The distribution of Alz-50 immunoreactivity in the hypothalamus and adjoining areas of Alzheimer's disease patients

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SUMMARY

The monoclonal antibody Alz-50 is directed against modified forms of tau proteins. Various hypotheses have been put forward concerning the meaning of Alz-50 staining in Alzheimer's disease brains. Cytoskeletal alterations are reported to occur exclusively in the cortex and the subcortical nuclei directly connected with the cortex. In addition, Alz-50 staining is presumed to be indicative of impending neuronal death. In order to test these hypotheses Alz-50 was applied to the hypothalamus and adjoining areas of five Alzheimer's disease patients of 40–90 years of age and five sex- and age-matched, non-demented controls. The results showed the following: (i) Alz-50 immunoreactivity is not restricted to Alzheimer's disease patients. Alz-50 immunoreactive beaded nerve fibres and patchy, granular cell bodies were observed in some hypothalamic nuclei of all controls with the exception of the youngest one. Dystrophic neurites were not only observed in all Alzheimer's disease hypothalami but also in that of the oldest control. (ii) In the hypothalamic area various nuclei had different Alz-50 staining patterns. Alz-50 staining could not, however, be related to neuronal death in the different nuclei. (iii) Histopathological changes in Alzheimer's disease patients are not restricted to the cortex or subcortical areas connected directly with the cortex. The present report indicates that the hypothalamus is considerably more affected in Alzheimer's disease than has often been assumed. However, these changes can also be found in non-demented old people.

INTRODUCTION

The mouse monoclonal antibody Alz-50 prepared by Wolozin *et al.* (1986) is directed against modified tau proteins with a mol. wt of 68 kDa (Ksiezak-Reding *et al.*, 1990; Lee *et al.*, 1991). For a certain time staining with this antibody was suggested to be specific for Alzheimer's disease (Wolozin *et al.*, 1986). However, recently Alz-50 staining was also found to be present in the hypothalamus of normal, non-demented controls. It appeared that in Alzheimer's disease hypothalami the staining of neurofibrillary tangles and dystrophic neurites could be distinguished from the staining patterns observed in controls (Byne *et al.*, 1991). Various hypotheses have been put forward with respect to the cytoskeletal alterations

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in the Alzheimer's disease brain. First, changes in Alzheimer's disease are often reported to occur only in cortical areas (including the hippocampus) and in those subcortical nuclei that send direct projections to these brain regions (Mann, 1985; German *et al.*, 1987). They would be observed most frequently in phylogenetically young association neocortices and related brain regions (archicortex, basolateral amygdala, nucleus basalis of Meynert) that have recently undergone integrated development during phylogeny (Rapoport, 1989). Secondly, immunolabelling with Alz-50 in Alzheimer's disease brains is believed to be indicative of impending neuronal death (Wolozin *et al.*, 1988). The hypothalamus is a unique structure to test the various hypotheses. It contains both nuclei with and without direct connections with the cerebral cortex and hippocampus. Moreover, clear differences in cell number changes of four hypothalamic nuclei can be observed both in the course of normal aging and in Alzheimer's disease. The suprachiasmatic nucleus generates and coordinates circadian rhythms (Rusak and Zucker, 1979). From the age of 80 years onwards this nucleus shows a decrease in total cell number and in neurons expressing vasopressin, one of its main peptide transmitters. In Alzheimer's disease, the decline in cell number is even more pronounced (Swaab *et al.*, 1985, 1987). The sexually dimorphic nucleus embedded in the chiasmatic grey matter is twice as large in males as in females (Swaab and Fliers, 1985) and is identical to the intermediate nucleus as described by Braak and Braak (1987). In Alzheimer's disease, the pattern of cell loss in this cell group is similar to that during normal aging. This goes for both genders (Swaab and Hofman, 1988). Neurons in the supraoptic and paraventricular nucleus produce vasopressin or oxytocin, which are conducted to the neurohypophysis. These nuclei do not show any change in total cell number with aging or Alzheimer's disease (Goudsmit *et al.*, 1990).

