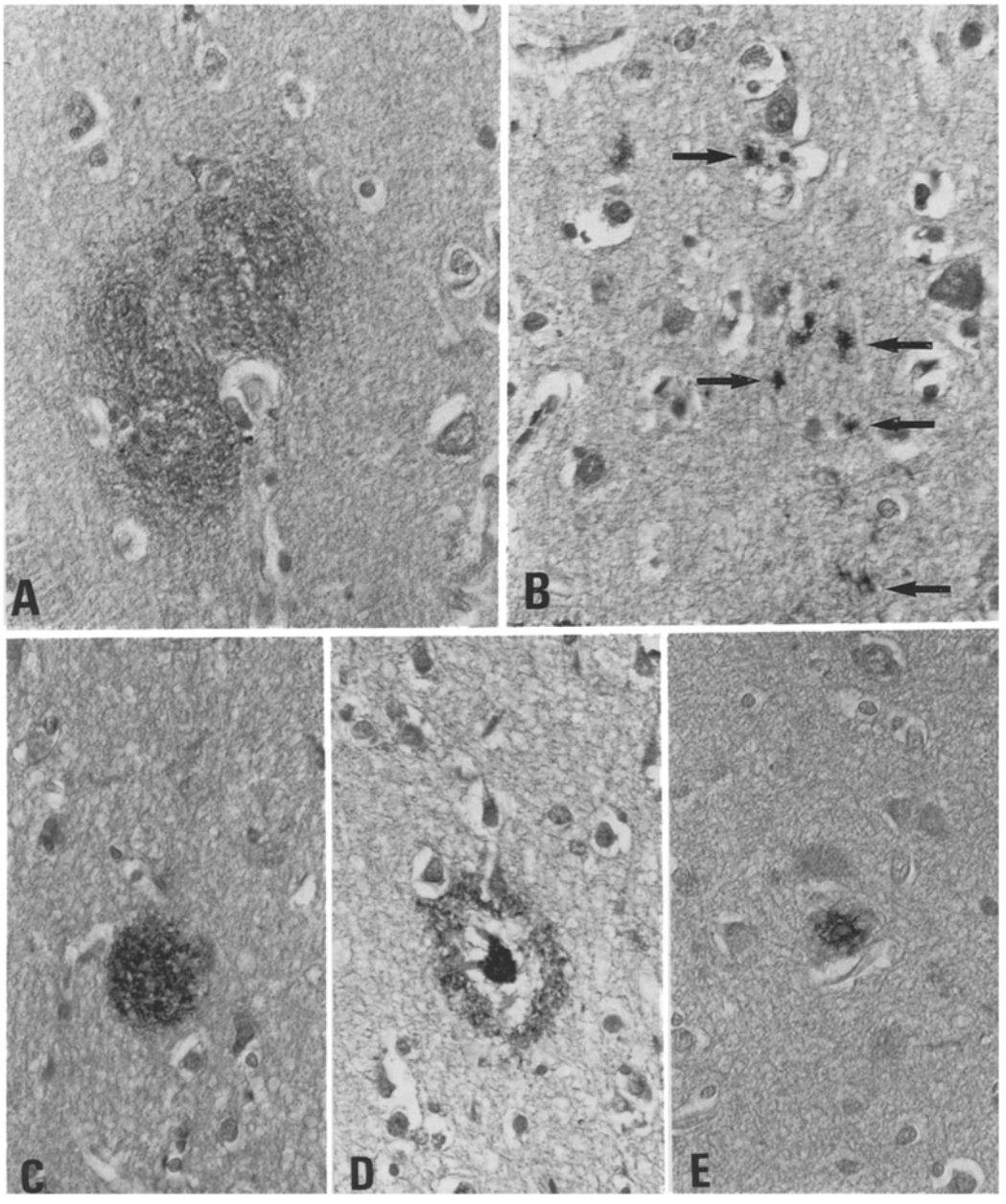

Fig. 1. A – E. βA4 immunoreactivity in senile dementia of Alzheimer type (SDAT) temporal neocortex shows various subtypes of βA4 deposits (see Table 1). **A** Diffuse deposits; **B** stellate deposits (*arrows*); **C** primitive plaques; **D** classic plaques; **E** compact plaques. ×250

