Research report

Early cytoskeletal changes as shown by Alz-50 are not accompanied by decreased neuronal activity

A. Salehi ^{a,b,*}, J.A.P. Van de Nes ^{a,c}, M.A. Hofman ^a, N.K. Gonatas ^d, D.F. Swaab ^a^a Graduate School Neurosciences Amsterdam, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands^b Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran^c Valerius Clinic, Department of Psychiatry, Free University, Amsterdam, The Netherlands^d Department of Pathology and Laboratory Medicine, Division of Neuropathology, University of Pennsylvania, Philadelphia, PA 19104, USA

Accepted 10 January 1995

Abstract

The nucleus tuberalis lateralis (NTL) is located in the basolateral part of the hypothalamus and is only present as a well-delineated nucleus in human and higher primates. In Alzheimer's disease (AD), NTL neurons show strong early cytoskeletal alterations, as revealed by the antibody Alz-50, but practically no senile plaques or neurofibrillary tangles. To study whether the activity of NTL neurons decreases when cytoskeletal changes appear, i.e., during aging and in AD, we applied a polyclonal antibody raised against the medial cisternae of the Golgi apparatus (GA). The size of the GA and the cell profile of NTL neurons, two established parameters for neuronal activity, were measured by an image analysis system. No significant change in the size of the profiles of the GA or of the neurons was observed in this nucleus during aging or AD. Earlier studies have shown that there is no decrease in cell number in the NTL in AD. We conclude that in the NTL an early hallmark of AD, i.e., cytoskeletal changes as stained by Alz-50, does not correlate with decreased neuronal activity, as reflected by the size of the GA, nor with a decrease in cell number. In addition, we found that the very early occurring and abundant presence of lipofuscin in NTL neurons does not go together with decreased neuronal activity.

Keywords: Alzheimer; Hypothalamus; Nucleus tuberalis; Alz-50; Golgi apparatus; Lipofuscin

1. Introduction

The nucleus tuberalis lateralis (NTL) constitutes one of the most prominent hypothalamic nuclei in higher primates and human [4] whereas its existence in subprimates is subject to controversy [10]. A well-delineated NTL has not been observed in lower species.

The NTL shows pronounced cell loss in Huntington's disease [20] dementia with argyrophilic grains [3] malignant neuroleptic syndrome [15] and Kallmann's syndrome [18]. In contrast, the number of NTL neurons does not decrease in normal aging or Alzheimer's disease (AD) [21]. The NTL in AD is characterized by extreme early cytoskeletal changes as shown by the

intense staining of dystrophic neurites and cell bodies by the antibody Alz-50. However, practically no senile plaques (SPs) or neurofibrillary tangles (NFTs) are found in this nucleus [19,35,37,38].

An inverse relationship between cytoskeletal alterations and neuronal activity was suggested by a number of observations. During aging and AD, the supraoptic (SON) and paraventricular nucleus (PVN) that show an increase in the size of the Golgi apparatus (GA) which is consistent with increased neuronal activity [23] show hardly any Alz-50-staining [35,38]. On the other hand, the nucleus basalis of Meynert in which we found a clear decrease in the size of the GA [30] stains heavily with Alz-50 [35,38]. In view of the extreme early cytoskeletal changes in the NTL in AD and even some old control patients, the question was studied whether a decrease would be present in the GA size of NTL neurons during aging and even more so in AD. In this study, the size of the GA and neuronal profiles was

* Corresponding author. Address: Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands. Fax: (31) (20) 6961006.

determined as a measure of neuronal activity. Earlier studies have indicated that these two parameters are reliable and sensitive indices of increased [16,23,34] or decreased neuronal activity [30]. It has also been suggested that the measurement of the neuronal profile is a good parameter for neuronal activity in the rat [17,43] and human [9]. These two activity parameters were related to the presence of an early neuropathological marker of AD, i.e., Alz-50-stained cytoskeletal changes and to cell number in the NTL [21]. The conclusion of this study is that in AD early cytoskeletal changes, as indicated by Alz-50-staining, are not accompanied by a decreased neuronal activity, as determined by the size of the GA or neuronal profile area.

2. Materials and methods

2.1. Tissue preparation

Brains of 38 human subjects were obtained at autopsy (see Table 1 for details and clinico-pathological information). The material consisted of 24 non-demented (23–93 yr) controls without any primary neurological or psychiatric disorder and 14 AD patients (40–98 yr). The patients were clinically assessed and diagnosed as 'probable AD' by excluding other possible causes of dementia by history, physical examination and laboratory tests. All patients had a global deterioration scale (GDS) of 6–7 for severity of dementia [28].