This report is meant to give a detailed survey of the distribution of Alz-50 staining structures in the chiasmatic and tuberal region of the hypothalamus and adjoining structures. In addition, Alz-50 staining in the suprachiasmatic, sexually dimorphic, supraoptic and paraventricular nuclei is related to their measure of cell loss during aging and in Alzheimer's disease.

MATERIALS AND METHODS

Subjects and tissues

Research was carried out on the post-mortem hypothalamus of five Alzheimer's disease and five age- and sex-matched non-demented patients (*see* Table 1). All five Alzheimer's disease patients had undergone a thorough clinical examination and neuropathological verification. The demented patients had a Global Deterioration Scale of 6–7 for severity of dementia (Reisberg *et al.*, 1982). The controls had no neurological or psychiatric disorder (*see* Table 1). The neuropathological diagnosis 'Alzheimer's disease' was made on the occurrence of many senile plaques, neurofibrillary tangles and a disorganized fibre pattern (dystrophic neurites) in Bodian and Congo stains of the hippocampus, the superior frontal (area 10) or orbito-frontal cortex (area 11), the superior temporal cortex (area 22) or the cortex of the temporal pole (area 38), the superior (area 7) and inferior parietal cortex (area 40) and the cortex of the occipital lobe (areas 17 and 18). In order to exclude parkinsonian changes the substantia nigra was also examined. Notably, the oldest control (patient 5) was clinically non-demented and neuropathologically not diagnosed as an Alzheimer's disease patient since the Alzheimer's disease changes were largely restricted to the hippocampus and temporal cortex and hardly found in the parietal, frontal and occipital cortex.

The hypothalamic block had already been dissected coronally between the lamina terminalis and the middle of the mammillary bodies and cut in series of 6 μ m-thin frontal sections which were stored at room temperature. Each fiftieth section had been stained with thionin (0.1% thionin in acetate, pH 4) for general orientation,

TABLE 1. CLINICAL AND PATHOLOGICAL INFORMATION ON CONTROLS AND ALZHEIMER PATIENTS

	Age (years)	Sex (M/F)	Brain weight (g)	Post-mortem delay (h)	Fixation duration (days)	Diagnosis, cause of death
Controls						
1	37	M	1510	48	46	Alcohol and drug 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
Alzheimer's disease patients						
6	40	M	1370	3	28	AD, cachexia
7	45	M	1130	4	119	AD, cachexia
8	56	F	1180	24	48	AD, bronchopneumonia
9	70	F	780	14	34	AD, epileptic insults
10	90	F	1060	3	38	AD, dehydration

AD = Alzheimer's disease.

while the suprachiasmatic nucleus had been visualized by means of an antiserum against vasopressin (Truus), since this nucleus cannot be recognized in a thionine staining (Swaab *et al.*, 1985). The thionine- and anti-vasopressin-stained sections had previously been used for morphometrics on cell number changes in the suprachiasmatic (Swaab *et al.*, 1985, 1987), sexually dimorphic (Swaab and Hofman, 1988), supraoptic and paraventricular nuclei (Goudsmit *et al.*, 1990).

Immunohistochemistry

The antibody used was an IgG mouse monoclonal (P-42) which was cloned from Alz-50 (IgM) following class switching. Alz-50 (IgG) immunolabelling was based upon the method of Wolozin *et al.* (1986) with a modification in the colouring step. Sections were mounted on chrome-alum gelatin coated glass slides by means of 0.5% bovine serum albumin (Sigma). Following removal of paraffin, hydration and rinsing in 0.05 M Tris/0.15 M NaCl (Merck) at pH 7.6 [Tris buffered saline (TBS)] for 3×10 min, the sections were incubated in 3% Perhydrol (Merck) and 0.2% Triton X-100 (Sigma) in TBS for 30 min. They were then washed in TBS (3×10 min), and incubated in 5% non-fat dried milk (Elk, Campina BV, Eindhoven, The Netherlands) in TBS at room temperature for 1 h in order to block non-specific antibody binding. Subsequently, the sections were incubated in Alz-50 which had been diluted 1:100 in a 5% Elk-TBS solution at room temperature for 1 h and then overnight at 4°C. The sections were washed in TBS (3×10 min), incubated in peroxidase-conjugated rabbit anti-mouse immunoglobulins (DAKO P260) in a dilution of 1:100 in 5% Elk in TBS for 1 h at room temperature and washed in TBS (3×10 min). Then, they were incubated in 0.05 mg/ml 3-3'-diaminobenzidine tetrahydrochloride (Sigma)/TBS with 0.5% nickel ammonium sulphate as a staining enhancer to which 0.01% Perhydrol had been added immediately before use. Following staining for 30 min the sections were washed in TBS (2×5 min), and finally dehydrated and covered with Entellan. The sequence resulted in a black precipitate with minimal background staining.