mortem delay ($r = -0.21$). The scattergram of BTS and density of βA4 deposits (Fig. 2) showed that case 3 [11] lay outside the main group of the scatter points. When excluding case 3, correlation was significant at $P < 0.01$ (Table 3). BTS was significantly correlated with the density of neuritic SP and of NFT stained by the Bodian's method associated with luxol fast blue in all cases, included case 3 (Table 1). This correlation remained significant even when excluding the cases devoid of lesions stained by Bodian's method associated with luxol fast blue (SP: $n = 20$, $r = -0.45$, $P < 0.05$, and NFT: $n = 20$, $r = -0.61$, $P < 0.01$). This indicated that the significant correlation between the intellectual status and the density of the neuritic alterations was not a spurious effect of the statistical weight of the normal cases, and was valid even for AD cases of varying degrees of severity. Neuritic SP and NFT were never observed in the absence of the

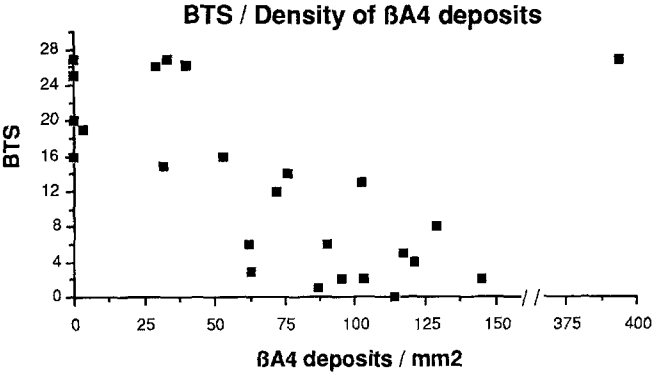

Fig. 2. Intellectual status (Blessed test score; BTS [4]) as a function of the density of the βA4 deposits; the lower the score, the more affected was the patient. βA4 deposits/mm^2: density of the βA4 deposits, whatever their subtype, assessed in ten neocortical columns (see Methods) in each case

Table 2. Intellectual status (Blessed test score; BTS), density of βA4 deposits, neuritic senile plaques and neurofibrillary tangles

Case no.	Age (years)	Brain weight[a] (g)	PMD[b] (h)	BTS[c]	Density (n/mm²)[d]		
					βA4 deposits	Neuritic SP	NFT
1	83	1050	9	27	0	0	0
2	85	1120	22	27	33	0	0
3[e]	88	1200	5	27	397	0	0
4	86	1190	45	26	29	2	0
5	94	1125	19	26	41	0.3	1
6	90	1175	26	25	0	0	0
7	86	1150	24	20	0	0	0
8	89	1160	14	19	3	0	0.8
9	88	1220	25	16	0	0	0
10	91	1120	23	16	53	1	4
11	99	1170	57	15	32	0.3	0
12	88	1170	12	14	76	2	2
13	97	1120	24	13	102	6	1
14	92	960	18	12	72	2	0
15	88	1150	57	8	129	25	57
16	89	1305	34	6	62	1	1
17	93	980	12	6	90	7	5
18	97	1100	7	5	117	12	54
19	82	1165	31	4	121	21	45
20	80	1130	42	3	63	20	45
21	75	825	24	2	95	22	44
22	89	1050	29	2	103	3	5
23	98	910	18	2	145	4	33
24	93	1130	5	1	87	2	16
25	93	1100	55	1	87	8	29
26	71	1050	8	0	114	6	35
r = (BTS/density)[f]							
$n = 26$ (with case 3)					nd[g]	−0.56[h]	−0.67[h]
$n = 25$ (without case 3)					−0.80[h]	−0.55[h]	−0.66[h]

