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Journal of Chemical Neuroanatomy 23 (2002) 279–290

Journal of CHEMICAL NEUROANATOMY

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The intralaminar nuclei assigned to the medial pain system and other components of this system are early and progressively affected by the Alzheimer's disease-related cytoskeletal pathology

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Received 1 November 2001; received in revised form 8 February 2002; accepted 11 February 2002

Abstract

The intralaminar nuclei of the human thalamus are integrated into the ascending reticular activating system and into limbic, oculomotor and somatomotor loops. In addition, some of them also represent important components of the medial pain system. We examined the occurrence and severity of the Alzheimer's disease (AD)-related cytoskeletal pathology and β-amyloidosis in the seven intralaminar nuclei (central lateral nucleus, CL; central medial nucleus, CEM; centromedian nucleus, CM; cucullar nucleus, CU; paracentral nucleus, PC; parafascicular nucleus, PF; subparafascicular nucleus, SPF) in 27 autopsy cases at different stages of the cortical neurofibrillary pathology (cortical NFT/NT-stages I–VI) and β-amyloidosis (cortical phases 1–4). The CEM, CL, PF, and SPF are slightly affected at stage II (corresponding to preclinical AD). They are markedly involved at stages III and IV (i.e. incipient AD) and severely affected at stages V and VI (i.e. clinical AD). In the PC and CU, the cytoskeletal pathology is mild at stage III, marked at stage IV, and severe at stages V–VI, whereas the CM is only mildly affected at stages IV–VI. In all of the intralaminar nuclei, deposits of the protein β-amyloid occur for the first time during the final phase of cortical β-amyloidosis. Functionally, the cytoskeletal pathology encountered in the intralaminar nuclei may contribute to the memory and affective symptoms, attention deficits, and dysfunctions related to horizontal saccades and smooth pursuits seen in AD patients. Equally important, however, are the findings that the cytoskeletal pathology developing within the intralaminar nuclei assigned to the medial pain system (CEM, CL, CU, PC, PF) as well as within other components of this system begins already during the preclinical or incipient phases of AD. Given this fact, the question arises as to whether non-discriminative aspects mediated by the medial pain system could be employed to identify individuals in the very earliest stages of AD.

Keywords: Alzheimer's disease; β-amyloid; Cytoskeletal pathology; Medial pain system; Tau protein

1. Introduction

The neuropathological hallmarks of Alzheimer's disease (AD) are alterations of the neuronal cytoskeleton and extracellular deposits of the protein β-amyloid within the neuropil and the walls of cerebral and meningeal blood vessels (Braak and Braak, 1991a,b; Probst et al., 1991; Hardy and Higgins, 1992; Hyman and Trojanowski, 1997). Presumably, the gradual aggregation of an abnormal cytoskeletal tau protein that is immunopositive in reactions with the antibody AT8 leads to the formation of argyrophilic neurofibrillary tangles (NFTs) in nerve cell perikarya and neuropil threads (NTs) in dendrites (Braak and Braak, 1991a,b; Braak et al., 1994; Braak and Braak 1997). Whereas the evolution of the AD-related cortical neurofibrillary pathology follows a constant sequence of six stages

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0891-0618/02/$ - see front matter
PII: S0891-0618(02)00007-8

Table 1
Case number, age, gender (F = female, M = male), and neuropathological evaluation of the cortical AD-related pathology in the cerebral cortex of the cases studied

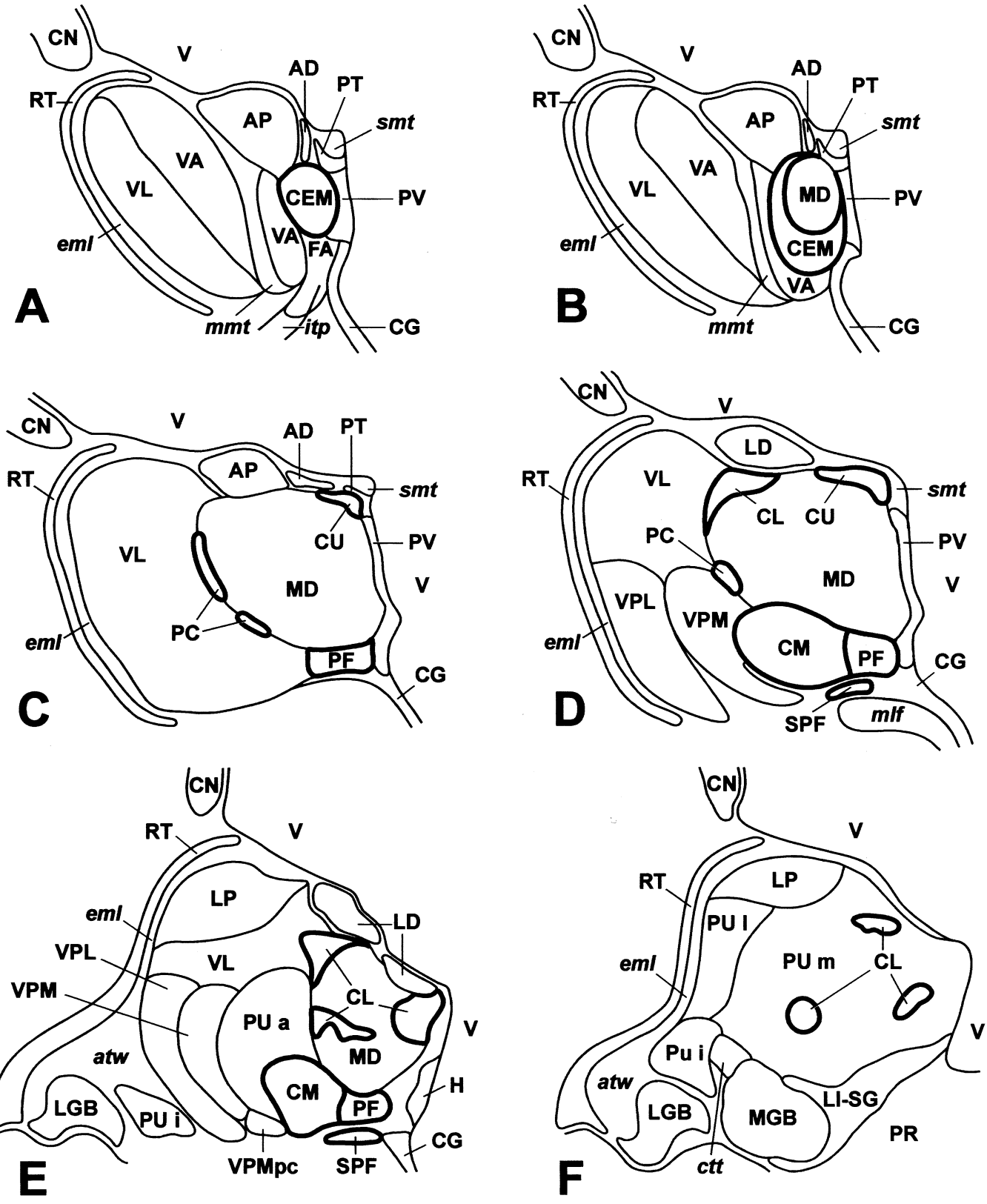
Case	Age	Gender	NFT/NT	β-Amyloid
1	57	M	I	0
2	73	F	I	0
3	77	M	I	0
4	79	M	I	1
5	80	F	I	0
6	73	M	II	0
7	75	F	II	0
8	77	M	II	0
9	80	M	II	0
10	88	M	II	3
11	65	M	III	4
12	76	F	III	2
13	83	M	III	0
14	84	M	III	4
15	78	F	IV	3
16	78	F	IV	4
17	88	F	IV	4
18	90	F	IV	0
19	80	F	V	4
20	85	F	V	4
21	86	F	V	4
22	86	F	V	4
23	86	M	V	4
24	89	M	V	4
25	69	F	VI	4
26	88	M	VI	4
27	91	F	VI	4