RESULTS

A post-mortem delay of 45 h (patient 3) at most did not appear to be critical for Alz-50 staining in the hypothalamus. Also, Alz-50 immunoreactivity appeared to remain unaffected after formalin fixation for a period of 119 days (patient 7). Separately, we also examined the influence of the duration of fixation on tissues that were kept in formalin for a considerably longer period. In samples of the temporal and frontal cortex of Alzheimer's disease patient 6, which were kept in formalin for 471 days, the Alz-50 staining remained

the same. However, the Alz-50 staining of the temporal and frontal cortex of Alzheimer's disease patients 7 and 10, which we kept formalin-fixed for more than 4 years, revealed considerably fewer dystrophic neurites, perikarya and plaque-like densities.

Alz-50 staining in the hypothalamus (Tables 2, 3)

Different Alz-50 staining structures were observed: (i) beaded fibres; (ii) patchy, granular perikarya; (iii) dystrophic neurites; (iv) diffusely stained cell bodies. Beaded fibres (f) and patchy, granular perikarya (p) were characteristic of controls, but they also occurred in Alzheimer's disease hypothalami (*see* Table 2). Alz-50 staining of dystrophic neurites (D) and non-granular cell bodies (P) was typical of Alzheimer's disease, although it also occurred in controls, especially in the oldest one, patient 5 (*see* Table 3). Only occasionally did we have to decide somewhat arbitrarily which staining pattern (beaded fibres versus dystrophic neurites; patchy granular perikarya versus diffusely stained cell bodies) belonged to the control staining type or the Alzheimer's disease staining type.

Control staining (see Table 2)

Control patient was immunonegative except for the median eminence. In control patients 2–5 and in all Alzheimer's disease hypothalami, a clear staining pattern of straight, often thin, beaded fibres and small-sized neurons was observed (control staining). Alz-50 control staining was observed in the chiasmatic grey, tuberal grey, periventricular area, ventromedial hypothalamic nucleus, tuberomammillary nucleus, tubero-infundibular nucleus, median eminence, the paraventricular nucleus in particular in its medial part along the third ventricle but also scattered throughout this nucleus, the stria terminalis, all four subnuclei of the bed nucleus of the stria terminalis and Calleja islands in the basal forebrain. Staining of beaded fibres in the ventromedial hypothalamic nucleus region was so intense that it could be recognized with the unaided eye as a small, black stained line. The dorsomedial hypothalamic nucleus was immunonegative or contained only a negligible amount of fibres (patients 3 and 5), while in the nucleus accumbens too few patchy, granular cell bodies were found to score. Such a staining pattern was not observed after application of other monoclonal IgG antibodies, e.g. PFU-83 (anti-CRH).

Alzheimer's disease staining (see Table 3)

In the Alzheimer's disease hypothalami and control subject 5, control staining was found together with dystrophic neurites and diffusely stained cell bodies (Alzheimer's disease staining), which represented most of the Alz-50 staining structures. Various nuclei tended to be variably affected by Alzheimer's disease changes ranging from none (supraoptic nucleus) to numerous (lateral tuberal nucleus). However, the lateral tuberal nucleus in patient 6, the paraventricular nucleus and periventricular area in patient 9, and the ventromedial hypothalamic nucleus in patients 7 and 10 appeared to deviate from the general pattern. The lateral tuberal nucleus in patient 6 (most often + + / + + +) and the ventromedial hypothalamic nucleus in patients 7 and 10 (+ / + +) were less (+) or hardly involved ($\pm$), respectively, while by exception in the paraventricular nucleus and periventricular area of Alzheimer's disease patient 9 dystrophic neurites were found. Notably, the hypothalamus of the oldest control (patient 5) did show not only Alz-50 stained structures observed in younger controls, but also dystrophic neurites and diffusely stained perikarya found in Alzheimer's disease affected brains. Interestingly, this non-