Table 1
Clinico-pathological information of controls and AD patients

Autopsy	M/F	Age (yr)	Postmortem delay (h)	Fixation time (days)	Brain weight (g)	Cause of death clinical diagnosis
Controls						
(1) 84258	F	23	< 8	NR	NR	Hepatitis B, acute myelocytic leukaemia
(2) 85303	M	28	NR	44	NR	Acute myocardial infarction
(3) 84018	F	36	96	51	1424	Multiple fractures
(4) 84248	M	37	39	35	1370	Pneumonia
(5) 81267	M	43	23	53	1260	Non-Hodgkin lymphoma
(6) 883114	M	49	< 24	49	1309	Hepatic failure
(7) 93026	M	50	NR	53	1646	Urothelial carcinoma
(8) 92047	M	54	14	31	1410	Bronchogenic carcinoma
(9) 9246	F	54	13	31	1080	Traffic accident
(10) 82161	F	57	35	45	1220	Mitral stenosis
(11) 4724	F	59	53	34	1350	Emphysema
(12) 80087	F	60	24	198	1110	Acute leukaemia
(13) 9347	M	70	NR	29	1454	Lung carcinoma
(14) 4725	F	72	53	21	1200	Endocarditis lenta
(15) 9341	F	73	8	38	1344	Lung embolism, asthmatic bronchitis, pneumonia
(16) 85267	F	81	NR	NR	1210	Hepatocellular carcinoma, pneumonia, alcohol intoxication
(17) 9075	M	82	5.3	28	1268	Ureter carcinoma with metastasis, hydronephrosis
(18) 81064	M	83	22	42	1280	Diverticulitis, myocardial infarction
(19) 82175	M	85	16	44	1400	Chronic myelocytic leukaemia, pneumonia
(20) 811 00	F	88	11	35	1030	Basolioma of left ear, heart failure
(21) 81033	F	90	13	48	1110	Pneumonia
(22) 83179	F	91	48	28	1060	Respiratory insufficiency
(23) 9312	M	92	11	32	1380	Myocardial infarction
(24) 85330	F	93	24	32	1020	Aspiration
Mean $\pm$ SEM		64.5 $\pm$ 4.4	< 27.0 $\pm$ 5.0	46.3 $\pm$ 7.8	1269.8 $\pm$ 34	
AD cases						
(25) 89166	M	40	3	28	1370	AD, cachexia
(26) 86364	M	46	4	119	1130	AD, cachexia
(27) 89503	F	49	NR	NR	NR	AD
(28) 89345	M	52	23	102	1296	Familial AD, bronchopneumonia
(29) 9192	F	54	3.25	NR	1055	AD, cachexia
(30) 850050	F	56	24	48	1180	AD, bronchopneumonia
(31) 88252	F	66	3	30	1250	AD, cachexia and sepsis
(32) 83170	F	70	14	34	780	AD, status epilepticus
(33) 88330	M	78	3.15	31	1240	AD, meningitis
(34) 88272	F	84	4.5	31	1000	AD, cachexia
(35) 84050	F	90	2	33	860	AD, anaemia, caecum adenocarcinoma
(36) 86004	F	90	3	38	1060	AD, dehydration
(37) 86001	M	97	5	40	1260	AD, dehydration
(38) 91084	F	98	3.5	31	940	AD, decompensio cordis
Mean $\pm$ SEM		69.3 $\pm$ 5.4	7.3 $\pm$ 2.2	48.6 $\pm$ 10.5	1109.3 $\pm$ 49.6	

M, male; F, female; NR, not recorded.

AD was diagnosed clinically according to NINCDS-ADRDA criteria [27]. The clinical diagnosis of AD was neuropathologically confirmed by F.C. Stam (The Netherlands Brain Bank), W. Kamphorst (Free University) or D. Troost (Academic Medical Centre), all in Amsterdam, The Netherlands. The neuropathological examination showed extensive neocortical and hippocampal SPs, NFTs and dystrophic neurites. The following brain areas were studied: gyrus orbitalis, gyrus temporalis superior, hippocampus, corpus striatum, substantia nigra, thalamus, locus coeruleus, gyrus cinguli and cerebellum. The hypothalamus containing the NTL was dissected and fixed in 4% formaldehyde at room temperature for ~1 month. The fixed hypothalamus was dehydrated in graded ethanols, embedded in paraffin and cut serially in 6- μ m-thin frontal sections. Sections were mounted on chrome-aluminum-sulphate-coated slides, deparaffinized, hydrated and stained either by thionine (4%) or immunocytochemically. Every 50th section was stained with thionine for anatomical orientation.