[a] Brain weight: weight of brain before fixation
[b] PMD: post-mortem delay
[c] The lower the score, the more affected was the patient
[d] Number of lesions counted in ten neocortical columns; βA4 deposits: All deposits, whatever their subtype, immunostained by the βA4 antibody; neuritic SP: senile plaques stained by Bodian's silver method; NFT: neurofibrillary tangles stained by Bodian's silver method
[e] Case 3 has been separately published ([11], case no. 2944)
[f] r: Correlation coefficient of Pearson for the relationship between the intellectual status (BTS) and the density of the changes: βA4 deposits, neuritic plaques and neurofibrillary tangles
[g] nd: Not done since density of βA4 deposits of the series of 26 cases did not fit a Gaussian distribution: density of βA4 deposits was exceptionally high in case 3, a non demented patient
[h] $P < 0.01$.

βA4 deposits (Table 2) and were numerous only in the presence of a high density of βA4 deposits (Fig. 3). On the other hand, the highest number of βA4 deposits was found in the neocortex of case 3, in the absence of neuritic SP and NFT.

Intellectual status (BTS) and density of the morphological subtypes of βA4 deposits.

Diffuse deposits, primitive plaques and classic plaques were observed in the 22 cases with deposits, even in the case 8 which exhibited the lowest density of βA4 deposits.

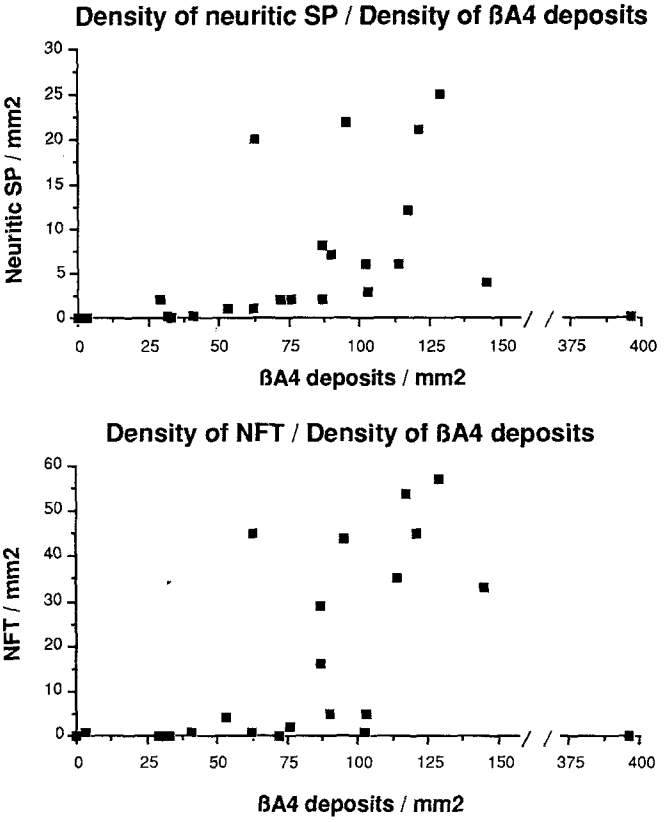

Fig. 3. Density of neuritic senile plaques (SP) and of neurofibrillary tangles (NFT) as a function of the density of the βA4 deposits. Neuritic SP/mm², NFT/mm²: density of SP and of NFT stained by the Bodian's method and assessed in ten neocortical columns. βA4 deposits/mm²: density of the βA4 deposits, whatever their subtype, assessed in ten neocortical columns

Stellate deposits were observed in 19 out of the 22 cases. Compact plaques were present in the assessed area of only 9 cases, and were rare (Table 3). BTS was always significantly correlated with the density of stellate deposits, primitive plaques and classic plaques (Table 3), and was significantly correlated with the density of the diffuse deposits after exclusion of case 3 (Table 3). The number of compact plaques was too low to look for a correlation.

