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Comparison of β -protein/A4 deposits and Alz-50-stained cytoskeletal changes in the hypothalamus and adjoining areas of Alzheimer's disease patients: amorphic plaques and cytoskeletal changes occur independently

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Abstract Alzheimer's disease is characterized neuropathologically by senile plaques and cytoskeletal changes. It has been proposed that amorphic plaques would locally induce anterograde propagation of cytoskeletal changes in consecutive neurons followed by amorphic plaque deposition at their axonal terminals. The Alzheimer changes would spread in this way along neural pathways. To test the 'primary amyloid anatomical cascade hypothesis', Congo red staining, β -protein/A4 (A β) antiserum and Alz-50, which recognizes cytoskeletal changes, were applied to the hypothalamus and adjoining brain areas of five Alzheimer's disease patients of 40–90 years of age and five age- and sex-matched controls. The results showed that (1) virtually all A β plaques in the hypothalamus were of the Congo red-negative amorphic type; (2) amorphic plaques and Alz-50-stained cytoskeletal changes were observed not only in all Alzheimer's disease patients but also in a non-demented, 90-year-old control subject; (3) the density of amorphic plaques in the hypothalamus was unrelated to the duration of the dementia; (4) the density of amorphic plaques was unrelated to that of Alz-50-stained cytoskeletal changes; (5) double-labeling with anti-A β and Alz-50 did not show an evident topical relationship between amorphic plaque deposition and the occurrence of cytoskeletal changes; and (6) the distribution of A β and Alz-50 staining in five brain areas, for which

essential anatomical information is available, did not support the primary amyloid anatomical cascade hypothesis. Amorphic plaques and cytoskeletal changes rather occur independently.

Key words Alzheimer's disease · Alz-50 · β -protein · Cytoskeletal changes · Human hypothalamus

Introduction

β -protein [10] or A4-protein fibril (A β) deposition [19] in the neuropil has often been thought to be the primary and crucial event in the pathogenesis of Alzheimer's disease (AD). One of the hypotheses based on this idea is the 'primary amyloid anatomical cascade hypothesis' put forward by Hardy in 1992 [11]. This hypothesis states that amorphic plaques locally induce neurons to the development and anterograde propagation of cytoskeletal changes that accumulate in perikarya and dystrophic neurites. Subsequently, amorphic plaques would be formed at their axonal terminals. Amorphic plaques stain with A β antibodies, but they do not have a congophilic core nor a corona of silver-stained dystrophic neurites as observed in neuritic and classic senile plaques (SPs) [25, 35]. Dystrophic neurites are short, thickened, curly or kinky processes – termed 'neuropil threads' if they are localized in the neuropil outside SPs [7] –, which are visualized by means of sensitive silver stainings [7] or antibodies such as anti-tau Alz-50 [17, 41].

The hypothalamus in AD patients is a suitable brain structure to test the 'primary amyloid anatomical cascade hypothesis'. Firstly, the hypothalamus affected by AD was reported to contain many A β -positive Congo red-negative amorphic plaques that did not contain epitopes corresponding to regions of the β -amyloid precursor protein other than A β [31]. Secondly, in the hypothalamus of AD patients the few neurofibrillary tangles (NFTs) and neuritic and classic SPs observed with conventional silver stainings [13, 14, 26, 28] are outnumbered by Alz-50-stained cytoskeletal changes in perikarya and dystrophic

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neurites [37]. Thirdly, various hypothalamic and adjoining nuclei appeared to react in very different ways during the course of AD, ranging from a total lack of staining with Alz-50 at one end to a high density of Alz-50-stained cytoskeletal changes at the other end of the spectrum [37].

The aim of the present study was to test the 'primary amyloid anatomical cascade hypothesis' by comparison of the density and distribution of amorphous plaques and Alz-50-stained cytoskeletal changes in various hypothalamic and adjoining nuclei in AD patients.