NFT/NT: Stages I–VI in the development of the cortical Alzheimer's disease-related neurofibrillary pathology (Braak and Braak 1991a, 1997). β-amyloid: Phases 0–4 in the development of the cortical Alzheimer's disease-related β-amyloidosis according to Thal et al. (2000).

(cortical NFT/NT-stages I–VI) (Braak and Braak, 1991a, 1997; Hyman and Trojanowski, 1997), AD-related cortical β-amyloidosis passes through four developmental phases (Thal et al., 2000). In cortical NFT/NT-stages I and II, the transentorhinal and entorhinal regions are the only territories marked by neurofibrillary lesions. In stages II and IV, the involvement in both of these areas is severe, in the hippocampus moderate, and in a number of neocortical association areas mild. In the end stages V and VI, the neocortical association areas show severe neurofibrillary changes, while the primary motor field, the primary sensory fields, and their belt areas remain more or less spared (Braak and Braak, 1991a, 1997; Hyman and Trojanowski, 1997). The first β-amyloid deposits observable in the medial temporal lobe consistently appear in the basal neocortex. Thereafter, in phase two, additional deposits occur in the entorhinal region and CA1 sector of the hippocampus. Subsequently, in the third phase, the AD-related β-amyloidosis involves the presubiculum and the fascia dentata. In the final phase 4, the CA4 sector of the hippocampus joins the medial temporal lobe regions affected by β-amyloid deposits (Thal et al., 2000).

AD cannot be diagnosed clinically until patients are in the final stages of the disease process (Price, 1998), and tangible progress with respect to effective early diagnostic and/or monitoring strategies based on assessment of neuropsychological dysfunctions remains elusive (Price, 1998; Schofield and Mayeux, 1998). Accordingly, additional symptoms and quantitative neurophysiological methods for their evaluation need to be identified. Prior to doing so, however, it remains to be determined, first, whether brain structures subserving the functions in question undergo the cytoskeletal changes early, thereby reflecting the disease process from the preclinical stage to beyond the incipient, and, finally, into the clinical phase of AD (Braak and Braak, 1991a; Bancher et al., 1993; Braak and Braak, 1997; Grober et al., 1999), and if so, second, whether the evolution of this pathology in correlates with the cortical NFT/NT-stages I–VI.

Fig. 1. Schematized frontal sections cut perpendicular to Forel's intercommissural axis showing the intralaminar nuclei of the human thalamus. (A) Rostral pole of the human thalamus at the level of the inferior thalamic peduncle (*itp*) showing the rostral pole of the central medial nucleus (CEM). (B) Frontal section through the thalamus at the level of the rostral pole of the mediodorsal nuclei (MD) and the mid-portions of the central medial nucleus (CEM). (C) Frontal section through the mid-level of the human thalamus with the cucullar (CU) and paracentral nuclei (PC), together with the rostral pole of the parafascicular nucleus (PF). (D) Frontal section through the caudal thalamus at the level of the medial longitudinal fascicle (*mlf*) with the central lateral (CL) and paracentral nuclei (PC), the cucullar nucleus (CU), as well as the centromedian (CM), parafascicular (PF), and subparafascicular nuclei (SPF). (E) Frontal section through the caudal thalamus at the level of the habenular nuclei (H), with the centromedian (CM), parafascicular (PF), and subparafascicular nuclei (SPF), as well as the caudal portions of the central lateral nucleus (CL). (F) Section through the caudal thalamus at the level of the pretectum showing the most caudally located portions of the central lateral nucleus (CL). (AD, anterodorsal nucleus; AP, anteroprincipal nucleus; *atw*, *triangular area of Wernicke*; CEM, central medial nucleus; CG, central gray; CL, central lateral nucleus; CM, centromedian nucleus; CN, caudate nucleus; *ctt*, *corticotectal tract*; CU, cucullar nucleus; *eml*, *external medullary lamina*; FA, fasciculosus nucleus; H, habenular nuclei; *itp*, *inferior thalamic peduncle*; LD, laterodorsal nucleus; LGB, lateral geniculate body; LI-SG, limitans-suprageniculate-complex; LP, lateral posterior nucleus; MD, mediodorsal nuclei; MGB, medial geniculate body; *mmt*, *mammillothalamic tract (Vicq d'Azyr)*; PC, paracentral nucleus; PF, parafascicular nucleus; PR, pretectum; PT, parataenial nucleus; PU a, pulvinar, anterior nucleus; PU i, pulvinar, inferior nucleus; PU l, pulvinar, lateral nucleus; PU m, pulvinar, medial nucleus; PV, paraventricular nuclei; RT, reticular nucleus; *smt*, *stria medullaris thalami*; SPF, subparafascicular nucleus; V, ventricle; VA, ventral anterior nucleus; VL, ventral lateral nucleus; VPL, ventral posterior lateral nucleus; VPM, ventral posterior medial nucleus; VPMpc, ventral posterior medial nucleus, parvocellular part).


A
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ctt

Fig. 1.

The intralaminar nuclei of the human thalamus are comprised of seven nuclei, which contribute to neural circuits of the medial pain, limbic, oculomotor, somatomotor, and ascending reticular activating systems. The present study aims at drawing attention to the early and progressive involvement on the part of the thalamic intralaminar components of the medial pain system during the evolution of the AD-related cytoskeletal pathology.

2. Material and methods

The brains of 27 individuals (14 females and 13 males; mean age 80.0±8.0 years) (Table 1) supplied by affiliated institutes for pathology were fixed in a 4% buffered aqueous solution of formaldehyde by immersion. Blocks of tissue including the thalamus were excised from the left hemispheres of all 27 brains, embedded in polyethylene glycol (PEG 1000, Merck, Darmstadt, Germany), and cut perpendicular to Forel's intercommissural axis into uninterrupted series of 100 μm frontal sections (Braak and Braak, 1991a,b).