TABLE 2. STAINING OF PATCHY, GRANULAR PERIKARYA AND BEADED FIBRES IN THE HYPOTHALAMUS OF CONTROLS AND ALZHEIMER'S DISEASE PATIENTS BY ALZ-50

	<i>Control patients</i>					<i>Alzheimer's disease patients</i>				
	1	2	3	4	5	6	7	8	9	10
<i>Anterior hypothalamus</i>										
SON	—	—	—	—	—	—	—	—	—	—
PVN	—	+p, f	±p	±p	±p, f	±p	—	—	+p, f	±p, f
SDN	—	—	—	—	—	—	—	—	—	—
SCN	—	—	—	—	—	—	—	—	—	—
CG	—	+f	+f	—	±f, p	±f, p	±f	±f, p	—	±f, p
PVA	—	±p, f	±p	±p	+p, f	±p	±p	—	±p, f	±p, f
ME	+f	NA	+++f	—	+++f	+++f	+++f	+++f	NA	±f
<i>Mediobasal hypothalamus</i>										
DMH	—	—	—	—	—	—	—	—	—	—
VMH	—	+++f	+++f	+++f	+++f	+++f	+++f	+++f	±f	+++f
TMN	—	±p	±p, f	—	±p, f	—	±p, f	±p	—	±p
NTL	—	—	—	—	±p	±p	—	±p	±p	±p
NTI	—	±f	+++f, p	+f, p	±f, p	+p, f	±f, p	—	±f, p	—
TG	—	±f	±f, p	±f	±f, p	±f	±f	±f, p	—	±f, p
<i>Adjoining extrahypothalamic areas</i>										
NBM	—	—	—	—	±p	+p	+p	±p	±p	+p
DBB	—	—	—	—	±p	+p	±p	—	±p	±p
MSN	—	—	—	—	—	—	NA	—	—	—
LSN	—	—	—	—	—	—	—	—	NA	—
ST	—	+f	+f	+++f	—	±f	±f	—	—	—
BSTc	—	+f	±f	+++f, p	+f, p	±f	±f, p	—	—	+++f, p
BSTm	—	+f	±f	+++f	±f, p	±f	±f, p	—	±f	+f
BSTl	—	+f	+f	±f, p	±f	±f	+f, p	—	—	±f
BSTv	—	+f	+f	+++f	±f	+f	+f, p	—	±f	±f
ACC	—	—	—	—	—	—	—	—	—	—
CI	—	+++f	+++f	+++f	±f	+f	+f	—	+f	±f

NA = not available; f = beaded fibres; p = patchy, granular perikarya. The order of the letters is related to the relative density of the various Alz-50 positive structures. The first letter presents the highest relative density. — = no or negligible staining; ± = few; + = small amount; ++ = considerable amount; +++ = large amount. SON = supraoptic nucleus; PVN = paraventricular nucleus; SDN = sexually dimorphic nucleus; SCN = suprachiasmatic nucleus; CG = chiasmatic grey; PVA = periventricular area; ME = median eminence; DMH = dorsomedial hypothalamic nucleus; VMH = ventromedial hypothalamic nucleus; TMN = tuberomammillary nucleus; NTL = lateral tuberal nucleus; NTI = tubero-infundibular nucleus; TG = tuberal grey; NBM = nucleus basalis of Meynert; DBB = diagonal band of Broca; MSN = medial septal nucleus; LSN = lateral septal nucleus; ST = stria terminalis; BSTc = central sector of the bed nucleus; BSTm = medial sector of the bed nucleus; BSTl = lateral sector of the bed nucleus; BSTv = ventral sector of the bed nucleus (including the bed nucleus of the commissura anterior); ACC = nucleus accumbens; CI = Calleja islands.