The densities of the five subtypes were expressed as percentages of all the βA4 deposits in each case. The proportion of each subtype was not related to the BTS. When all cases were pooled, βA4 deposits were mostly made of diffuse deposits (43%; SEM, 3.4%), then in decreasing order: stellate deposits (26%; SEM, 3.2%), primitive plaques (18%; SEM, 1.7%), classic plaques (12%; SEM, 1.9%) and compact plaques (1%; SEM, 0.4%).

Laminar distribution of the various subtypes of βA4 deposits, the neuritic SP and the NFT

Since the mean location of stellate deposits, diffuse deposits, primitive plaques and classic plaques in the thickness of the neocortex was the same whatever the severity of the intellectual impairment (Fig. 4), results of the various cases were pooled. Compact plaques were too rare

Table 3. Intellectual status (BTS) and density of five morphological subtypes of βA4 deposits

Case no.	BTS	Number of βA4 deposits	Density (n/mm^2) of βA4 deposits[a]				
			Stellate deposits	Diffuse deposits	Primitive plaques	Classic plaques	Compact plaques
1	27	0					
2	27	21	0	29	3	2	0
3[a]	27	286	88	234	36	2	0
4	26	34	6	21	9	4	2
5	26	31	16	8	4	12	1
6	25	0					
7	20	0					
8[b]	19	17	4	3	0.5	0.5	0
9	16	0					
10	16	45	0	42	18	3	0
11	15	16	5	16	2	14	0
12	14	50	36	31	15	9	0
13	13	62	23	46	23	7	0
14	12	60	31	28	26	13	0
15	8	105	7	95	31	8	1
16	6	58	53	25	31	6	4
17	6	85	41	44	14	16	1
18	5	80	53	38	32	16	5
19	4	107	50	65	15	28	0
20	3	34	0	38	27	25	3
21	2	115	55	76	24	18	0
22	2	81	43	45	33	8	2
23	2	91	51	88	35	14	4
24	1	64	10	34	21	15	0
25	1	74	40	42	27	3	0
26	0	86	41	31	16	18	0
r = (BTS/density)							
n = 26 (with case 3)			−0.40[d]	nd[c]	−0.61[e]	−0.66[e]	nd[c]
n = 25 (without case 3)			−0.69[e]	−0.65[e]	−0.78[e]	−0.65[e]	nd[c]

[a] Density: distribution of βA4 deposits was assessed in two neocortical columns

[b] βA4 deposits were few in case 18: counts have been performed in six columns in order to have at least 15 βA4 deposits

[c] nd: Not done since compact plaques were too rare to be included in statistics and density of diffuse βA4 deposits of the series of 26 cases did not fit a Gaussian distribution: density of diffuse βA4 deposits was exceptionally high in case 3, a non-demented patient

[d] $P < 0.05$

[e] $P < 0.01$

to be taken into account. Any type of βA4 deposit could be observed in a given layer of the neocortex. Nevertheless, deposits were distributed differently within the depth of the neocortex according to their subtype (Fig. 5): densities of stellate deposits and diffuse deposits were the highest in layer I and in layers III and IV. Densities of primitive plaques and classic plaques were the highest in layers II and III. This last distribution was similar to the distribution of the neuritic SP.

Discussion

βA4 deposits can be observed in intellectually normal cases in the absence of any changes detectable by silver impregnation or tau immunocytochemistry. On the contrary, the demented cases exhibit both βA4 deposits and argyrophilic- or tau-positive neurites [27, 28, 36, 39]. In the present study, we have shown in addition that neuritic SP and NFT were never observed in the absence of βA4 deposits and that the density of neuritic SP and of NFT were noticeable only in those cases with a high density of βA4 deposits. This is also the case using anti-tau antibodies in the same series [10], confirming that βA4 deposits appear before any neuritic alteration as seen by these techniques [10, 15, 33, 34].