Lipofuscin was visualized in NTL neurons on the basis of autofluorescence in unstained embedded sections using a fluorescent microscope (Axioskop Zeiss, Germany), UV light (BP365.11 nm) and a barrier filter (P397).

2.2. Immunocytochemistry

The following antibodies were used in this study: (1) a polyclonal antibody against MG-160; and (2) Alz-50. The polyclonal antibody was raised against immunoaffinity purified MG-160, a sialoglycoprotein of medial cisternae of the rat neuronal GA. The antibody is able to recognize the medial cisternae of the GA of neurons and glia in formalin-fixed paraffin-embedded tissue of the human brain [6]. The specificity of this antibody for GA membranes had already been established [12]. Microwave pretreatment was used to obtain optimal retrieval of the antigen. For details on the staining method, see Salehi et al. (1994) [30].

Briefly, the sections were incubated with anti MG-160 1:800 for 1 h at room temperature followed by overnight incubation at 4°C. Subsequently, the sections were washed in Tris-buffered saline and incubated with biotinylated goat anti-rabbit (Vector Laboratories) 1:500 for 30 min and finally with avidin-biotin coupled to peroxidase (Vector Laboratories) 1:1500 for 1 h. As a chromogen, 3-3'-diaminobenzidine (Sigma) was used. Staining enhancement was obtained by adding ammonium nickel sulphate (2.2 mg/ml).

Alz-50 is a monoclonal IgG antibody (clone p-42) probably directed against the N-terminus of tau [11,22] and considered to be a marker of early cytoskeletal changes in AD. Details of the Alz-50-staining method have been reported elsewhere [38]. The intensity of

Fig. 1. Schematic representation of tuberal region of hypothalamus (mamillary part). F, fornix; IC, internal capsule; OT, optic tract; III, third ventricle; CM, corpus mammillare.

Alz-50-staining in sections adjacent to those stained with the antibody against MG-160 was scored based on the relative density of (1) dystrophic neurites and (2) stained cell bodies. Dystrophic neurites refers to thick kinky unbeaded fibres [35]. As an internal positive control staining, a NTL section that showed intensive Alz-50-staining was taken in each staining run.

2.3. Morphometry

In the mamillary region, the NTL is bordered laterally by the optic tract, dorsomedially by the descending fornix, medially by the mamillary body, dorsally by the tuberomammillary nucleus (TM) and ventrally by the pia matter of the tuber cinereum (Fig. 1). NTL neurons are composed of a single medium sized neuronal cell body with a polygonal or triangular shape, an eccentrically located nucleus and large and densely packed lipofuscin granules. The NTL cells can easily be distinguished from the surrounding large (40 μ m) TM neurons which are characterized by an irregularly bordered perikaryon and a nucleus containing a large nucleolus [7,10,31]. To measure the size of the GA and the neuronal cell profile, an IBAS-KAT image analysis system (Kontron) was used. The details of the morphometrical and sampling methods have been described elsewhere [30]. Briefly, in each section the analysis consisted of 6 steps. (1) Outlining the NTL using a 2.5 $\times$ objective followed by superimposing a grid over the NTL profile. (2) Sampling the fields in this grid. (3) Saving the coordinates of these fields in a scanning stage in a 2.5 $\times$ objective. (4) Loading the selected fields in a 40 $\times$ objective. (5) Making a mask for the stained GA profile in each cell followed by outlining of the neurons using a 40 $\times$ objective. (6) In every selected field, all NTL neurons containing a nucleolus

Fig. 3. Immunocytochemical staining of GA in NTL and TM neuron. Note clear difference in GA size (86364). Scale bar, 30 μ m.

were outlined manually and the IBAS measured the size of the mask (GA) and the size of the cell body profile of the neurons.

2.4. Statistical methods

Differences among the groups were tested using the Student's *t* test. The Pearson's correlation coefficient was used to study the associations between various parameters. Furthermore, one-way ANOVA was used to compare four different groups. The critical level for statistical significance was 5%.

3. Results

As shown in Table 1, there were no significant differences in age ($P > 0.5$) or fixation time ($P > 0.5$) between controls and AD patients. Furthermore, although there was a significant difference in post-mortem delay ($P < 0.01$) and brain weight ($P < 0.01$), no significant correlation was found between the size of the GA or cell profile area and postmortem delay and brain weight in controls ($P > 0.05$) and AD patients ($P > 0.05$), indicating that this can not have affected our data.