Four collections of free-floating sections were processed with different staining techniques. The first collection, consisting of the first, eleventh, twenty-first, etc. free-floating sections, was stained both for Nissl material (Darrow red) as well as for lipofuscin granules (aldehyde-fuchsin) (Braak and Braak, 1991a). The intralaminar nuclei of the thalamus were localized in these aldehyde-fuchsin Darrow red-stained sections as recommended by the 'Michigan School of Anatomy' (Jones, 1985; Hirai and Jones, 1989) and previous authors (Hassler 1982; Heinsen et al., 1996; Morel et al., 1997) by using a stereomicroscope at final magnification of 10:1.

The collection of the second, twelfth, twenty-second, etc. sections was treated with the modified Gallyas silver-iodide technique to show AD-related NFTs/NTs (Braak and Braak, 1991b), while a third collection underwent staining with the modified silver pyridine Campbell–Switzer method for purposes of identifying β-amyloid depositions (Braak and Braak, 1991b; Thal et al., 2000). Immunocytochemistry was performed on the fourth collection of sections using the monoclonal antibody AT8 (Innogenetics, Ghent, Belgium) to render visible the abnormally phosphorylated cytoskeletal tau protein (Braak et al., 1994). Incubation of free-floating sections was carried out for 18 h at 4 °C (antibody dilution 1:2000). Incubation with the second biotinylated antibody (anti-mouse IgG) was performed for 2 h. Immunoreactions were visualized by means of the ABC complex (Vectastain, Vector Laboratories, Burlingame, CA) and 3,3-diaminobenzidine-tetra-HCl/H_2O_2 (DAB D7679, Sigma, Taufkirchen, Germany). The severity of the AD-related cortical neurofibrillary pathology was classified according to the Braak staging procedure (Braak and Braak, 1991a, 1997; Hyman and Trojanowski, 1997), whereas the classification of the AD-related β-amyloidosis within the cerebral cortex was performed as described recently by Thal et al. (2000) (Table 1).

An average of two Gallyas-impregnated sections, two AT8-immunostained sections, and three Campbell–Switzer-stained sections through each of the seven intralaminar nuclei were studied per case. The severity of the AD-related pathology in these nuclei was assessed semiquantitively at a final magnification of 10:1 (0 = not discernible; 1 = mild; 2 = moderate; 3 = severe) and blind to classification of the cortical AD-related pathology.

We used the Kruskal and Wallis H-test to determine whether the severity of the cytoskeletal pathology is dependent upon the cortical NFT/NT-stages and to see if the severity of the β-amyloid deposition correlates with the Thal phases 1–4 of cortical β-amyloidosis. Our working hypothesis that the severity of the pathology within the thalamic intralaminar nuclei gradually increases with the growing severity of the cortical pathology was tested by means of a nonparametric trend analysis. The correlation between the severity of the AT8-immunoreactive cytoskeletal pathology and that of the neurofibrillary pathology was described using Kendalls's rank correlation coefficient tau (t) (Bortz et al., 1990).

3. Results

3.1. Anatomical remarks

The fibrous layer of the internal medullary lamina surrounds the mediodorsal nuclei (MD) of the human thalamus at its rostral, lateral, dorsal, and caudal borders. It harbors nerve cells, which owing to their location, are subsumed under the term 'intralaminar nuclei'. These nuclear grays are divided into a rostral and a caudal group. The rostral intralaminar group consists of the central medial (CEM), paracentral (PC), cucullar (CU), and central lateral nuclei (CL) (Fig. 1A–F). The CEM covers the anterior pole and anteroventral portion of the MD (Fig. 1A, B), while the PC, a thin band of disconnected nerve cell clusters, separates the MD from the adjacent ventral lateral nucleus (Fig. 1C, D). The CU abuts on the dorsomedial portion of the MD (Fig. 1C, D). Rostrally, the CL forms a dorsolateral extension of the PC (Fig. 1D) and disintegrates caudally into numerous islands of neurons located between the caudal portions of the MD and the rostral parts of the medial subnucleus of the pulvinar (Fig. 1E, F). The caudal intralaminar group consists of three nuclei: centromedian (CM), parafascicular (PF), and subpar-

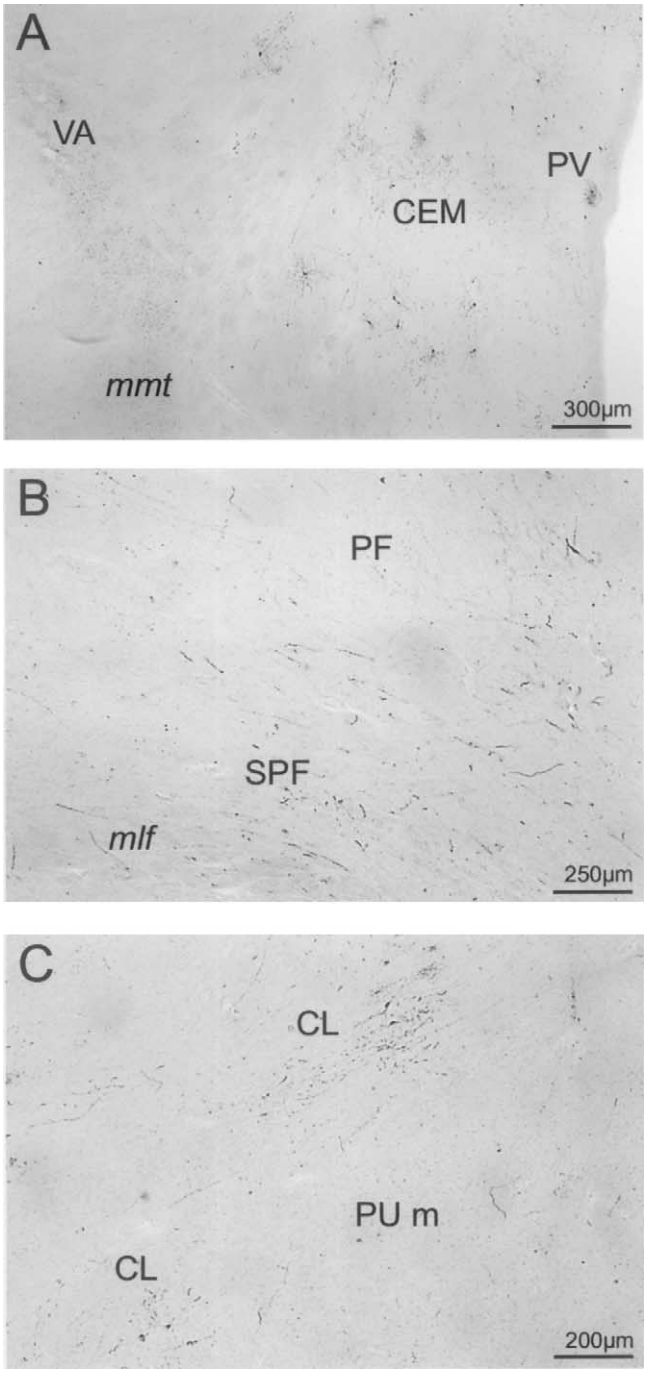

Fig. 2. Mild AD-related immunopositive cytoskeletal changes in the thalamic intralaminar nuclei at cortical NFT/NT-stage II (case 6, Tables 1 and 2). (A–C) PEG sections, AT8 immunostaining, 100 µm. For topographical orientation see Fig. 1. (A) Central medial nucleus (CEM). (B) Parafascicular nucleus (PF), subparafascicular nucleus (SPF). (C) Central lateral nucleus (CL). *mlf,* medial longitudinal fascicle; *mmt*, mammillothalamic tract (Vicq d'Azyr); PU m, pulvinar, medial nucleus; PV, paraventricular nuclei; VA, ventral anterior nucleus.