Materials and methods

Subjects and tissues

For this study the hypothalamus from five AD and five non-demented, age- and sex-matched control patients was used. The clinicopathological information on these patients is given in Table 1. The demented patients had been thoroughly examined in a clinical setting and were scored 6–7 for severity of dementia according to the Global Deterioration Scale (maximum score: 7) [23]. The controls had no primary neurological or psychiatric disorder. The clinical diagnosis of AD was confirmed neuropathologically on the basis of the presence of many SPs, NFTs and dystrophic neurites in Bodian- and Congo red-stained sections from the hippocampus and cortex (frontal area 10 or 11; temporal area 22 or the temporal pole; parietal area 7 or 40; occipital area 17 and 18). To exclude Parkinson changes the substantia nigra was also examined. Notably, the oldest control patient (no. 5) was clinically non-demented and neuropathologically not diagnosed as having AD since the presence of dystrophic neurites, NFTs and SPs were largely restricted to the hippocampus and temporal cortex and rarely seen in the parietal, frontal and occipital cortex.

The formalin-fixed, paraffin-embedded hypothalami was cut coronally into 6- μ m-thick sections, which were stored at room temperature. Every 50th section was stained with thionin (0.1% thionin in acetate, pH 4) for general orientation. In addition, the suprachiasmatic nucleus (SCN) was visualized using anti-vasopressin [33] since this brain structure cannot be recognized in 6- μ m-thin sections stained with thionin. Schematic drawings of the chiasmatic and tuberal region of the human hypothalamus in adults are given in Fig. 1.

Congo red staining and antibodies used

Congo red was used to detect apple-green birefringence to visualize β -amyloid cores (i.e., amyloid and classic SPs).

The following antibodies were used: 1G102 (Dako), directed against amino acid sequence 8–17 of the A β peptide (for information on the staining specificity see [2]); SP28 (generously obtained from Dr. B. Frangione, N.Y.) raised against amino acid sequence 1–28 of the A β peptide and recognizing amino acids 20–28 (for information of the staining specificity see [8]) – double staining with rabbit antiserum SP28 and mouse monoclonal Alz-50 was easier to perform than that with mouse monoclonal A β antibody 1G102 – ; and monoclonal Alz-50 antibody (P-42, kindly donated by Abbott Laboratories, Chicago, Ill.), a mouse IgG clone isotype from the original IgM monoclonal produced by Wolozin et al. [41]. Alz-50 (IgG) had the same staining characteristics as the IgM clone [38, 39].

Staining protocols

Antibody 1G102 was used exclusively for single staining; SP28 was used for single staining and for double-labeling with Alz-50. Before staining with SP28 or 1G102, sections were pretreated with 98–100% formic acid (Merck) for 20 min followed by five rinses in tap water for 10 min to improve A β immunoreactivity [15]. Staining with SP28 (1:200) was visualized using the peroxidase-antiperoxidase (PAP) technique [32]. Staining with 1G102 was visualized using a two-step peroxidase method using rabbit anti-mouse immunoglobulins (Dako, P260). Alz-50 single staining (1:100) was performed as for A β antibody 1G102 without formic acid pretreatment (see [37]). Single staining with SP28, 1G102 or Alz-50 was carried out with 0.05% mg/ml 3,3'-diaminobenzidine tetrahydrochloride (DAB; Merck) or DAB enhanced with 0.25% nickel ammonium sulphate (DAB-Ni), which gave a black precipitate. Selected sections were double-labeled with SP28 and Alz-50. Following formic acid pretreatment, SP28 (dilution 1:200) was used in the first sequence and Alz-50 (1:75) in the second. A β staining was visualized with DAB only, while Alz-50 staining was achieved with DAB-Ni. In between the staining sequences, sections were incubated with methanol-hydrogen peroxide series according to Axelsson and Van Leeuwen [3] to block residual horseradish peroxidase (HRP) activity of the first staining sequence. Following this step the yellow-brown staining of the SPs did not change to black, proving that no HRP activity was left.

Results

For specimens that were formalin-fixed for a period of up to 119 days (patient 7) or were obtained after a post-mortem delay up to 24 h (patient 8), an intense A β and Alz-50 staining was seen in the hypothalamus of AD pa-

Table 1 Clinico-pathological information

	Age (y)	Demen dur(y)	Sex (M/F)	Brain wght(g)	Delay (h)	Fix dur(d)	Diagnosis, causes of death
Controls:							
1	37		M	1510	48	46	Alcohol & drugs abuse
2	43		M	1260	23	53	Non-Hodgkin lymphoma
3	57		F	1220	45	35	Mitral stenosis
4	72		F	1200	21	53	Endocarditis lenta
5	90		F	1110	13	48	Post-operative infections
AD Patients:							
6	40	5–6	M	1370	3	28	AD, cachexia
7	45	10	M	1130	4	119	Familial AD, cachexia
8	56	4–5	F	1180	24	48	AD, bronchopneumonia
9	70	12	F	780	14	34	AD, epileptic insults
10	90	8	F	1060	3	38	AD, dehydration