3.1. Immunocytochemistry

Immunocytochemical staining with MG-160 revealed in all patients a very large and intense staining

of the GA of TM neurons and a small and less intensive staining of the GA in NTL neurons (Fig. 2). This difference in the GA size between the NTL and TM neurons was even more pronounced than the difference in cell size (Fig. 3). The GA of NTL neurons was mainly situated around the nucleus. The rest of the cytoplasm contained large amounts of lipofuscin which did not stain with the antibody against MG-160 (Fig. 2).

The monoclonal antibody Alz-50 stained in NTL neurons of AD patients and some old non-demented control subjects dystrophic neurites and cell bodies (Fig. 4).

Lipofuscin was abundantly present in NTL neurons of controls from the youngest age (23 yr) onwards and also in both, young and old AD patients (Fig. 5A–D). Lipofuscin appeared to be slightly more abundant in older than in younger controls. No difference was found in the amount of lipofuscin between controls and AD patients (Table 2).

3.2. Morphometry

Two parameters were quantified by image analysis.

(1) The cross-sectional area of the GA: no significant difference in the size of the GA was found between young controls and young AD patients ($P = 0.68$) or between old controls and old AD patients ($P = 0.31$). No significant change was found between young and old controls ($P = 0.19$) or young and old AD patients ($P = 0.77$) (Fig. 6). Three very high values were found

Fig. 2. Immunocytochemical staining of GA in (A) young control (93026) and (B) old control (9347) and (C) young AD patient (86364) and (D) old AD patient (86004). Note similarity between GA of cells of four groups. Scale bar, 30 μ m.

for the GA area in the NTL of subjects of > 90 yr, i.e., two in the controls 83179 and 81033 (91 and 90 yr old, respectively) and another in the AD patients 84050 (90 yr old) (Table 1) for which no explanation can be given at present.

There was no clear relationship between the size of the GA and the severity of the cytoskeletal alterations in AD as shown by Alz-50-staining (Table 3). Intense Alz-50-staining occurred together with a large- (35), small- (34 and 36) or with a medium-sized GA (38).

(2) The cross-sectional area of the NTL neurons: no significant differences in cell profile area were found between young controls and young AD patients ($P = 0.58$) or old controls and old AD patients ($P = 0.43$)

(Table 4). In addition, no change in cell profile size was found between young controls and young AD patients ($P = 0.58$) or old controls and old AD patients ($P = 0.44$) (Table 4). Furthermore, no difference was observed in this parameter between young and old controls ($P = 0.94$) (Fig. 7).

4. Discussion

In the present paper we have shown that in AD there is no correlation between the presence of early cytoskeletal alterations in NTL neurons, as stained by Alz-50, and neuronal activity, as judged by the size of

Fig. 4. Immunocytochemical staining of NTL by Alz-50 in an AD patient (88252). Note prominent staining of perikarya and dystrophic neurites. Scale bar, 0.5 mm (A) and 30 μ m (B). Arrows indicate borders of NTL.

Fig. 5. Lipofuscin autofluorescence in NTL of (A) young control (84258), (B) old control (4725) and (C) young AD patient (89345) and (D) old AD patient (91084). Note similarity in autofluorescence between controls and AD patients. Scale bar, 35 μ m.

Table 2
Lipofuscin in NTL neurons

Patient	M/F	Age (yr)	Lipofuscin entire NTL	Cytoplasm
Controls				
1	F	23	+++	+++
2	M	28	++	++
3	F	36	+	++
4	M	37	+++	+++
5	M	43	+++	+++
6	M	49	++++	++++
7	M	50	++	+
8	M	53	+++	+++
9	F	54	++++	++++
10	F	57	++++	++++
11	F	59	++++	++
12	F	60	++	+
13	M	70	+++	+++
14	F	72	+++	+++
15	F	73	+++	++
16	F	82	++++	++++
17	M	82	++++	++++
18	M	83	++	+++
19	M	85	+++	++++
20	F	88	+++	++++
21	F	90	++++	++++
22	F	91	+++	+++
23	M	92	++++	++++
24	F	93	ND	ND
AD				
25	M	40	++	++
26	M	46	+++	++++
27	F	49	++	++
28	M	52	++++	++++
29	F	54	++++	++++
30	F	56	+	+
31	F	66	+++	++
32	F	70	++	++++
33	F	78	++++	+++
34	F	84	+++	++++
35	F	90	+++	++++
36	F	90	+++	++++
37	M	97	++	+
38	F	98	++++	++++

M, male; F, female.

Entire, proportion of neurons containing lipofuscin.

Cytoplasm, proportion of cytoplasm area which is occupied by lipofuscin.

+, few positive cells or small part of cytoplasm.

++, considerable number of neurons or part of cytoplasm.

+++, large number of neurons or large part of cytoplasm.