afascicular nucleus (SPF). The CM abuts medially on the PF (Fig. 1C–E), and both are separated from the SPF by a thin sheet of white matter (Fig. 1D, E) (Hassler, 1982; Jones, 1985; Hirai and Jones 1989; Bentivoglio et al., 1993; Groenewegen and Berendse, 1994; Heinsen et al., 1996; Morel et al., 1997).

3.2. AT8-immunoreactive cytoskeletal changes

The CEM, CL, PF, and SPF consistently begin to exhibit AT8-immunoreactive cytoskeletal pathology at cortical NFT/NT-stage II (preclinical AD) (Fig. 2). They are markedly affected at stages III–IV (incipient AD) and severely involved at stages V–VI (clinical AD) (Fig. 3, Table 2). The PC and CU at stages III and IV exhibit slight immunoreactive cytoskeletal changes (Fig. 3) and at stages V and VI sustain severe damage (Fig. 4, Table 2). In the CM, the immunoreactive cytoskeletal pathology appears for the first time with regularity at stage IV and remains mild at stages V–VI (Fig. 4, Table 2). In all of the intralaminar nuclei, the severity of the immmunoreactive lesions correlates with the progression of the cortical NFT/NT-stages I–VI, which is characterized by a linear trend (Table 3).

3.3. AD-related neurofibrillary tangles and neuropil threads

The CEM and the CL constantly show isolated NFTs/NTS in cases at cortical stage II. In cases at stages III and IV, both nuclei are conspicuously affected, and the SPF and PF display a few NFTs/NTs (Table 2). The PC and CU are inconsistently involved at stage III and, at stage IV, exhibit slight neurofibrillary changes. At cortical stages V–VI, the CEM, CL, CU, and SPF typically are filled with NFTs/NTs, while the PC and PF suffer severe damage (Fig. 5, Table 2). The CM displays NFTs/NTs for the first time with regularity at stage V and remains slightly involved in stage VI cases (Fig. 5, Table 2). In all of the nuclei studied, the severity of the neurofibrillary pathology steadily increases with the growing intensity of the AT8-immunoreactive changes there (Tables 2 and 3) and correlates significantly with the cortical stages I–VI. This correlation is characterized by a linear trend (Table 3).

3.4. β-amyloid deposits

Consistent β-amyloid deposition is seen in the CM one stage, in the PC, CU, CL, and PF two stages, and in the CEM and SPF three cortical NFT/NT-stages after they become affected by the AD-related cytoskeletal pathology (Fig. 6). This consistent involvement coincides with phase 4 in the development of cortical β-amyloidosis according to Thal et al. (2000) (Table 2). The severity of the β-amyloidosis in all seven intralaminar nuclei is linearly linked to the progression of the cortical phases 1–4 and cortical NFT/NT-stages I–VI (Table 3).