AD: Alzheimer's disease; fix dur(d): fixation duration (in days); delay (h): post-mortem delay (in hours); M: male; F: female; demen dur(y): dementia duration (in years); brain wght(g): brain weight (in grams)

Fig. 1 Chiasmatic and tuberal region of the hypothalamus in the human adult (based upon [6], with kind permission from Elsevier Science). The diagrams show the main landmarks and nuclear gray areas that are encountered as coronal sections are traced antero-posteriorly. The distance between sections is 800 μ m (*AC* anterior commissure, *AN* accessory neurosecretory nucleus, *CM* corpus mamillare, *CU* cuneate nucleus, *DBB* nucleus of the diagonal band (of Broca), *DMH* dorsomedial nucleus, *FM* fasciculus mamillo-thalamica, *FO* fornix, *CG* chiasmatic gray, *TG* tuberal gray, *NTI* infundibular nucleus, *SDN* sexually dimorphic nucleus, *OC* optic chiasm, *OT* optic tract, *PVA* periventricular nucleus, *PH* posterior hypothalamic nucleus, *PM* postero-medial nucleus, *PVN* paraventricular nucleus, *RE* retrochiasmatic nucleus, *SCN* suprachiasmatic nucleus, *SON* supraoptic nucleus, *BST* nucleus of the stria terminalis, *SU* subthalamic nucleus, *TH* thalamus, *NTL* lateral tuberal nucleus, *TMN* tuberomammillary nucleus, *UN* uncinate nucleus, *VMH* ventromedial nucleus)

tients and controls. Controls 1 and 3 with longer post-mortem delays, 48 and 45 h, respectively, did not show A β immunoreactivity. The negative A β staining in these subjects was not due to the effect of postmortem decom-

position since we obtained a good A β staining in the hypothalamus of a 61-year-old AD patient with a post-mortem delay of 84 h.

Morphology of the A β staining

Three types of A β deposits were distinguished. In the first, reactive vessels were found only in the nucleus accumbens (ACC) and the central nucleus of the bed nucleus of the stria terminalis (BSTc) of AD patient 7, and in the tubero-mamillary nucleus (TMN) of AD patient 9. These A β deposits showed a apple-green birefringence with Congo red, representing congophilic angiopathy. In the second, congophilic SP staining was so rare that they could not be scored, but the brain regions containing such SPs are indicated with an asterisk in Table 2. In the third type, most of the A β immunoreactivity was found in A β -positive, Congo red-negative amorphous plaques. The following data concern this type of plaque.

Table 2 Alzheimer's disease staining pattern of A β deposits

ACC nucleus accumbens, BST bed nucleus of the stria terminalis, BSTc central sector of the bed nucleus, BSTl lateral sector of the bed nucleus, BSTm medial sector of the bed nucleus, CG chiasmatic gray, CI Calleja islands (in the nucleus accumbens), DBB diagonal band of Broca, DMH dorsomedial hypothalamic nucleus, NBM nucleus basalis of Meynert, NTI tubero-infundibular nucleus, NTL lateral tuberal nucleus, PVN paraventricular nucleus, SCN suprachiasmatic nucleus, SDS sexually dimorphic nucleus, SON supraoptic nucleus, TG tuberal gray, TMN tuberomammillary nucleus, VMH ventromedial hypothalamic nucleus

*: Congophilic cores were present but their amount was too low to score.
 NA: not available; -: not or negligible staining; $\pm$: few; +: small amount; ++: considerable amount; +++: large amount