++++, all neurons of nucleus or entire cytoplasm.

ND, not determined.

the profiles of the GA and neurons. Earlier studies of the NTL in AD have shown that in spite of the extremely prominent Alz-50-staining in perikarya and dystrophic neurites, there was no decrease in cell number [21]. In contrast to AD, the NTL appears to be susceptible to severe cell loss, e.g., in Huntington's disease [20] or in the case of dementia with argyrophilic grains [3].

Various observations have suggested an inverse rela-

Relation between GA area and age in controls and AD patients

Fig. 6. Graph depicting mean GA size per subject in controls and AD patients. There is no significant change with age and no difference between controls and AD subjects. Bar, 1 SEM.

Table 3
Relationship between neuronal activity (GA size) and Alz-50-staining in NTL neurons of AD patients

Patient	Age (yr)	GA size *	Alz-50-staining	
			P	D
25	40	116.2	±	+
26	46	36.9	+	+
27	49	68.6	ND	ND
28	52	136.0	+	±
29	54	140.0	+	±
30	56	138.0	+	+++
31	66	51.0	++	++
32	70	42.0	+++	+++
33	78	97.0	-	-
34	84	68.0	±	-
35	90	298.0	++	+++
36	90	46.0	++	++
37	97	52.0	++	+++
38	98	104.0	+++	+++

P, perikarya; D, dystrophic neurites.

* % of mean of all AD patients.

-, no or negligible staining; ±, few Alz-50-positive perikarya or dystrophic neurites/

+, small amount; ++, considerable amount; +++, large amount.

Table 4
Mean area of GA and neuronal profiles in NTL

Group	Subjects (n)	NTL ○	
		GA area (μm²)/cell	Cell area (μm²)
Young control	12	36.0 ± 5.8	221.5 ± 8.6
Old control	12	53.2 ± 1.0	222.8 ± 14.7
Young AD	6	40.0 ± 6.6	234.0 ± 28.6
Old AD	8	35.7 ± 11.0	204.0 ± 18.2
Statistics *			
P		NS	NS

○ Values are given as mean ± SEM.

* One-way ANOVA; young, < 65 yr old; old, ≥ 65 yr old.

NS, not significant.

Fig. 7. Graph showing mean values of neuronal profiles per subject in controls and AD patients. There is no change with age and no difference between controls and AD patients. Bar, 1 SEM.

tionship between neuronal activity and cytoskeletal alterations. It has been reported that the PVN and SON of the hypothalamus are very stable in aging and AD. No change was found in these nuclei in total [13,14] or oxytocin cell numbers [41] whereas the number of vasopressin expressing neurons in the PVN was even increased in aging [40]. In AD, these nuclei are not stained by different antibodies used to reveal AD pathology, e.g., 60e (against NFT), tau-1 (against microtubule-associated protein tau), 3–39 (against ubiquitin) or Alz-50 [35,38]. The SON and PVN are activated in the course of aging as appeared, e.g., from a clear increase in the GA size in controls and AD patients [23]. In contrast to the SON and PVN which do not seem to be vulnerable to NFT formation, the nucleus basalis of Meynert of AD patients that is known to contain NFT showed both a heavy staining with Alz-50 [38] and a significant decrease in the size of the GA [30]. These data suggested that the increased activity of the PVN and SON may make these neurons less vulnerable to the changes of AD, a hypothesis that has been paraphrased before as ‘use it or lose it’ [33].

Alz-50 recognizes a soluble 68-kDa intraneuronal protein (A68) in AD brains which is not detectable by immunoblotting techniques in normal brains [42]. It has been proposed that expression of Alz-50 immunoreactivity is an early manifestation of AD pathology preceding the onset of neurofibrillary degeneration as shown by silver-staining [2,36] or impending cell death [1]. However, despite the prominent immunoreactivity with Alz-50 in AD, the NTL neurons showed no decrease of the size of the GA, of the cell or of neuron

numbers. These findings suggested that neuronal activity, as judged by the size of the GA and neuronal profile area, is preserved in the NTL. It should be noted that the NTL does not show NFTs [21]. It is, therefore, tempting to speculate that the persistent neuronal activity of NTL neurons in aging and AD is a factor in their resistance to develop NFT.