The densities of thioflavin S-stained SP, those of neuritic SP and of NFT, revealed either by silver methods or by anti-tau and anti-PHF antibodies, are significantly correlated with the intellectual status [4, 10, 14, 15, 24]. The present study also shows that the density of stellate βA4 deposits, primitive βA4 plaques and classic βA4 plaques was also significantly correlated with the severity of the intellectual impairment. Primitive βA4 plaques and classic βA4 plaques are indeed usually associated with neuritic alterations. When the relationship between the density of diffuse deposits and the neuritic plaques on one hand, and the intellectual status on the other hand, was studied, case 3 was an outlier: this case exhibited the highest density of βA4 deposits, mostly of the diffuse type, without any neuritic degeneration and NFT, as far as Bodian's method and anti-tau antibodies were concerned. This patient fulfilled all clinical and neuropathological criteria of a non-demented elderly person [11], suggesting that large amounts of βA4 deposits, particularly diffuse deposits, do not necessarily induce an intellectual impairment. Nevertheless, she could have been at a incipient stage of AD which might have led, by a continuous or remitting process, to the development of the full range of lesions and to the signs and symptoms of the disease.

A quantitative analysis carried out in the cerebral cortex of six Down's syndrome patients has shown that the diffuse or preamyloid βA4 deposits prevail over neuritic SP in young adult with this syndrome, whereas the proportion is reversed in the aged patients [18], suggesting that the diffuse deposits are the earliest lesions and evolve into neuritic SP in the course of the disease [18, 20, 26]. This was not the case in the present study, performed in SDAT. When the densities of the subtypes of βA4 deposits were expressed as percentages of all the deposits in each case, the proportion of each of the five subtypes were not related to the intellectual status.

Some preferential location of the different subtypes of βA4 deposits has been described in the cerebral cortex on qualitative grounds. For some authors [28], layer I of the temporal neocortex exhibits stellate deposits; layers III and IV show numerous stellate deposits, diffuse deposits and primitive plaques; and layers V and VI contain more numerous classic and compact plaques. In other studies, classic plaques are numerous in layers II and III of the occipital cortex [7], whereas diffuse deposits seem more numerous in layers V and VI of the frontal cortex [20] and of the occipital cortex [7]. These discrepancies could be due to regional variations. In our quantitative study performed in Brodmann's area 22, the finding of a