	Controls					Alzheimer's disease patients				
	1	2	3	4	5	6	7	8	9	10
Chiasmatic region of the hypothalamus										
SON	-	-	-	-	-	-	-	-	-	-
CG	-	-	-	-	++*	+	+	++	++	++*
SDN	-	-	-	-	+	-	$\pm$	$\pm$	$\pm$	$\pm$
SCN	-	-	-	-	-	-	-	-	-	-
PVN	-	-	-	-	-	-	-	-	-	-
PVA	-	-	-	-	-	-	-	-	-	-
ME	-	NA	-	-	-	-	-	-	NA	-
Tuberal region of the hypothalamus										
DMH	-	-	-	-	$\pm$	$\pm$	$\pm$	$\pm$	-	$\pm$
VMH	-	-	-	-	+	+	++	++	+	++
TG	-	-	-	-	++	+	+	+++	+	+
TMN	-	-	-	-	+	+++*	++	+	$\pm$	+
NTL	-	-	-	-	$\pm$	+++*	++	$\pm$	$\pm$	+
NTI	-	-	-	-	-	$\pm$	$\pm$	-	-	$\pm$
Adjoining areas										
DBM	-	-	-	-	+	+	+	+	-	$\pm$
DBB	-	-	-	-	+	+	+	$\pm$	-	-
BSTc	-	-	-	-	$\pm$	+++*	++	+	$\pm$	$\pm$
BSTl	-	-	-	-	$\pm$ *	+++*	+++	++	$\pm$	$\pm$
BSTm	-	-	-	-	$\pm$	+	+	++	-	+
ACC	-	-	-	-	+	+++	+++	+++*	++	++
CI	-	-	-	-	+	++	++	+	+	+

Amorphous plaque staining (Table 2)

A β staining using SP28 or 1G102 was similar. Amorphous plaques were observed in all AD patients and in the oldest, 90-year-old control subject. Amorphous plaques were found in the chiasmatic gray (CG), sexually dimorphic nucleus (SDN), tuberal gray (TG), dorsomedial hypothalamic nucleus (DMH), ventromedial hypothalamic nucleus (VMH), tubero-infundibular nucleus (NTI), TMN and nucleus tuberalis lateralis (NTL) in the hypothalamus, and in all subnuclei of the bed nucleus of the stria terminalis (BST), the nucleus basalis of Meynert (NBM) and diagonal band of Broca (DBB) in the basal forebrain, the nucleus accumbens (ACC) and islands of Calleja (CI) in the ACC. The supraoptic nucleus (SON), SCN, paraventricular nucleus (PVN), periventricular area (PVA) and median eminence (ME) were devoid of A β staining. The same held true for white matter, i.e., the anterior commissure, internal capsule and fornix.

A β staining could be subdivided into four different types. In the first, in the chiasmatic region (CG, SDN), tuberal region (TG, DMH, VMH, NTI, TMN) and the lateral (BSTl) and medial subnucleus of the BST (BSTm), most amorphous plaques were well demarcated and intensively stained in the younger AD patients (nos. 6 and 7 aged 40 and 45 years, respectively). In the second type, the same areas often had vague borders and a fine granular or dust-like aspect in the three older AD patients (nos. 8–10) and in the non-demented, 90-year-old control (no. 5). In the third type of A β staining, the NTL and BSTc of

patients 8–10 and of control no. 5 showed no or only small amounts of amorphous plaques in the periphery of the nuclei. In the fourth type, the NTL and BSTc of AD patients 6 and 7 showed, in addition, many intensively stained amorphous plaques scattered throughout the nuclei (Fig. 2c, d).

In contrast to the presence of different types of amorphous plaque types in the hypothalamus and BST, amorphous plaque morphology was similar in the ventral striatum or basal forebrain of all AD patients, and of control no. 5. In addition, the ACC and CI showed considerable amounts of patchy or ill-bordered globular amorphous plaques. The ACC-CI region was, therefore, easily distinguishable (Fig. 3d). The NBM and DBB in the basal forebrain showed only a small (cases 5–7) or a negligible or very low amounts (cases 8–10) of amorphous plaques (Fig. 3d).

Alz-50 immunoreactivity (Table 3)

Application of Alz-50 revealed the presence of cytoskeletal changes, i.e., diffusely stained perikarya and dystrophic neurites. This type of Alz-50 staining was observed in all AD patients and in the 90-year-old non-demented control. Classic NFT-shaped structures were rarely observed. Recognition of these structures was obscured by the intense diffuse cell body staining and because of their globoid shape. The density of Alz-50-stained cytoskeletal changes seen using the double-labeling method was not different from that using Alz-50 single

Table 3 Alz-50 staining of dystrophic neurites (D) and perikarya (P)