Since there is also staining of some cell bodies and with Alz-50 in other regions of the hypothalamus of non-demented young controls [5,29,35,38,39], one may rise the question whether the NTL-staining by Alz-50 is really due to cytoskeletal alterations and not to ‘control staining’. This is not the case since (1) in young controls NTL neurons are not stained with Alz-50, [5,35,38,39]. As we have shown recently, in other hypothalamic regions, the neurons which are stained by Alz-50 in non-demented controls, are also stained by antibodies directed toward somatostatin 15–28 [39]. The NTL of controls does not stain with Alz-50 and not with antibodies directed against somatostatin 15–28 [39] and (2) in AD patients not only Alz-50 but also other antibodies show cytoskeletal changes in this nucleus, e.g., antibodies against paired helical filaments, tau (i.e., 60e and tau-1) or anti-ubiquitin (3–39) [35]. Dystrophic neurites were, however, most conspicuous after Alz-50-staining which was the reason to continue with this antibody [35]. The prominent staining of the NTL by Alz-50 in AD is, thus, considered to show early cytoskeletal alterations.

As has been mentioned before [32] and confirmed in the present paper, numerous lipofuscin granules are present in NTL neurons from the earliest age (23 yr in our study) onwards. NTL neurons in controls contained no more lipofuscin than those in AD patients. This is in accordance with previous studies in which no difference in amount of lipofuscin was reported between controls and AD patients in the pyramidal cells of the Ammon’s horn, Purkinje cells and neurons of the dentate gyrus and inferior olivary nucleus [24,26]. It is interesting to note that high amounts of this ‘aging pigment’ were present in NTL neurons from early adulthood onwards (Table 2) and that there was only a slight increase in the amount with aging. In addition, the high amount of lipofuscin in NTL neurons does not correlate with signs of decreased metabolic activity of the NTL neurons or cell death (present study and [19]) and is in contrast with the suggestion of Mann [25] that accumulation of lipofuscin in neurons might be harmful for their metabolic activity. Our data are, however, in agreement with a report that indicate that lipofuscin does not necessarily affect the functional activity of the cell [8].

The main conclusion of the present study is that in AD the size of the GA as a parameter of neuronal activity is not decreased during the occurrence of early cytoskeletal changes as revealed by Alz-50.

Acknowledgements

Brains were obtained from The Netherlands Brain Bank, Amsterdam, The Netherlands (coordinator R. Ravid). Alz-50 was kindly donated by Abbott laboratories, Chicago, IL. We wish to thank C.W. Pool for his considerable help with the image analysis system, H.P.H. Kremer for his valuable comments, H. Stoffels for drawing the figures and G. van der Meulen for his photographic work. This work was partly supported by a grant from the Shahid Beheshti University of Medical Sciences, Tehran, Iran (A. Salehi), by the Stimuleringsprogramma Gerontologisch Onderzoek of the Ministry of Education and Science of The Netherlands and by the National Institutes of Health (NS05572) and an award from the Amyotrophic Lateral Sclerosis Society (both N.K. Gonatas).