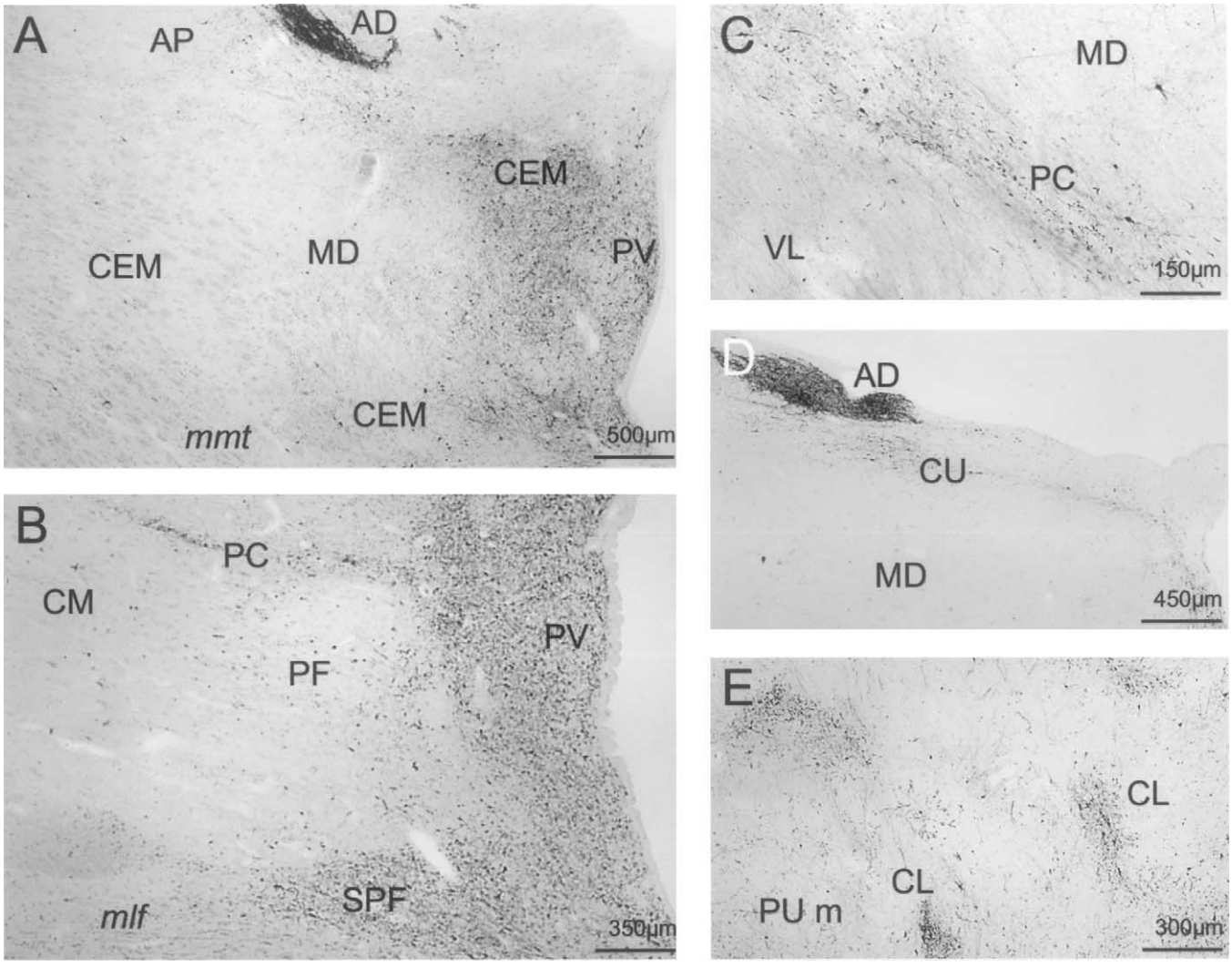

Fig. 3. AD-related immunopositive cytoskeletal changes in the thalamic intralaminar nuclei at cortical NFT/NT-stage IV (case 15, Tables 1 and 2). (A–E) PEG sections, AT8 immunostaining, 100 µm. For topographical orientation see Fig. 1. (A) Central medial nucleus (CEM). (B) Centromedian nucleus (CM), parafascicular nucleus (PF), subparafascicular nucleus (SPF). (C) Paracentral nucleus (PC). (D) Cucullar nucleus (CU). (E) Central lateral nucleus (CL). AD, anterodorsal nucleus; AP, anteroprincipal nucleus; MD, mediodorsal nuclei; *mlf* medial longitudinal fascicle; *mmt* mammillothalamic tract (Vicq d'Azyr); PU m, pulvinar, medial nucleus; PV, paraventricular nuclei; VL, ventral lateral nucleus.

4. Discussion

All of the thalamic intralaminar nuclei become progressively affected by the AD-related cytoskeletal pathology and by β-amyloidosis while the pathology in the cerebral cortex is evolving. Our finding that these same nuclei undergo the cytoskeletal changes prior to β-amyloid deposition indicates that the amyloid cascade hypothesis, which postulates that β-amyloid deposits are a prerequisite for the formation of the cytoskeletal alterations (Hardy and Higgins, 1992), does not apply here. The severity of the AT8-immunoreactive cytoskeletal pathology in all of the intralaminar nuclei examined correlates with that of the neurofibrillary lesions. This fact, along with the finding that apart from the CEM and CL, which exhibit both types of lesions at stage II, all of the intralaminar nuclear grays display AT8-immunopositive lesions earlier than the AD-related neurofibrillary pathology, reinforces the position that the argyrophilic material underlying the AD-related neurofibrillary pathology originates from the aggregation of the abnormally phosphorylated τ protein (Braak et al., 1994).

All of the rostral intralaminar nuclei belong to the ascending activating reticular system (Fig. 7) that modulates attention intensity and the level of arousal by influencing the activity of the cerebral cortex (Martin et al., 1990; Heckers et al., 1992; Bentivoglio et al., 1993;

Table 2
The severity of the cortical Alzheimer's disease-related neurofibrillary pathology

	CEM			PC			CU			CL			PF			CM			SPF		
	Aβ	AT8	NF	Aβ	AT8	NF	Aβ	AT8	NF	Aβ	AT8	NF	Aβ	AT8	NF	Aβ	AT8	NF	Aβ	AT8	NF
Stage I																					
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Median	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Range	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stage II																					
6	0	1	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0
7	0	1	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	1
8	0	1	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0
9	0	1	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	1
10	0	1	1	0	1	0	0	1	0	0	1	1	0	1	0	0	0	0	0	1	0
Median	0	1	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0
Range	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Stage III																					
11	2	3	2	2	1	2	2	1	2	2	2	2	2	2	1	2	1	0	2	3	2
12	0	2	1	0	1	0	0	1	0	0	1	1	0	2	1	0	0	0	0	2	1
13	0	2	2	0	2	1	0	2	1	0	2	2	0	1	1	0	0	0	0	2	2
14	2	3	3	2	2	1	2	1	1	2	2	2	3	2	1	1	0	0	1	2	2
Median	1	2	2	1	1	1	1	1	1	1	2	2	0	2	1	0	0	0	0	2	2
Range	2	1	2	2	1	2	2	1	2	2	1	1	3	1	0	2	1	0	2	1	1
Stage IV																					
15	2	3	3	2	2	2	2	2	2	2	2	2	2	2	1	2	1	0	2	3	2
16	1	3	2	1	2	2	1	2	2	2	3	2	3	2	1	3	1	0	2	3	2
17	1	3	2	1	2	1	1	2	1	1	3	1	3	2	1	2	1	0	1	3	2
18	0	3	2	0	1	1	0	1	1	0	3	2	0	2	2	0	1	0	0	3	2
Median	1	3	2	1	2	1	1	2	1	2	3	2	3	2	1	2	1	0	2	3	2
Range	2	0	1	2	1	1	2	1	1	2	1	1	3	0	1	3	0	0	2	0	0
Stage V																					
19	2	3	3	2	2	2	2	2	3	2	3	3	3	3	2	3	1	1	3	3	3
20	2	3	3	1	2	2	1	2	3	2	3	3	3	3	2	3	1	1	3	3	3
21	2	3	3	2	3	2	3	3	2	3	3	2	2	3	2	3	1	1	2	3	3
22	2	3	3	2	3	2	2	3	3	2	3	3	3	3	2	3	1	1	3	3	3
23	2	3	3	2	3	2	2	3	3	2	3	3	2	3	2	3	2	1	2	3	3
24	2	3	3	2	3	2	2	3	3	2	3	3	3	3	2	3	1	1	3	3	3
Median	2	3	3	2	3	2	2	3	3	2	3	3	3	3	2	3	1	1	3	3	3
Range	0	0	0	1	1	0	2	1	1	1	0	1	1	0	0	0	1	0	1	0	0
Stage VI																					
25	2	3	3	3	3	3	2	3	3	3	3	3	3	3	2	3	2	1	3	3	3
26	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	1	1	3	3	3
27	3	–	2	3	–	2	3	–	3	3	–	3	2	–	2	2	–	1	2	–	3
Median	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	1	1	3	3	3
Range	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	1	0	1	0	0

(Aβ, β-amyloid deposits; AT8, nerve cells immunoreactive to the antibody AT8; NF, neurofibrillary tangles and neuropil threads) in the intralaminar nuclei of the human thalamus at cortical NFT/NT-stages I–VI (0 = not discernible, 1 = mild, 2 = moderate, 3 = severe. The case numbers correspond to the cases specified in Table 1 (CEM, central medial nucleus; PC, paracentral nucleus; CU, cucullar nucleus; CL, central lateral nucleus; PF, parafascicular nucleus; CM, centromedian nucleus; SPF, subparafascicular nucleus).