demented old control showed more Alzheimer's disease changes in the hypothalamus than in the orbital frontal cortex (data not shown) and adjoining extra-hypothalamic areas, while the lateral tuberal nucleus contained markedly more Alzheimer's disease changes (++) than the nucleus basalis of Meynert (±). In addition, the extent of Alzheimer's disease changes in the tuberal region of the hypothalamus of this control was virtually similar to that of Alzheimer's disease patient 7. Note that the staining of Alzheimer's disease changes in the suprachiasmatic, sexually dimorphic, medial and lateral septal nuclei was observed exclusively in the Alzheimer's disease hypothalami. The supraoptic nucleus was always devoid of Alz-50 staining. The same holds true for white matter, i.e. the

TABLE 3. STAINING OF DYSTROPHIC NEURITES AND NON-GRANULAR PERIKARYA IN THE HYPOTHALAMUS OF CONTROLS AND ALZHEIMER'S DISEASE PATIENTS BY ALZ-50

	<i>Control patients</i>					<i>Alzheimer's disease patients</i>				
	1	2	3	4	5	6	7	8	9	10
<i>Anterior hypothalamus</i>										
SON	—	—	—	—	—	—	—	—	—	—
PVN	—	±P	—	—	+P	±P	—	—	++P, D	+P
SDN	—	—	—	—	—	+D, P	±D, P	±D, P	++D, P	+D, P
SCN	—	—	—	—	—	+P, D	±P	±P	+D, P	+P
CG	—	—	—	—	±D, P	±D, P	±D, P	±P	+D, P	±D, P
PVA	—	—	—	—	±P	—	—	—	+P	—
ME	—	—	—	—	—	—	—	—	—	—
<i>Mediobasal hypothalamus</i>										
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
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	—	—	±P	—	±P	—	±P	—	±P	—
TG	—	—	—	—	±D, P	+D, P	±D, P	±P	+D, P	+D, P
<i>Adjoining extrahypothalamic areas</i>										
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
MSN	—	—	—	—	—	+D, P	NA	+D, P	+D, P	+D, P
LSN	—	—	—	—	—	±D, P	—	+D, P	NA	±D, P
ST	—	—	—	—	—	+D	—	±D	+D	+D
BSTc	—	—	—	—	±D, P	++D, P	—	+D, P	+D, P	±D, P
BSTm	—	—	—	—	±P	+D, P	±P	+D, P	±D, P	++D, P
BSTl	—	—	—	—	±D	+D, P	—	±D, P	±D, P	+D, P
BSTv	—	—	—	—	±P, D	+D, 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	±D, P	+D, P	±P	+D, P

D = dystrophic neuritis; P = diffusely stained cell bodies of non-granular perikarya. The order of the letters is related to the relative density of the various Alz-50 positive structures. The first letter presents the highest relative density. See legend to Table 2 for explanation of symbols and definitions of abbreviations.

anterior commissure, fornix and internal capsule. Recognition of neurofibrillary tangle-like structures was difficult or occasionally even impossible because these structures were obscured by the intense diffuse cell body staining and because of their globoid shape. They are included in the non-granular perikaryal staining.

DISCUSSION

Using Alz-50, the studied brain material revealed four staining characteristics, i.e. (i) patchy, granular cell bodies, (ii) diffusely labelled perikarya, (iii) beaded fibres, and (iv) dystrophic neurites. In Alzheimer's disease and in the oldest, 90-year-old control, the short, often thickened, curly or kinky dystrophic neurites could easily be distinguished from the straight, often thin, beaded fibres observed in the younger controls (i.e. patients 2–4, aged 43, 57, 72 years, respectively). Patchy, granular and entirely stained cell bodies