	Controls					Alzheimer's disease patients				
	1	2	3	4	5	6	7	8	9	10
Chiasmatic region of the hypothalamus										
SON	—	—	—	—	—	—	—	—	—	—
CG	—	—	—	—	±D,P	+D,P	±D,P	±P	++D,P	±D,P
SDN	—	—	—	—	—	+D,P	±D,P	±D,P	++D,P	+D,P
SCN	—	—	—	—	—	+P,D	±P	±P	+D,P	+P
PVN	—	—	—	—	—	—	—	—	+P,D	—
PVA	—	—	—	—	—	—	—	—	+P	—
ME	—	NA	—	—	—	—	—	—	NA	—
Tuberal region of the hypothalamus										
DMH	—	—	—	—	±D,P	+D,P	±D,P	±D,P	+D,P	+D,P
VMH	—	—	—	—	±D,P	+D,P	±D,P	++D,P	+D,P	±D,P
TG	—	—	—	—	±D,P	+D,P	±D,P	±P	++D,P	+D,P
TMN	—	—	—	—	±D,P	+++D,P	+D,P	++D,P	++D,P	+D,P
NTL	—	—	—	—	+D,P	±D,P	+D,P	+++D,P	+++D,P	+++D,P
NTI	—	—	—	—	—	—	—	—	—	—
Adjoining areas										
NBM	—	—	—	—	±D,P	++D,P	++D,P	++D,P	++D,P	++D,P
DBB	—	—	—	—	±D,P	++D,P	+D,P	+D,P	++D,P	++D,P
BSTc	—	—	—	—	±D,P	+D,P	±P	+D,P	+D,P	+D,P
BSTl	—	—	—	—	±D	±D,P	—	±D,P	±D,P	+D,P
BSTm	—	—	—	—	±P	+D,P	±P	+D,P	±D,P	+D,P
ACC	—	—	—	—	±P,D	±P,D	±P,D	±D,P	+D,P	+D,P
CI	—	—	—	—	+P,D	±P,D	±P	+D,P	±P	+D,P

The order of the letters D or P is related to the relative density of the Alz-50 positive dystrophic neurites of perikarya, respectively. The first letter presents the highest relative density. For abbreviations see Table 2. NA: not available; —: not or negligible staining; ±: few; +: small amount; ++: considerable amount; +++: large amount

staining. There was a large spectrum in the density of cytoskeletal changes per nucleus, ranging from none in the SON to many in the NTL of AD patients. The NTL of the younger AD patients (cases 6 and 7) showed, however, low amounts of Alz-50-reactive cytoskeletal changes (Fig. 3a), while the older ones (cases 8–10) showed many such changes (Fig. 3b). The amount of Alz-50-stained cytoskeletal changes in the ventral striatum (ACC, CI) of AD patients 6 and 7 was only slightly less than that in AD patients 8–10. In other nuclei no difference was found in the density of Alz-50-stained cytoskeletal changes between the younger and older AD patients.

Staining with Alz-50 in controls gave a pattern of mainly patchy granular perikarya and beaded fibers in particular areas in the hypothalamus (e.g., PVA, PVN, VMH, NTI, ME and all BST nuclei), as reported previously [40]. This staining in the controls is due to cross-reaction with a subpopulation of somatostatinergic neurons [41]. Distinction between the Alz-50 staining in controls and the cytoskeletal changes in AD patients was not difficult.

Comparison of the distribution of A β (SP28) and Alz-50 staining (cf. Tables 2, 3)

Amorphous plaque density did not correlate to that of Alz-50-stained cytoskeletal changes. The hypothalamus and BST of the 45-year-old AD patient (no. 7) showed the highest numbers of A β deposits among the AD patients, while this brain region revealed the lowest number of Alz-

50-stained cytoskeletal changes. Note, moreover, that AD patient 9 had amounts of A β deposits that were as low as those observed in the hypothalamus of the non-demented control no. 5, while the density of Alz-50-stained cytoskeletal changes ranked among the higher ones.

Many brain areas showed both amorphous plaques and cytoskeletal changes. However, there were quite a few exceptions to this rule. The SCN remained entirely negative for A β immunoreactivity, while in all AD patients it contained some Alz-50-stained cytoskeletal changes (cf. Fig. 3c). The PVN and PVA were devoid of amorphous plaques in all AD patients. On the other hand, the PVN and PVA of AD patient 9 showed some Alz-50-stained cell bodies and dystrophic neurites. The NTI showed a few amorphous plaques in AD patients 6, 7 and 10, while Alz-50-immunoreactive cytoskeletal changes were never observed in that nucleus of any of the AD patients. The SON and ME did not show staining with anti-A β or Alz-50.