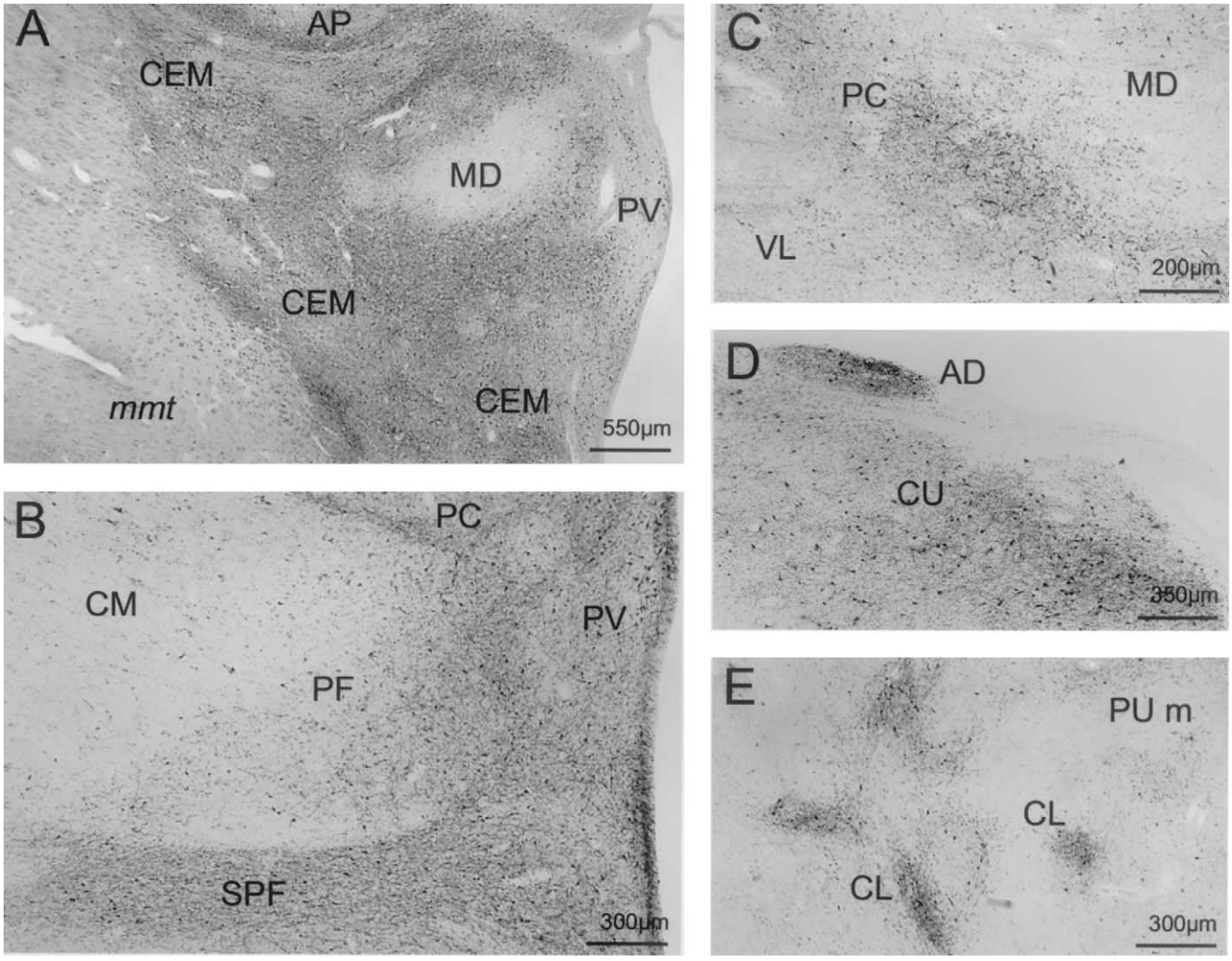

Fig. 4. AD-related immunopositive cytoskeletal alterations in the thalamic intralaminar nuclei at cortical NFT/NT-stage VI (case 21, Tables 1 and 2). (A–E) PEG sections, AT8 immunostaining, 100 µm. For topographical orientation see Fig. 1. (A) Central medial nucleus (CEM). (B) Centromedian nucleus (CM), parafascicular nucleus (PF), subparafascicular nucleus (SPF). (C) Paracentral nucleus (PC). (D) Cucullar nucleus (CU). (E) Central lateral nucleus (CL). AD, anterdorsal nucleus; AP, anteroprincipal nucleus; MD, mediodorsal nuclei; *mmt*, mammillothalamic tract (Vicq d'Azyr); PU m, pulvinar, medial nucleus; PV, paraventricular nuclei; VL, ventral lateral nucleus.

Groenewegen and Berendse, 1994; Kinomura et al., 1996; Mesulam, 1998). Together with the PF, the rostral intralaminar nuclei also are integrated into loops influencing smooth pursuit eye movements and horizontal saccades (Fig. 7). Furthermore, along with the SPF, they are involved in limbic loops (Fig. 7) and perform functions assigned to this system, such as affective and motivational behavior, memory and learning capacities (Leigh and Zee, 1991; Sadikot et al., 1992; Groenewegen and Berendse, 1994; Rüb et al., 2002). As such, the cytoskeletal pathology in this subset of intralaminar thalamic nuclei may contribute to the memory and affective dysfunctions (Förstl and Kurz, 1999; Perry and Hodges, 1999), the dysfunctional smooth pursuit eye movements and deficiencies in horizontal saccades (Fletcher and Sharpe, 1986, 1988; Zaccara et al., 1992), and the attention deficits (Perry and Hodges, 1999; Gainotti et al., 2001) encountered in AD patients.

The CM plays an important role in somatomotor loops (Alexander et al., 1990; Sadikot et al., 1992 Heinsen et al., 1996) (Fig. 7). Nonetheless, because it is only mildly affected by the neurofibrillary pathology even at stages V–VI, we regard the CM's contribution

Table 3
Results of the statistical analyses performed

	CEM	PC	CU	CL	PF	CM	SPF
AT8: *H* corr	23.68	22.35	22.56	23.85	24.42	21.49	24.05
P	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.001	< 0.0005
AT8: *H* lin	11.75	15.50	15.85	14.49	15.98	12.44	12.94
P	< 0.001	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005
NFTs/NTs: *H* corr	22.74	22.78	23.97	23.95	25.03	26.00	25.05
P	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005
NFTs/NTs: *H* lin	15.10	18.06	18.13	18.75	17.68	10.81	18.85
P	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.005	< 0.0005
Aβ: *H* corr	21.55	20.97	20.97	21.66	22.69	22.44	21.53
P	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005
Aβ: *H* lin	8.19	8.19	8.19	7.94	7.45	7.53	7.86
P	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.01	< 0.01
AT8-NFTs/NTs: τ	0.80	0.83	0.84	0.87	0.87	0.85	0.82
P	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.01	< 0.005

Line 1: value of the test statistic *H* corr of the one-way analysis of variance for the AT8-immunopositive nerve cells and significance level of *H* corr; line 2: value of the test statistic *H* lin from the trend analyses for the AT8-immunopositive nerve cells and significance level of *H* lin; line 3: value of the test statistic *H* corr of the one-way analysis of variance for the NFTs/NTs and significance level of *H* corr; line 4: value of the test statistic *H* lin from the trend analyses for the NFTs/NTs and significance level of *H* lin; line 5: value of the test statistic *H* corr of the one-way analysis of variance for the β-amyloid deposits and significance level of *H* corr; line 6: value of the test statistic *H* lin from the trend analyses for the β-amyloid deposits and significance level of *H* lin; line 7: value of Kendall's rank correlation tau between the severity of AT8-immunopositive lesions and the severity of the neurofibrillary pathology and significance level of Kendall's rank correlation tau) (CEM, central medial nucleus; PC, paracentral nucleus; CU, cucullar nucleus; CL, central lateral nucleus; PF, parafascicular nucleus; CM, centromedian nucleus; SPF, subparafascicular nucleus).

to the late-appearing somatomotor deficits in AD (Franssen et al., 1993; Förstl and Kurz, 1999) to be minor.