occurred in control as well as in Alzheimer's disease hypothalamic areas so that the mere occurrence of these changes does not discriminate between normal aging and Alzheimer's disease. The patchy granular staining was more apparent in controls, while in Alzheimer's disease diffusely stained cell bodies appeared more frequently. Moreover, the overall Alz-50 staining of the neurons is more impressive in Alzheimer's disease than in controls. These findings might lead to the presumption that beaded fibres and patchy, granular cells at one end and dystrophic neurites and entirely black-stained perikarya and neurofibrillary tangle-bearing neurons at the other end of the spectrum are an expression of the same pathogenetic process. This is, however, most probably not the case since in control hypothalami at the ultrastructural level the reaction product was associated with vesicles, while in Alzheimer's disease it was related to vesicles and filaments (Byne *et al.*, 1991). This suggests a reaction with different antigens. The finding of Swaab *et al.* (1992) may point to the same direction since the staining of neurons and thin, beaded fibres in the paraventricular nucleus of controls by means of Alz-50 was not observed when two other anti-tau antibodies (tau-1, 60e) were used instead of Alz-50. Moreover, recently we also found Alz-50 control staining in the hypothalamic area of 3- and 1-year-old child and a 2-day-old neonate (unpublished data). Alz-50 staining at such young ages excludes the possibility that this staining pattern might be related in any way to an early stage of Alzheimer's disease changes. Therefore, in Alzheimer's disease hypothalami stained with Alz-50, the control staining pattern should be distinguished from the Alzheimer's disease changes. Swaab *et al.* (1992) noted that the Alzheimer's disease changes stained with Alz-50 were basically similar to that with other anti-tau antibodies (tau-1, 60e) and anti-ubiquitin antiserum (3-39), thus supporting the idea that cytoskeletal changes were stained. The relative density of Alzheimer's disease changes for the different antibodies varied, however, per patient and per hypothalamic nucleus but not in any systematic way. This reinforces the idea that the Alz-50 staining of Alzheimer's disease changes concerns that of the cytoskeletal alterations. As yet, insight into the evolution of the neuronal changes cannot be given. Patchy, granular neurons or diffusely stained cell bodies are found in controls as well as Alzheimer's disease patients. In addition, the relative density of patchy, granular perikarya in respect to that of the entirely black-stained cell bodies in the oldest control was not different from that in the Alzheimer's disease hypothalami. Moreover, in Alzheimer's disease hypothalami in some nuclei, i.e. the suprachiasmatic, sexually dimorphic, medial and lateral septal nuclei, only diffusely stained perikarya were observed, whereas they were negative in all controls. Besides, Alz-50 did not appear to be an appropriate tool for the visualization of neurofibrillary tangles in the hypothalamus since these structures were often masked by the diffuse cell body staining. Yet, notwithstanding the difficulties in the interpretation of Alz-50 immunolabelling in the hypothalamic area, Alz-50 staining in this brain region might be useful for the discrimination between Alzheimer's disease and normal aging, as we observed Alz-50 staining of both dystrophic neurites and neurons in the suprachiasmatic, sexually dimorphic, paraventricular, medial and lateral septal nuclei in Alzheimer's disease patients but not in controls.

The report of Byne *et al.* (1991) on Alz-50 immunoreactivity in the hypothalamus was mainly concerned with the control staining pattern of beaded fibres and cell bodies, which they observed in the periventricular area, medial part of the paraventricular nucleus, lateral part of the tuberal grey, ventromedial hypothalamic nucleus, tubero-infundibular nucleus and median eminence. In addition to these observations, which we confirmed, we also

found beaded fibres and patchy granular cell bodies in the chiasmatic grey and medial part of the tuberal grey. In addition, not only did we find the reported cell body staining in the periventricular region of the paraventricular nucleus, but also staining scattered throughout the paraventricular nucleus (*see* Fig. 1E). Byne *et al.* (1991) paid little attention to the occurrence of dystrophic neurites and Alz-50 positive cell bodies with and without neurofibrillary tangles in Alzheimer's disease affected hypothalami. However, most of the Alz-50 immunolabelling in the Alzheimer's disease hypothalamic area is represented by that of the Alzheimer's disease changes, in particular that of the dystrophic neurites. The abundant presence of these structures is in sharp contrast to the sparse occurrence of senile plaques and neurofibrillary tangles in the tuberomammillary, ventromedial and dorsomedial hypothalamic nuclei and tuberal grey visualized by means of thioflavine S and silver stains (Hirano and Zimmerman, 1962; Ishii, 1966; Rudelli *et al.*, 1984; Saper and German, 1987). The presence of structures resembling dystrophic neurites in this brain area of Alzheimer's disease patients has never been reported.

