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SPECIAL ISSUE

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Correlations between mental state and quantitative neuropathology in the Vienna Longitudinal Study on Dementia

Abstract Quantitative clinicopathological correlation studies are one way to address the question of the relevance of morphological abnormalities in Alzheimer's dementia (AD). This paper summarizes results of the Vienna Longitudinal Study on Dementia obtained during the past few years and presents a critical discussion on the relevance of clinicopathological correlation studies for the pathogenesis of AD. Plotting of psychometric test scores against the numbers of plaques, tangles and neuropil threads in various cortical areas shows that significant correlations are due primarily to very high lesion counts in severely demented patients. These data indicate that neocortical neurofibrillary pathology can be considered an end-stage marker in the pathology of AD. On the other hand, the topographical staging of neuritic Alzheimer changes proposed by Braak and Braak (1991) appears to be a better reflection of the progression of the degenerative process than numerical lesion counts; there is a linear correlation between the Braak stages and Mini-Mental State scores in 122 aged individuals. Significant correlations are further obtained between the severity of dementia and the levels of a number of synaptic proteins including synaptophysin and the chromogranins. Taken together, our data suggest that none of the classical AD lesions, plaques and tangles, play a central role in the pathogenesis of dementia, a fact that is supported by a molecular biological study showing that there is no close relationship between these lesions and the neurons undergoing degeneration in AD. Whereas neuritic pathology is a useful histopathological marker for the diagnosis and staging of AD, the major correlate of cognitive deficits is the loss of corticocortical and subcorticocortical connections reflected by a depletion of synapses. This pathology may be induced by a mismetabolism of the β-amyloid precursor proteins or their interaction with cytoskeletal proteins related to neuronal degeneration.

Key words Alzheimer's dementia · Clinicopathological correlations · Neurofibrillary tangles · Senile plaques · Pathogenesis · Synapse Poss · Apoptosis

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Introduction

One of the key questions in basic research on Alzheimer's disease (AD) concerns the relevance of morphological, biochemical or molecular genetic changes in the pathogenesis of the dementia syndrome. Major current research efforts try to assess which of the many abnormalities described in AD patients may be *causally* involved in the genesis of the neurodegenerative process. Strategies aiming at this goal include the study of animal models, transgenic animals, and cell culture systems in which the role of given biochemical or genetic factors can be studied individually. These approaches, however, have obvious limitations and cannot usually be performed on human material.

Clinicopathological studies correlating the severity of the dementing syndrome and quantitative measures of structural and biochemical changes are one approach to obtain helpful information on the role of a given abnormality. A number of papers have addressed this question and significant correlations for senile plaques (SP), neurofibrillary tangles (NFT) and various other morphological and biochemical parameters have been found (Crystal et al. 1988; Terry et al. 1991; Price et al. 1991; McKee et al. 1991; Arriagada et al. 1992; DeKosky et al. 1992; Jellinger et al. 1992; Berg et al. 1993; Braak et al. 1993;

Kazee et al. 1993; Samuel et al. 1994a, b; Bierer et al. 1995). The number of NFT has been shown to correlate inversely with cerebral metabolic rates of glucose as measured by PET (DeCarli et al. 1992). The results of the studies referred to are partly conflicting, and this is mostly due to methodological issues. Early work describing significant correlations between mental state and the numbers of SP and NFT has included variable numbers of non-demented controls (Blessed et al. 1968; Tomlinson and Henderson 1976; Wilcock and Esiri 1982), a procedure that is only justified if one considers AD and normal aging as a continuum. The inclusion of such controls may have contributed to the inflation of the correlations, thus limiting their relevance concerning the question of the significance of the lesions investigated in the pathogenesis of AD.

Since 1986 a prospective, longitudinal study on dementia in old age has been performed in Austria (Fischer et al. 1991; Jellinger et al. 1992). This paper reports some of our current results on the relationship between psychometric test scores and a number of structural and biochemical findings, and presents a critical discussion on the relevance of clinicopathological correlations for the diagnosis and the pathogenesis of AD.

Patients and methods

Patients are recruited from two major geriatric centres in Austria. The majority are chronic inpatients admitted to the neurological department of a large geriatric hospital in Vienna for various causes of dementia. They are included in the Vienna Prospective Study if they or their relatives give informed consent. The second group of patients is from the psychogeriatric department of a large county hospital in Linz, Upper Austria. Patients are admitted for various psychiatric problems. Only those patients in whom adequate clinical documentation is available, including psychometric testing, are entered into the study.

Clinical workup and psychometric testing

Patients admitted to both departments undergo extensive routine clinical workup including a detailed patient history (often informant based), mental state, neuropsychological testing, neurological examination and internal medical examination. Routine blood tests include thyroid function, vitamin B_{12} and folic acid levels. An EEG and cranial CT scan are carried out routinely. An MRI examination and Duplex ultrasound of the carotid arteries are performed in selected cases.