References

- [1] Al-Ghoul, W.M. and Miller, M.W., Transient expression of Alz-50 immunoreactivity in developing rat neocortex: a marker for naturally occurring neuronal death?, *Brain Res.*, 481 (1989) 361–367.
- [2] Bancher, C., Brunner, C., Lassmann, H., Budka, H., Jellinger, K., Wiche, G., Seitelberger, F., Grundke-Iqbal, I., Iqbal, K. and Wisniewski, H.M., Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease, *Brain Res.*, 477 (1989) 90–99.
- [3] Braak, H. and Braak, E., Cortical and subcortical argyrophilic grains characterize a disease associated with adult onset dementia, *Neuropathol. Appl. Neurobiol.*, 15 (1989) 13–26.
- [4] Braak, H. and Braak, E., Anatomy of the human hypothalamus (chiasmatic and tuberal region). In D.F. Swaab et al. (Eds.), *The Human Hypothalamus in Health and Disease, Progress in Brain Research, Vol 93*, Elsevier, Amsterdam, The Netherlands, 1992, pp. 3–16.
- [5] Byrne, W., Mattiace, L., Kress, Y. and Davies, P., Alz-50 immunoreactivity in the hypothalamus of the normal and Alzheimer human and the rat, *J. Comp. Neurol.*, 306 (1991) 602–612.
- [6] Croul, S., Mezitis, S.G.E., Stieber, A., Chen, Y., Gonatas, J.O., Goud, B. and Gonatas, N.K., Immunocytochemical visualization of the Golgi apparatus in several species, including human, and tissues with an antiserum against MG-160, a sialoglycoprotein of rat Golgi apparatus, *J. Histochem. Cytochem.*, 38 (1990) 957–963.
- [7] Daniel, P.M. and Prichard, M.M.L., The anatomy of the hypothalamus, *Acta Endocrinol.*, 80, Suppl. 201 (1975) 6–26.
- [8] Davies, I. and Fotheringham, A., The effect of lipofuscin on cellular function, *Mech. Ageing. Dev.*, 23 (1983) 347–356.
- [9] De Lacalle, S., Iraizoz, I. and Gonzalo, L., Differential changes in cell size and number in topographic subdivision of human basal nucleus in normal aging, *Neuroscience*, 43 (1991) 445–456.
- [10] Fujii, M., Cyto- and myeloarchitectural studies on the lateral tuberal nucleus in simian Callithricidae and Prosimiae, *Acta Anat.*, 114 (1982) 155–164.
- [11] Goedert, M., Spillantini, M.G. and Jakes, R., Localization of the Alz-50 epitope in recombinant human microtubule-associated protein tau, *Neurosci. Lett.*, 126 (1991) 149–154.
- [12] Gonatas, J.O., Mezitis, S.G.E., Stieber, A., Fleischer, B. and Gonatas, N.K., MG-160: a novel sialoglycoprotein of the medial cisternae of the Golgi apparatus, *J. Biol. Chem.*, 264 (1989) 646–653.
- [13] Goudsmit, E., Hofman, M.A., Fliers, E. and Swaab, D.F., The supraoptic and paraventricular nuclei of the human hypothalamus in relation to sex, age and Alzheimer's disease, *Neurobiol. Aging*, 11 (1990) 529–536.
- [14] Hofman, M.A., Fliers, E., Goudsmit, E. and Swaab, D.F., Morphometric analysis of the suprachiasmatic and paraventricular nuclei in the human brain: sex differences and age-dependent changes, *J. Anat.*, 160 (1988) 127–143.
- [15] Horn, E., Lach, B., Lapiere, Y. and Hrdina, P., Hypothalamic pathology in the neuroleptic malignant syndrome, *Am. J. Psychiatry*, 145 (1988) 617–620.
- [16] Jongkind, J.F. and Swaab, D.F., The distribution of thiamine diphosphate-phosphohydrolase in the neurosecretory nuclei of the rat following osmotic stress, *Histochemie*, 11 (1967) 319–324.
- [17] Kalimo, H., Ultrastructural studies on the hypothalamic neurosecretory neurons of the rat. III. Paraventricular and supraoptic neurons during lactation and dehydration, *Cell Tiss. Res.*, 163 (1975) 151–168.
- [18] Kovacs, K. and Sheehan, H.L., Pituitary changes in Kallmann's syndrome. A histologic, immunocytologic, ultrastructural, and immunoelectron microscopic study, *Fertil. Steril.*, 37 (1982) 83–89.
- [19] Kremer, H.P.H., The hypothalamic lateral tuberal nucleus: normal anatomy and changes in neurological diseases. In D.F. Swaab et al. (Eds.), *The Human Hypothalamus in Health and Disease, Progress in Brain Research, Vol 93*, Elsevier, Amsterdam, The Netherlands, 1992 pp. 249–261.
- [20] Kremer, H.P.H., Roos, R.A.C., Dingjan, G., Marani, E. and Bots, G.T.A.M., Atrophy of the hypothalamic lateral tuberal nucleus in Huntington's disease, *J. Neuropathol. Exp. Neurol.*, 49 (1990) 371–382.
- [21] Kremer, B., Swaab, D., Bots, G., Fisser, B., Ravid, R. and Roos, R.A.C., The hypothalamic lateral tuberal nucleus in Alzheimer's disease, *Ann. Neurol.*, 29 (1991) 279–284.
- [22] Ksiazek-Reding, H., Binder, L.I. and Yen, S.-H., Alzheimer's disease proteins (A68) share epitopes with tau but show distinct biochemical properties, *J. Neurosci. Res.*, 25 (1990) 420–430.
- [23] Lucassen, P.J., Ravid, R., Gonatas, N.K. and Swaab, D.F., Activation of human supraoptic and paraventricular nucleus neurons with aging and in Alzheimer's disease as judged from increasing size of the Golgi apparatus, *Brain Res.*, 632 (1993) 105–113.
- [24] Mann, D.M.A. and Yates, P.O., Lipoprotein pigments. Their relationship to ageing in the human nervous system. I. The lipofuscin content of nerve cells, *Brain*, 97 (1974) 481–488.
- [25] Mann, D.M.A. and Sinclair, K.G.A., The quantitative assessment of lipofuscin pigment, cytoplasmic RNA and nucleolar volume in senile dementia, *Neuropathol. Appl. Neurobiol.*, 4 (1978) 129–135.
- [26] Mann, D.M.A., Yates, P.O. and Marcyniuk, B., Changes in nerve cells of the nucleus basalis of Meynert in Alzheimer's disease and their relationship to ageing and to the accumulation of lipofuscin pigment, *Mech. Ageing Dev.*, 25 (1984) 189–204.
- [27] McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D. and Stadlan, E., Clinical diagnosis of Alzheimer's disease: report of NINCDS-ADRDA Work Group under the Auspices of Department of Health and Human Service Task Force on Alzheimer's Disease, *Neurology*, 34 (1984) 939–944.
- [28] Reisberg, B., Ferris, S.H., de Leon, M.J. and Crook, T., The global deterioration scale for assessment of primary degenerative dementia, *Am. J. Psychiatry*, 139 (1982) 1136–1139.
- [29] Rye, D.B., Leverenz, J., Greenberg, S.G., Davies, P. and Saper, C.B., The distribution of Alz-50 immunoreactivity in the normal human brain, *Neuroscience*, 56 (1993) 109–127.