The SDN, DMH, BSTl and BSTm tended to show both amorphous plaques and cytoskeletal changes in the AD patients, but there were some exceptions. The SDN of AD patient 6 did not show amorphous plaques, but showed Alz-50-reactive cell bodies and dystrophic neurites; the SDN of control no. 5 contained amorphous plaques but no Alz-50-stained cytoskeletal changes (cf. Fig. 3e, f). The DMH and BSTm of AD patient 9 contained some Alz-50-stained cytoskeletal changes, while amorphous plaques were not observed. On the other hand, the BSTl of AD patient 7 showed no Alz-50-stained cytoskeletal changes but contained many amorphous plaques.

Fig. 2 **a** Alzheimer patient 6, 40 years of age; SP28/Alz-50 staining. Anti-A β (SP28) staining in the tuberal gray (TG) shows a low number of mainly large, intensely stained amorphous plaques (ap). The amounts of Alz-50-stained perikarya (p) and dystrophic neurites (dn) are low. There is no clear local relationship between the distribution of amorphous plaques and cytoskeletal changes. **b** Alzheimer patient 8, 56 years of age; anti-A β (1G102) staining. Staining of amorphous plaques in the tuberal gray (TG) is characterized by large amounts of often faintly granular and small deposits. **c** Alzheimer patient 7, 45 years of age; SP28/Alz-50 staining. The central part of the bed nucleus of the stria terminalis (BSTc) can be easily delineated from the lateral sector of the bed nucleus (indicated with an asterisk). Arrows indicate faint granular β /A4-reactive garland-like A β deposits in the rim of the nucleus. Amorphous plaques in the BSTc of AD patients are often more spheroid and more intensively stained with anti-A β . Cytoskeletal changes are not observed. **d** Alzheimer patient 9, 70 years of age, anti-A β (1G102) staining. Again, the central sector of the bed nucleus of the stria terminalis (BSTc) is obviously distinguishable from the surroundings, as indicated by arrows. Only in the periphery of the nucleus were some A β deposits present. The surrounding nuclei are: 1 the caudate nucleus, 2 the lateral, and 3 the medial part of the bed nucleus of the stria terminalis (CG chiasmatic gray, IC internal capsule, lv lateral ventricle)

Double-labeling with anti-A β and Alz-50 did not show any obvious local topical relationship between amorphous plaques and cytoskeletal changes.

Discussion

The hypothalamus and adjoining areas of AD patients have been reported to be affected merely by minute amounts of classic and amyloid SPs stained by conventional thioflavin S [26, 28] and Congo red staining [30].

Fig. 3 **a** Alzheimer patient 6, 40 years of age; SP28/Alz-50 staining. The nucleus tuberalis lateralis (NTL) of this young patient shows many amorphous plaques (ap) and few Alz-50-stained cytoskeletal changes. Most of the amorphous plaques are not intimately related to the few Alz-50-positive cell bodies or dystrophic neurites. **b** Alzheimer patient 10, 90 years of age; SP28/Alz-50 staining. The nucleus tuberalis lateralis (NTL) of this old patient stains heavily with Alz-50-stained cytoskeletal changes, but the amount of amorphous plaques (ap) is very low. These findings contrast those observed in **a**. **c** Alzheimer patient 9, 70 years of age; SP28/Alz-50 staining. The suprachiasmatic nucleus (SCN) is indicated by arrows. It contains some perikarya (p) and dystrophic neurites (dn). However, A β -reactive

deposits are not observed. **d** Alzheimer patient 9, 70 years of age; SP 28/Alz-50 staining. The nucleus accumbens (ACC) shows a high density of amorphous plaques (ap). The adjoining nucleus basalis of Meynert (NBM) contains many cell bodies (p) and dystrophic neurites (dn), but amorphous plaques are not found. **e** Alzheimer patient 6, 40 years of age, SP28/Alz-50 staining. The sexual dimorphic nucleus (SDN) contains some perikarya (p) and dystrophic neurites (dn). Amorphous plaques (ap) are found in the surrounding chiasmatic gray, but not within the SDN itself. **f** Non-demented control no. 5, 90 years of age; 1G102 staining. The SDN, indicated by arrows, does contain amorphous plaques (ap), in contrast to that of Alzheimer patient 6 (cf. **e**)