In the introduction, we touched briefly upon the neuropsychological approach to AD diagnostics and monitoring. Recently, we discussed in greater detail the potential of neurophysiological tests involving vertical and horizontal saccades for preclinical or early screening purposes and for the clinical monitoring of both the disease process and patient response to drug regimens (Rüb et al., 2001a,b). Here, we want to point to the pivotal role of specific thalamic intralaminar nuclei in events related to the experience and processing of pain, which, according to recent studies, are altered in AD patients (Farrell et al., 1996; Fisher-Morris and Gellatly, 1997; Bendetti et al., 1999; Scherder et al., 1996; Pickering et al., 2000; Rainero et al., 2000; Scherder and Bouma, 2000).

The experience of pain incorporates: (1) sensory-discriminative, and (2) non-discriminative aspects, i.e. autonomic, emotional, and nocifensive reactions to painful stimuli (Farrell et al., 1996; Huffman and Kunik, 2000; Pickering et al., 2000). In AD patients, the sensory-discriminative aspects usually are preserved (Farrell et al., 1996; Bendetti et al., 1999; Huffman and Kunik, 2000), whereas the changes that occur are in the non-discriminative components of pain experience. These shifts include elevation of pain tolerance (Farrell et al., 1996; Bendetti et al., 1999; Scherder et al., 1996; Scherder and Bouma, 2000), decrease in pain affect (Scherder et al., 1996; Pickering et al., 2000; Scherder and Bouma, 2000), altered reactions to sudden painful stimuli, such as blunted autonomic reactions (Porter et al., 1996; Pickering et al., 2000; Rainero et al., 2000) or extinction of nocifensive reactions (Fisher-Morris and Gellatly, 1997). All of these non-discriminative aspects of pain are mediated by the 'medial pain system' (Jones, 1985; Bentivoglio et al., 1993; Vogt et al., 1993; Vogt and Sikes, 2000). The PF and the rostral intralaminar nuclei (CEM, CU, CL, PC) (Fig. 7), together with the parabrachial nuclei, periaqueductal gray, and anterior cingulate area, represent important building blocks within this system (Jones, 1985; Bentivoglio et al., 1993; Vogt et al., 1993; Vogt and Sikes, 2000) and, like the last three components (German et al., 1987; Braak and Braak, 1991a; Parvizi et al., 1998, 2000; Rüb et al., 2001c), are severely affected by cytoskeletal pathology in AD. The lesions in the parabrachial nuclei are seen already with regularity at cortical NFT/NT-stage I (Rüb et al., 2001c), in the CEM, CL, and PF at stage II, and in the CU, PC, and anterior cingulate cortex at stage III (Braak and Braak, 1991a), whereby the severity of the pathology at all of these sites increases linearly with the NFT/NT-staging sequence I–VI,