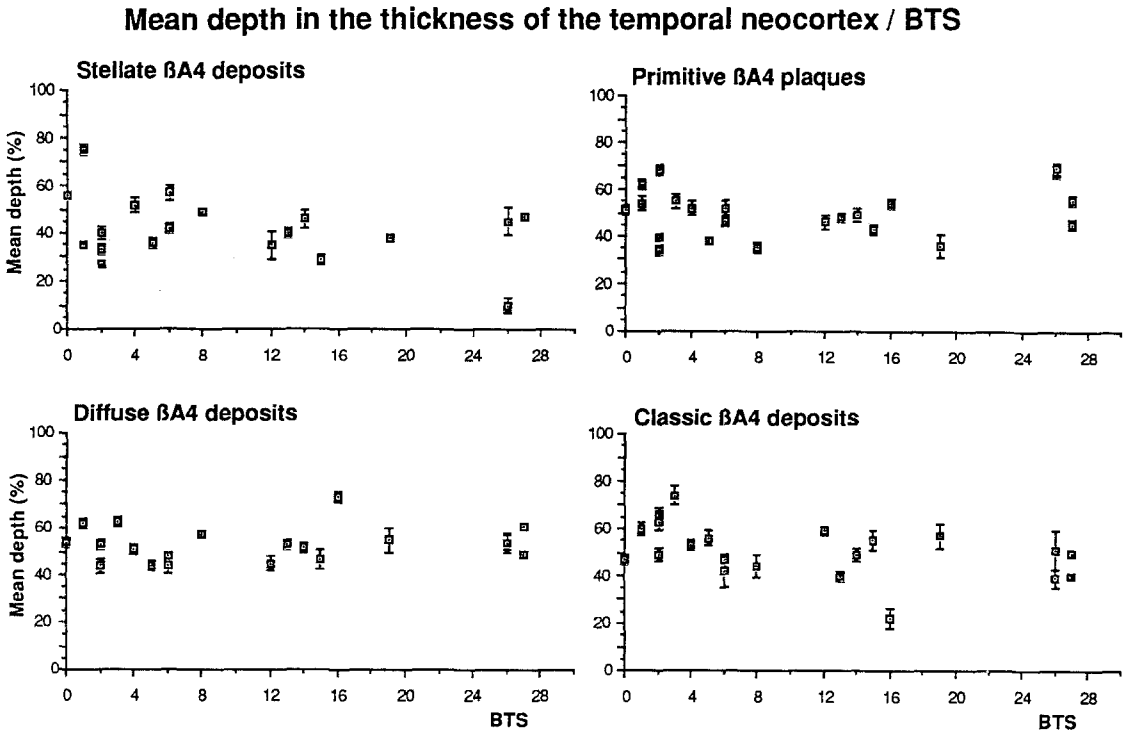

Fig. 4. Mean cortical depth of the various subtypes of βA4 deposits as a function of the intellectual status. Mean depth (%): mean depth of all the subtypes of βA4 deposits counted in each case, expressed as percentage of the whole thickness from the pial surface (1) to the white matter (100). Only the cases with > 2 SP on the neocortical sample which was examined are represented. Bars: $\pm$ 1 (SEM)