Our data confirm the presence of large amounts of amorphous plaques in these brain areas of AD patients, as reported by Standaert et al. [31]. In addition, we agree with Standaert et al. [31] that considerable A β staining in the hypothalamus is not restricted to AD dementia. Amorphous

plaque density in the hypothalamus of AD patient 9 who had suffered from AD for more than 12 years was similar to that in the 90-year-old non-demented control (no. 5). Our data also show, in addition, that anti-A β staining was not only entirely negative in the SON and PVN, but also

in the PVA, SCN and ME. Standaert et al. [31] reported a remarkable uniformity in amorphous plaque staining in the rest of the hypothalamus and BST between the AD patients that they studied, whose ages ranged from 65 to 88 years. We also found a similar A β immunostaining in the hypothalamus and BST of the three AD patients aged 56, 70 and 90 years (nos. 8–10) and of the 90-year-old non-demented control (no. 5). Almost all of the amorphous plaques had vague borders and were weakly stained. A distinctly different type of anti-A β staining, however, was found in the two younger AD patients, i.e. the 40-year-old (no. 6) and 45-year-old ones (no. 7). Most amorphous plaques in the hypothalamus and BST of these early-onset AD patients showed an intense staining and had clear borders (cf. Figs. 2 a–d; 3 a, b).

The combination of anti-A β and Alz-50 allowed a detailed survey of the local distribution and density of amorphous plaques and Alz-50-stained cytoskeletal changes and enabled us to test the hypothesis of primary amyloid local induction put forward by Hardy [11]. For this purpose information on connections in some of the hypothalamic and adjoining areas is essential. Hardy's hypothesis states that amorphous plaques are deposited at axonal terminals and locally induce the occurrence of cytoskeletal changes in dendritic processes of subsequent neurons. From the dendritic compartment cytoskeletal changes would proceed towards the perikaryon and the axon of the affected neurons, followed by amorphous plaque deposition at their axonal terminals, and so on.

The primary amyloid anatomical cascade hypothesis seems, at first sight, to be supported by the presence of amorphous plaques and Alz-50-stained cytoskeletal changes in the same brain areas, such as the ACC and CI. Cytoskeletal changes in the ACC occur in small type IV–V neurons with short ramifying axons and dendrites. These processes remain within the nucleus [5]. CI neurons have a poorly developed dendritic tree and a short unramified axon that does not leave the islands [22]. However, subsequent analysis of the individual differences does not support the hypothesis. In the ACC and CI of the 40- and 45-year-old AD patient (nos. 6 and 7) large amounts of amorphous plaques were found with only a very few Alz-50-stained cytoskeletal changes. In addition, the ACC of older AD patients aged 56, 70 and 90 years (nos. 8–10) contained fewer amorphous plaques, while cytoskeletal changes were present in similar or even higher densities than in the two younger AD patients. Moreover, the amorphous plaque densities in the CI of the 90-year-old control (no. 5) were similar to those in the 70- and 90-year-old AD patients (nos. 9 and 10), whereas Alz-50-stained cytoskeletal changes in the CI of the 90-year-old, non-demented control (no. 5) were even more pronounced than those of the 70-year-old AD patient.

The observations in the NBM also cannot be explained by the amyloid cascade hypothesis. Magnocellular cholinergic neurons in the NBM [12] have direct connections with the cerebral cortex and hippocampus in primates [21]. In AD, a functional deficit of these projections is likely to be partly responsible for the severe memory and

cognitive disturbances. Large cholinergic neurons undergo extensive atrophy in AD [1, 24, 29] and show many Alz-50-stained NFTs, cell bodies and dystrophic neurites in all seriously affected AD patients ([34, 37, 41], cf. Table 3). The amount of amorphous plaques is, however, very low or virtually absent in the NBM of AD patients. Amorphous plaque density in the AD patients was rather low and, in fact, in the same range of that in the 90-year-old non-demented subject (no. 5), while this control showed only a few Alz-50-stained cytoskeletal changes.