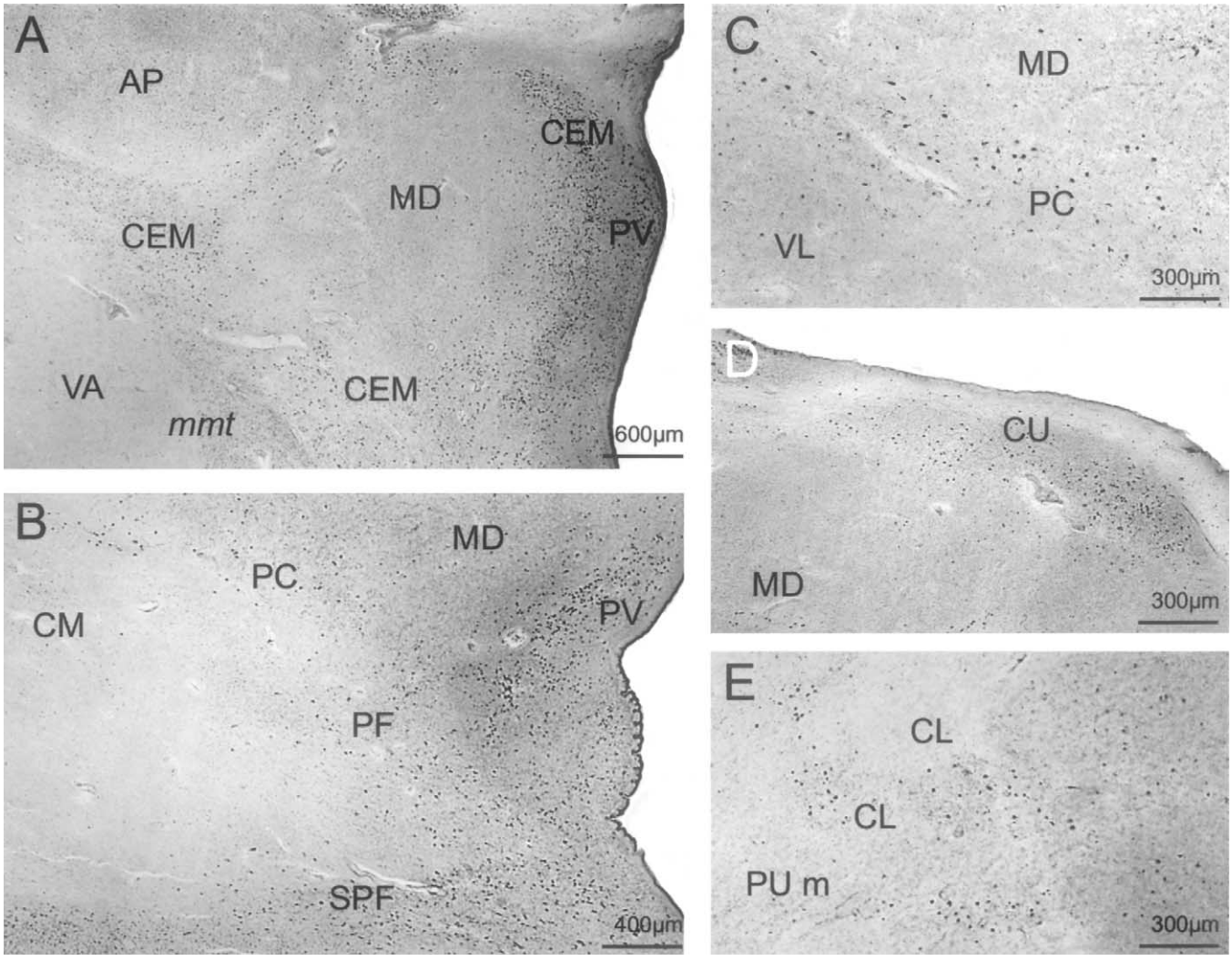

Fig. 5. AD-related argyrophilic neurofibrillary pathology in the thalamic intralaminar nuclei at cortical NFT/NT-stage VI (case 25, Tables 1 and 2). (A–E) PEG sections, modified silver-iodide Gallyas stain, 100 µm. For topographical orientation see Fig. 1. (A) Central medial nucleus (CEM). (B) Centromedian nucleus (CM), parafascicular nucleus (PF), subparafascicular nucleus (SPF). (C) Paracentral nucleus (PC). (D) Cucullar nucleus (CU). (E) Central lateral nucleus (CL). AP, anteroprincipal nucleus; MD, mediodorsal nuclei; *mmt*, mammillothalamic tract (Vicq d'Azyr); PU m, pulvinar, medial nucleus; PV, paraventricular nuclei; VA, ventral anterior nucleus; VL, ventral lateral nucleus.

thereby reflecting the clinical course of AD (Braak and Braak, 1991a; Bancher et al., 1993; Braak and Braak, 1997; Grober et al., 1999).

Clinicians agree that parameters capable of serving both as early diagnostic markers for AD and also as guidelines for monitoring its clinical progression and the effects of therapy are needed (Almkvist and Winblad, 1999). The findings by some studies that alterations in the experience of pain and reactions to pain become progressively aggravated with worsening cognitive impairment in AD (Bendetti et al., 1999; Huffman and Kunik, 2000) provide possible clues to the development of suitable parameters designed to fulfil these diagnostic demands. Currently, a number of methods are available that allow quantification of experience and reactions to painful stimuli within the framework of clinical and experimental pain assessment even in non-communicative patients (Huffman and Kunik, 2000). Given the progressive involvement of the intralaminar nuclei and other medial pain system components in the AD-related cytoskeletal pathology, and bearing in mind the alterations of the non-discriminative aspects of pain that take place in the course of the disease, ethically-based clinical studies specifically designed to improve the early diagnosis and progression of AD via assessment of dysfunctions within the medial pain system are needed. Should such studies prove effective, AD would be among the disorders of the nervous system capable of detection by

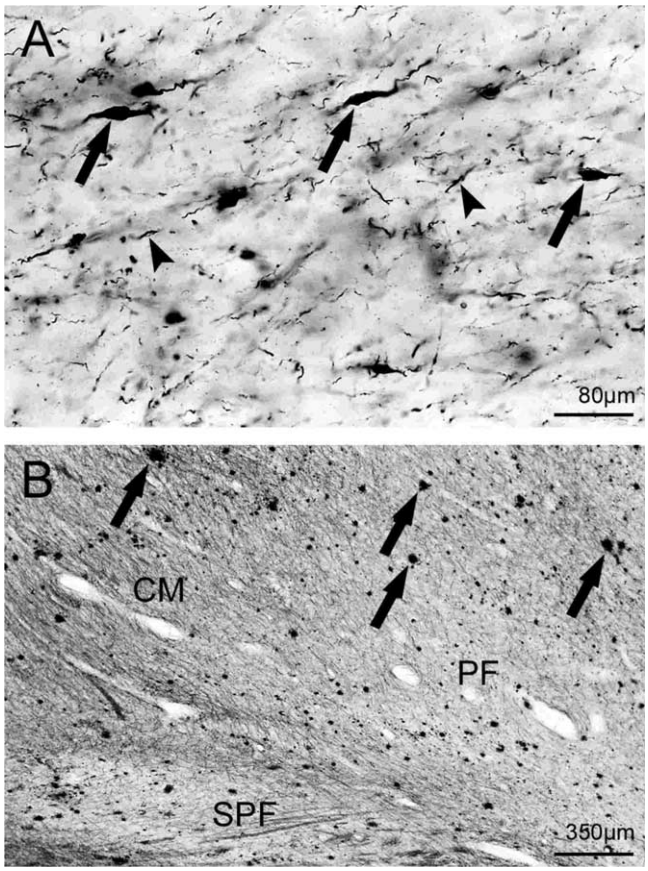

Fig. 6. (A) Immunoreactive cytoskeletal pathology in the subparafascicular nucleus (case, 14, Tables 1 and 2) shown at higher magnification. Arrows point to immunopositive nerve cell somata and arrowheads mark immunoreactive nerve cell processes (PEG section, AT8-immunostaining, 100 µm). B. AD-related β-amyloid deposits (arrows) in the caudal intralaminar nuclei (case 16, Tables 1 and 2) (PEG section, modified silver pyridine Campbell-Switzer staining, 100 µm) (CM, centromedian nucleus; PF, parafascicular nucleus; SPF, subparafascicular nucleus).

alarm signals dispatched by pain system components even prior to the manifestation of pathognomonic symptoms.

Acknowledgements

This study was supported by a grant from the Deutsche Forschungsgemeinschaft. The skillful assistance of Ms M. Lazar and B. Meseck-Selchow (processing of tissue sections and immunocytochemistry), M. Walter (technical support), and Ms I. Szász (graphics) is gratefully acknowledged.

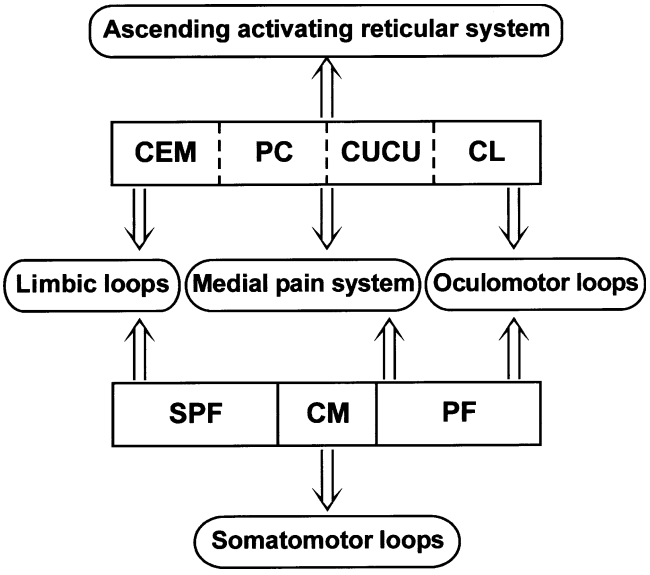

Fig. 7. Simplified block diagram demonstrating the involvement of the intralaminar nuclei in various functional circuits of the human central nervous system. CEM, central medial nucleus; CL, central lateral nucleus; CM, centromedian nucleus; CU, cucullar nucleus; PC, paracentral nucleus; PF, parafascicular nucleus; SPF, subparafascicular nucleus.

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