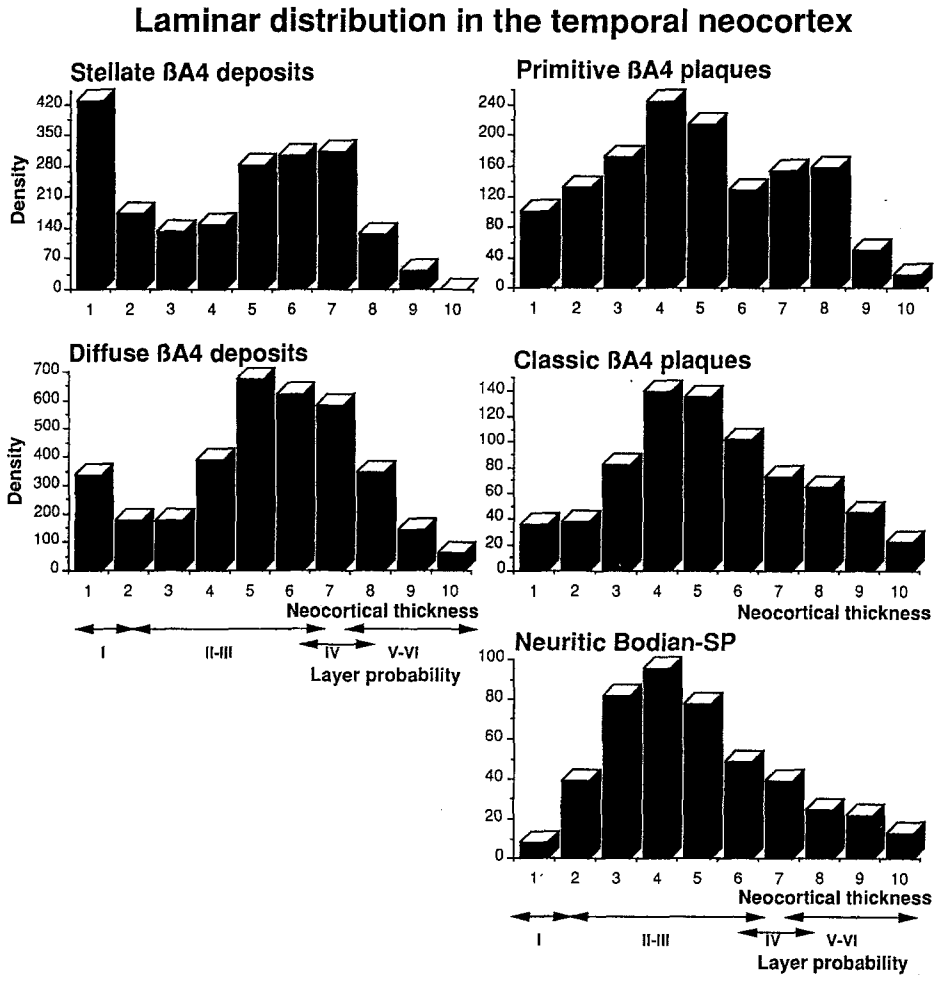

Fig. 5. Densities of subtypes of βA4 deposits, neuritic plaques and NFT as a function of their localization in the neocortical layers. Density: change densities (expressed as number per 260 fields of 143 x 215 µm) of the 26 cases were pooled. Neocortical thickness: ten-level scale of cortical depth expressed as number of standard field from the pial surface (1) to the white matter (10). Layer probability: the probability of neocortical layer at each level of the scale had been previously estimated [13]

constant mean neocortical depth of βA4 subtypes showed that the laminar distribution of the changes was the same whatever the severity of the intellectual impairment. Stellate and diffuse deposits predominated in the sub-pial layer (layer I) and in layers III and IV. Primitive and classic plaques were more numerous in layers II and III. Layers V and VI were less affected. Distributions of primitive and classic plaques were the same as that of SP stained by silver methods [7, 13, 25, 30, 31], by anti-tau and anti-PHF antibodies and by thioflavin S [10]. This differs from the distribution of NFT, more numerous in layers III and V [10, 25, 30]. The significance of the stellate deposits is not clear. Laminar distribution of stellate deposits does not support the suggestion that this subtype is produced by "grazing sections through the outermost edges" of classic or primitives plaques [20]. The emerging point was that neuritic SP did not predominate in those layers where diffuse and stellate deposits were prominent, whatever the severity of the intellectual deficit.

The relationship between the βA4 deposits and the neuritic component of SP does not seem to be direct: accumulation of βA4 protein appears to be a necessary but not sufficient condition for the formation of SP. βA4 deposits are associated with degenerating neurites shown by silver impregnation and tau immunocytochemistry only in some specific regions, such as the hippocampus or the neocortex, whereas they are devoid of argyrophilic- or tau-positive in the cerebellar cortex [5, 8, 22, 35, 41] and seldom in the striatum [6], suggesting that formation of degenerating neurites depends on regional [8, 38] or individual "susceptibility" to βA4 deposits [2]. Possible regulating factors have been suggested: impaired circulating monocytes/macrophages, microglial cell [19, 21, 22] and perhaps astrocytes [3, 19, 35], defect involving one or more steps of the posttranslational modification of the amyloid precursor protein [1, 22, 29].

In conclusion, large amounts of diffuse βA4 deposits do not necessarily induce an intellectual impairment. However, the density of such deposits is usually linked to the mental status. This is always the case for stellate deposits and for neuritic plaques, as well as for NFT. βA4 immunochemistry can not be used alone for the diagnosis of AD. A European multicenter study showed that the density of various types of lesions varied mainly with the staining method [16]. For diagnostic purpose, techniques for amyloid should be associated with techniques for neurofibrillary degeneration. The present results demonstrated that (1) the neocortical distribution of stellate and diffuse deposits and that of primitive and classic plaques did not vary during the course of SDAT; (2) these distributions were laminar; and (3) the neocortical distribution of diffuse and stellate βA4 deposits did not match that of amyloid deposits usually associated with abnormal argyrophilic neurites, i.e., classic and primitive βA4 plaques. These data suggest that accumulation of βA4 protein into diffuse deposits or into classic neuritic plaques depend on factors which are laminarly distributed in the neocortex.

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Note added in proof

Differential laminar distributions of A4 deposits has been also suggested by a previous study using computer imaging in the prefrontal cortex of four AD cases [43]. The authors found a predominance of the punctate (stellate) and the macular (primitive and diffuse) configurations in layer I. They did not find the pic of density in layer III for the ring and ring-with-core (classic plaques) configurations. Regional variations (temporal vs prefrontal cortices) as well as methodological differences could explain the discrepancies.