The BSTc and NTL are two other areas suitable for checking the amyloid cascade hypothesis. These nuclei have some anatomical characteristics in common. Both can be clearly delineated from the enveloping areas. The BSTc and NTL show a dense fiber staining with anti-somatostatin_{1–12} [9, 18, 36, 38, 40]. In addition, both contain major somatostatin neuron subpopulations, which are distributed throughout these nuclei and maintain an intensive connectivity within these nuclei [4, 9, 18, 20, 36]. Staining with anti-somatostatin_{1–12} revealed densely distributed beaded fiber pericellular basket-like terminations in the BSTc [18, 40] and NTL [36, 38]. Both the NTL and BSTc showed Congo red-negative and A β -reactive deposits in the periphery of the nuclei in all AD patients. In addition, in AD patients 6 and 7 considerable amounts of amorphous plaques were observed in the core of the BSTc (Fig. 2 c) and of the NTL (Fig. 3 a). On the basis of the primary amyloid anatomical cascade hypothesis, one would expect that both nuclei contained many Alz-50-reactive cell bodies and dystrophic neurites. However, while the NTL showed many cytoskeletal changes in AD patients 8–10, the BSTc rarely showed such changes in any of the AD patients. Therefore, local induction of cytoskeletal changes by amorphous plaques does not seem to be the explanation for the findings in the BSTc.

Individual differences in the NTL also fail to support Hardy's cascade hypothesis. In the younger AD patients the presence of many amorphous plaques was accompanied by a few cytoskeletal changes in this nucleus (Fig. 3 a). On the other hand, staining of cytoskeletal changes by Alz-50 in the NTL of AD patients 8–10 was so intensive that it could be easily seen with the naked eye, while there was only little amorphous plaque staining in this nucleus (Fig. 3 b). Since dystrophic neurites stained by Alz-50 were found to hardly cross the border of the NTL [16, 34, 37], the vast majority of Alz-50-stained affected neurons in this nucleus would, according to Hardy's cascade hypothesis, be locally induced only by amorphous plaques within the nucleus itself. The data from the NTL do not support this possibility and rather seem to show an inverse relationship between amorphous plaque deposition and the occurrence of cytoskeletal changes. It is also not possible to explain why the NTL of young AD patients aged 40 and 45 years showed only few Alz-50-stained cytoskeletal changes, while the amount of amorphous plaques was high. On the other hand, the NTL of the older AD patients aged 56–90 years revealed many cytoskeletal changes, while amorphous plaque density was low in this nucleus. The number of amorphous plaques in the NTL of these three

AD patients was as low as in the 90-year-old control. This is more of a problem since no relationship was apparent between Alz-50 or A β staining and disease duration. The occurrence of Alz-50-stained cell bodies and dystrophic neurites does not seem, therefore, to be locally induced by A β nor does it seem to propagate through the neurons affected by amorphous plaques.

In summary, the distribution of A β -reactive deposits and Alz-50-stained cytoskeletal changes appeared to be highly variable for (i) the two stainings, (ii) the various brain regions, and (iii) the different AD patients. The presence of a large number of amorphous plaques does not necessarily go together with many Alz-50-stained cytoskeletal changes. Amorphous plaque density in the CG and TG was high in the AD patients aged 56, 70 and 90 years, but the density of Alz-50-stained cytoskeletal changes did not differ in these nuclei between the two younger and the three older AD patients. The ACC contained many amorphous plaques, but few cytoskeletal changes. The reverse was found in the NBM, which showed many cytoskeletal changes, while amorphous plaques were sparse. In the NTL, the density of Alz-50-stained cytoskeletal changes tended to be inversely related to that of amorphous plaques and showed a cross-over in the course of aging. The ratio of Alz-50 and A β staining in the ACC-CI, NBM, NTL and BSTc did not support the primary amyloid anatomical cascade hypothesis. Also, the observation that in the NTL there was not any local relationship between amorphous plaques and cytoskeletal changes is not in favor of the hypothesis. Taken together, A β deposition and the occurrence of Alz-50-stained cytoskeletal changes rather seem to be independent phenomena. An alternative explanation is that amorphous plaque deposition is a risk factor rather than the causal factor for AD. The variable way in which the different hypothalamic and adjoining nuclei are affected by cytoskeletal changes or spared in AD and controls might be due to their connectivity or different innate life span.

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