In the Vienna cohort patients are exposed to extensive psychometric testing including the Mini-Mental State examination (MMS; Folstein et al. 1975), the Token Test (DeRenzi and Vignolo 1962; Orgass 1982), the Intracategorical Delayed Selective Reminding List Learning Procedure (Buschke and Fuld 1974) and the original naming subtest of the Aachen aphasia battery (Huber et al. 1982). This test battery is repeated every 6 months until the patients' deaths. Test results obtained less than 3 months prior to death are rejected for possible interference of terminal factors with the underlying dementing disorder. The average time between last testing and patient death is 6.8 months. The average age at death is 77.6 years (SD 9.0 years) and the mean duration between admission and death is 5.1 years (SD 1.9 years). Because of the ratio of female to male wards, women are overrepresented by 5 to 1. The MMS scores are in the range between 00 and 28 (Mean 12.4). Exclusion criteria are a history of head trauma, alcoholism, major psychiatric illness other than dementia, neurological diseases, such as multiple sclerosis or brain tumours, malignancies, severe cardiorespiratory disease and education of less than 5 years. Diagnosis of dementia is made according to DSM-III-R and ICD-9. A clinical diagnosis of probable or possible AD is made according to NINCDS-ADRDA criteria (McKhann et al. 1984).

In the Linz cohort patients are exposed to a similar clinical workup, including the above-mentioned neurophysiological and neuroimaging studies, and at least one MMS score.

Clinical diagnostic neuropathology

Brains are removed between 4 and 36 h (mean 18 h) post mortem and the left hemisphere is frozen for biochemical studies. For histology, multiple blocks of at least three neocortical areas (middle and superior frontal gyrus at the convexity, superior parietal cortex and inferior temporal gyrus), hippocampal formation at two coronal levels, basal ganglia, hypothalamus, midbrain, pons, medulla oblongata and cerebellum are embedded in paraffin. Sections are routinely stained with hematoxylin and eosin, cresyl-violet, luxol fast blue, and modified Bielschowksy's (Yamamoto and Hirano 1986) and Reusche's (Reusche 1991) silver stains. A definite diagnosis of AD is established by two neuropathologists (K. J and C. B.) according to four published sets of histopathological criteria including NIH (Khachaturian 1985), CERAD (Mirra et al. 1993), Tierney (Tierney et al. 1988) and the histopathological staging of neuritic Alzheimer lesions (Braak and Braak 1991). The diagnosis of vascular dementia is based on current criteria (Jellinger and Bancher 1994).

Immunohistochemistry

Immunostaining is performed on at least three neocortical areas and the hippocampal formation. Antibodies against the following antigens are used:

1. β-A4 (mouse monoclonal, 4G8; Kim et al. 1988) for the detection of amyloid deposits

2. Microtubule-associated protein tau (mouse monoclonal, tau-1, Binder et al. 1985) for the detection of paired helical filaments (PHF)-containing lesions, i.e. NFT, neuropil threads (NT) and the neuritic component of SP

3. Ubiquitin (mouse monoclonal, 3-39; Wang et al. 1984) for the detection of NFT at late maturation stages and cortical Lewy bodies.

Quantitative neuropathology

Lesion counts are performed by an observer blind to the clinical and histopathological diagnosis (H. L.) on slides stained with Bielschowsky and anti-tau. An area of 6 mm^2 at magnification × 250 is evaluated in frontal, parietal and temporal isocortices. The hippocampal sectors CA1, CA3 and the subiculum are scored in toto. Neuropil threads are counted in a 30 μm broad band spanning the entire cortical thickness at magnification × 1000.

Statistical evaluation

Statistical analysis is performed using the Statistical Package for the Social Sciences (Nie et al. 1975). Nonparametric Spearman correlation coefficients are calculated between test scores and lesion counts. The level of significance is 0.05 (two-tailed).

Quantitative immunoblotting

Quantitative immunoblots are performed on homogenates of fresh frozen standardized regions of frontal and temporal cortices. The following antisera/antibodies are used:

1. Synaptin/synaptophysin (rabbit polyclonal, courtesy of R. Jahn, Munich)
2. SV2 (10 H3) and p65 (mouse monoclonals)
3. Chromogranin A (rabbit antiserum; Fischer-Colbrie et al. 1989)
4. Secretogranin II (rabbit antiserum; Weiler et al. 1987)

Histochemical detection of DNA fragmentation

DNA fragmentation is visualized with the technique described by Gold et al. (1993, 1994) using terminal transferase as the detecting enzyme and digoxigenine labeled nucleotides. Bound digoxigenine is detected by immunohistochemistry (Lassmann et al. 1995).