- [30] Salehi, A., Lucassen, P.J., Pool, C.W., Gonatas, N.K., Ravid, R. and Swaab, D.F., Decreased neuronal activity in the nucleus basalis of Meynert in Alzheimer's disease as suggested by the size of the Golgi apparatus, *Neuroscience*, 4 (1994) 871–880.
- [31] Saper, C.B., Hypothalamus. In G. Paxinos (Ed.), *Human Nervous System*, Academic Press, New York, NY, 1990, pp. 389–413.
- [32] Streng, H., Über den nucleus tuberosus lateralis im Gehirn des Menschen. Eine pigmentarchitektonische Studie, *Z. Mikrosk.-Anat. Forsch.*, 89 (1975) 1043–1067.
- [33] Swaab, D.F., Brain aging and Alzheimer's disease: 'wear and tear' versus 'use it or lose it', *Neurobiol. Aging*, 12 (1991) 317–324.
- [34] Swaab, D.F. and Jongkind, J.F., Influence of gonadotropic hormones on the hypothalamic neurosecretory activity in the rat, *Neuroendocrinology*, 8 (1971) 36–47.
- [35] Swaab, D.F., Grundke-Iqbal, I., Iqbal, K., Kremer, H.P.H., Ravid, R. and Van de Nes, J.A.P., Tau and ubiquitin in human hypothalamus in aging and Alzheimer's disease, *Brain Res.*, 590 (1992) 239–249.
- [36] Trojanowski, J.Q., Schmidt, M.L., Shin, R.-W., Bramblett, G.T., Goedert, M. and Lee, V.M.-Y., PHF tau (A68): from pathological marker to potential mediator of neuronal dysfunction and degeneration in Alzheimer's disease, *Clin. Neurosci.*, 1 (1993) 184–191.
- [37] Ulfig, N. and Braak, H., Amyloid deposits and neurofibrillary changes in the hypothalamus tuberomammillary nucleus, *J. Neural. Transm. P-D Sect.*, 1 (1989) 143.
- [38] Van de Nes, J.A.P., Kamphorst, W., Ravid, R. and Swaab, D.F., The distribution of Alz-50 immunoreactivity in the hypothalamus and adjoining areas of Alzheimer's disease patients, *Brain*, 116 (1993) 103–115.
- [39] Van de Nes, J.A.P., Sluiter, A.A., Pool, C.W., Kamphorst, W., Ravid, R. and Swaab, D.F., The monoclonal antibody Alz-50, used to reveal cytoskeletal changes in Alzheimer's disease, also reacts with a large subpopulation of somatostatin neurons in the normal human hypothalamus and adjoining areas, *Brain Res.*, 655 (1994) 97–109.
- [40] Van der Woude, P.F., Goudsmith, E., Wierda, M., Purba, J.S., Hofman, M.A., Bogte, H. and Swaab, D.F., No vasopressin cell loss in the human hypothalamus in aging and Alzheimer's disease, *Neurobiol. Aging*, 16 (1995) 11–18.
- [41] Wierda, M., Goudsmit, E., Van der Woude, P.F., Purba, J.S., Hofman, M.A., Bogte, H. and Swaab, D.F., Oxytocin cell number in the human paraventricular nucleus remains constant with aging and Alzheimer's disease, *Neurobiol. Aging*, 12 (1991) 511–516.
- [42] Wolozin, B.L., Pruchnicki, A., Dickson, D.W. and Davies, P., A neuronal antigen in the brains of Alzheimer patients, *Science*, 232 (1986) 648–650.
- [43] Zambrano, D. and De Robertis, E., The effect of castration upon the ultrastructure of the rat hypothalamus. I Supraoptic and paraventricular nuclei, *Zeitschr. Zellforsch.*, 86 (1986) 487–498.