The intense and widespread staining of dystrophic neurites in the hypothalamus and adjoining structures has major implications for Mann's (1985) hypothesis, which was supported by German *et al.* (1987), for example. For, in contrast to their idea that in Alzheimer's disease cortical areas including the hippocampus and brain structures with direct cortical connections would preferably be affected, Braak and Braak (1990) recently reported the presence of fibrillary tangles in aspiny types IV and V local circuit neurons in the striatum. In addition, our data show cytoskeletal changes in hypothalamic and adjoining brain areas, direct cortical connections of which are unknown, i.e. paraventricular nucleus, chiasmatic grey (Saper, 1985), sexually dimorphic nucleus embedded in the chiasmatic grey and suprachiasmatic nucleus (Watts and Swanson, 1987; Watts *et al.*, 1987). The same holds true for Alz-50 staining cytoskeletal alterations in Calleja islands neurons in the basal forebrain (*see* Fig. 2F) which have a poorly developed dendritic tree and a short unramified axon that does not leave the island (Meyer *et al.*, 1989). These findings also imply that Alzheimer's disease pathology is not restricted to those brain areas that have undergone integrated development during phylogeny in relation to the association cortex, as was hypothesized by Rapoport (1989).

Another hypothesis on Alz-50 staining in Alzheimer's disease brains, proposed by Wolozin *et al.* (1988), states that it is predictive of impending neuronal death. However, our data on Alz-50 staining in the supraoptic, periventricular, suprachiasmatic and sexually dimorphic nuclei on the other hand, and our knowledge of the course of cell number changes in these nuclei both during aging and in Alzheimer's disease on the other, do not support this idea (*see* Table 4). Admittedly, Wolozin's idea seems to be established by the negative observations in the supraoptic nucleus of both Alzheimer's disease and control patients. His statement also appears to hold true for the presence of Alz-50 staining in the suprachiasmatic nucleus in Alzheimer's disease (although its number of Alz-50 positive neurons is small) and for the negative findings in the suprachiasmatic nucleus of controls until the age of 72 years. However, in contradiction with the idea that Alz-50 is a marker for cell death is the negative staining in the suprachiasmatic nucleus of the 90-year-old control subject, for at this age considerable neuron loss is observed (Swaab *et al.*, 1985, 1987). In addition, because the paraventricular nucleus has a stable cell number (Goudsmit *et al.*, 1990), according to Wolozin's hypothesis, no Alz-50 staining should be expected in this nucleus. Yet, Alz-50 staining was found in the paraventricular

FIG. 1. A, Alz-50 staining in Alzheimer's disease patient 8, aged 56 years. The supraoptic nucleus (SON) is devoid of staining in contrast to the tuberal grey (TG), in which Alz-50 positive neurites are observed. B, Alz-50 staining in Alzheimer's disease patient 6, aged 40 years. The suprachiasmatic nucleus is bordered by arrow heads. It contains a small number of dystrophic neurites (D) and entirely black stained perikarya (P). *Indicates a blood vessel. C, Alz-50 staining in Alzheimer's disease patient 9, aged 70 years. The sexually dimorphic nucleus which is embedded in the chiasmatic grey (CG) is bordered by arrow heads. It shows diffusely stained perikarya (P) and dystrophic neurites (D). D, Alz-50 staining in the lateral tuberal nucleus of Alzheimer's disease patient 10, aged 90 years. This is one of the most heavily Alz-50 stained nuclei found in the hypothalamus of an Alzheimer's disease patient. It is full of dystrophic neurites and diffusely stained cell bodies. E, Alz-50 staining in the paraventricular nucleus of control patient 5, aged 90 years. In this figure one thin, beaded nerve fibre (f), one patchy, granular perikarya (p) and one entirely stained perikarya (P) are shown. Unstained magnocellular neurons of the paraventricular nucleus can be recognized in the background. F, Alz-50 staining in Alzheimer's disease patient 9, aged 70 years. In the paraventricular nucleus of this patient, staining of dystrophic neurites (D) is observed in addition to beaded fibres (f), diffusely black (P) and patchy, granular neurons (p). III indicates the third ventricle. The bars indicate 100 μ m.

FIG. 2. A, B, Alzheimer's disease patient 6, aged 40 years. Among the neuropil fibres in the nucleus basalis of Meynert stained with the Bodian staining dystrophic neurites (D) are normal appearing fibres. Using Alz-50, only altered fibres are stained, thus providing an easier interpretation of the cytoskeletal pathology. C. In the ventromedial nucleus of a control (patient 4, aged 72 years), a dense pattern of Alz-50 positive thin beaded fibres was found. D. In Alzheimer's disease patient 8, aged 56 years, the fibre pattern seen in Fig. 2c is not present but dystrophic neurites (D) and diffusely stained perikarya are apparent. f indicates a thin, beaded fibre. E. A similar beaded nerve fibre pattern to that seen in Fig. 2c is observed in Calleja islands in control subject 4, aged 72 years. F. In contrast, in a Calleja island of Alzheimer's disease patient 7, aged 45 years, practically no beaded nerve fibres are observed, but dystrophic neurites (D) and diffusely stained perikarya (P) are present. The bar, which represents 100 μ m, applies to all figures.

nucleus of Alzheimer's disease patients 6, 9, 10 and control patients 2–5. Finally, the presence of staining of dystrophic neurites and cell bodies in the sexually dimorphic nucleus of Alzheimer's disease patients and the negative staining in control subjects is not in

TABLE 4. ALZ-50 POSITIVE STAINING IN THE HYPOTHALAMUS OF CONTROLS AND ALZHEIMER PATIENTS IN RELATION TO CELL LOSS

	Aging			Alzheimer's disease		
	Cell no.	Expected staining (a)	Result	Cell no.	Expected staining (a)	Result
SON	c (1)	n	n	c (1)	n	n
PVN	c (1)	n	n or +	c (1)	n	n or +
SCN	c-↓ (2, 3)	n-+	n-n	↓↓ (2, 3)	++	+
SDN	↓ (4)	+	n	↓ (4)	+	+

(a) = on the basis of the theory that Alz-50 staining indicates impending neuronal death. c = a constant cell number; ↓ = a decrease in cell number; ↓↓ = a marked decrease in cell number; n = no staining of Alz-50 positive structures; c-↓ = a constant cell number up to 80 years, decreasing after this age; n-+ = no staining of Alz-50 positive structures up to 80 years, but staining found after this age; n-n = no staining of Alz-50 positive structures at any age. + = staining of Alz-50 positive structures; ++ = a massive staining of Alz-50 positive structures; (1) = Goudsmit *et al.*, 1990; (2, 3) = Swaab *et al.*, 1985, 1987; (4) = Swaab and Hofman, 1988. See Table 2 for definitions of abbreviations.

agreement with the similar decline in cell counts in this nucleus during aging and in Alzheimer's disease (Swaab and Hofman, 1988). In conclusion, Alz-50 immunoreactivity appears to be related to the Alzheimer's disease process but not to impending neuronal cell death. This conclusion is reinforced by the findings of Kremer *et al.* (1991) in the lateral tuberal nucleus of Alzheimer's disease patients with an age range of 45–64 years, which was so full of dystrophic neurites and cell bodies stained with Alz-50 that it could even be seen with the unaided eye. Yet, the neuron number did not decrease in Alzheimer's disease.

In summary, the various nuclei in the hypothalamus and adjoining brain areas react in a different way to the Alzheimer's disease process with lack of staining in the supraoptic nucleus at one end and intense Alz-50 staining the lateral tuberal nucleus at the other end of the spectrum. Also, the different hypothalamic nuclei are not involved simultaneously in the Alzheimer's disease process, as appeared, for example, from the ventromedial hypothalamic nucleus in the hypothalamus of Alzheimer's disease patient 10 which was full of control staining of beaded fibres while its neighbouring areas showed considerable amounts of Alzheimer's disease changes, nor does there seem to be a consistent sequence in which the hypothalamic nuclei are affected. In the lateral tuberal nucleus of the non-demented control patient 5, considerably more Alz-50 staining dystrophic neurites and cell bodies were observed than in that of the severely affected Alzheimer's disease patient 6. In addition, Alz-50 staining of cytoskeletal Alzheimer's disease changes did not appear to be indicative of impending neuronal death, nor to be restricted to the cortex and subcortical areas directly connected with the cortex. A limitation of this argument is, of course, that the connections have only been studied in animal experiments. An alternative explanation of the variable way in which the different hypothalamic nuclei are affected or spared in Alzheimer's disease and controls might be their connectivity or different innate life span.

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