Results

Correlations between lesion counts and psychometric test scores in the Vienna Longitudinal Study on Dementia

Statistical correlations between the performance of 19 histopathologically confirmed AD patients on four psychometric test scales and lesion counts in three isocortical and three allocortical areas are shown in Table 1. The number of SP as seen with the Bielschowsky method can be equated approximately to the total number of SP, i.e. neuritic plaques (NP) and amyloid deposits. The number of SP immunostained with tau-1 corresponds to those plaques having a neuritic component containing PHF (Barcikowska et al. 1989). The number of NFT immunostained with tau-1 corresponds to all intracellular tangles, excluding pretangles (Bancher et al. 1991).

Table 1 shows that statistical correlations of different significance levels between lesion counts and psychometric scores can be found for almost all AD changes in three neocortical areas if patients at the end stage of the disease, who were not testable due to severe dementia (MMS: 00), are included (total 6 patients). Correlations are strongest for PHF-containing lesions (NFT, NP and NT) in parietal and frontal isocortices, and somewhat weaker in the temporal isocortex. There is no clear difference in the significance levels between the four psychometric scales used in isocortical areas. Correlations are weaker in the subiculum and the hippocampal formation. The psychometric scale focusing on short-term memory (Intracategorical Delayed Selective Reminding) shows a strong correlation with lesion counts in the CA1 sector of the hippocampus, a structure that is known to be involved in short-term information storage and retrieval (Markowitsch 1994).

Table 1 Spearman nonparametric correlation coefficients between neuropsychological test scores and histopathological lesion counts in Alzheimer's disease (AD)

		MMS		NAM		TT		IDSR	
		All	Testable	All	Testable	All	Testable	All	Testable
Frontal	SP	–0.477*	0.197	–0.501*	0.399+	–0.486	0.455	–0.394	0.686++
	SP-tau	–0.679****	–0.012	–0.608***	0.275	–0.574**	0.410	–0.547*	0.487
	NFT	–0.565***	0.507+	–0.796****	–0.306	–0.644***	0.488	–0.778****	–0.177
	NT	–0.622***	0.034	–0.569**	0.177	–0.510*	0.359	–0.632***	0.016
Parietal	SP	–0.681***	–0.276	–0.705***	0.214	–0.777****	–0.144	–0.753***	0.074
	SP-tau	–0.824****	–0.338	–0.771***	–0.089	–0.788****	–0.180	–0.796****	0.000
	NFT	–0.686***	0.068	–0.760***	0.126	–0.631*	0.791+	–0.728***	0.206
	NT	–0.710***	0.094	–0.822****	–0.273	–0.843****	–0.376	–0.744***	0.132
Temporal	SP	–0.608***	–0.267	–0.423*	0.392	–0.591**	0.021	–0.424*	0.313
	SP-tau	–0.649****	–0.274	–0.435*	0.229	–0.500*	0.122	–0.478*	0.235
	NFT	–0.553**	–0.044	–0.485*	0.287	–0.509*	0.274	–0.580**	–0.066
	NT	–0.523**	–0.117	–0.451*	0.238	–0.517*	0.153	–0.498*	0.163
Subiculum	SP	–0.230	0.532+	–0.461*	0.483+	–0.423*	0.609+	–0.565**	0.205
	SP-tau	–0.474*	0.166	–0.624***	0.089	–0.557*	0.165	–0.564*	0.241
	NFT	–0.445*	0.024	–0.330	0.400	–0.396	0.285	–0.395	0.287
	NT	–0.462*	0.105	–0.417*	0.294	–0.432*	0.274	–0.362	0.303
CA1	SP	–0.451	0.164	–0.787***	0.293	–0.774***	0.396	–0.818****	–0.099
	NFT	–0.522*	0.611	–0.754***	0.371	–0.709**	0.667	–0.760***	0.174
CA3	SP	–0.589*	0.039	–0.878****	–0.393	–0.724**	0.664	–0.792***	–0.133
	NFT	–0.561*	0.764+	–0.745***	0.525	–0.714**	0.752+	–0.712**	0.564

All: 19 patients with histopathologically confirmed AD
Testable: the 13 patients testable with the MMS (MMS > 00)

SP: plaques seen in sections stained with the Bielschowsky method
SP-tau, NFT, NT: neuritic plaques, neurofibrillary tangles and neuropil threads seen in sections immunostained for tau
MMS Mini-Mental State; NAM Object naming; TT Token Test; IDSR Intracategorical Delayed Selective Reminding (verbal list learning)

*, **, ***, ****: significant at level 0.05, 0.01, 0.005, 0.001 in expected direction (higher score goes with lower lesion count) +, ++: significant at level 0.05, 0.01 in opposite direction (higher score goes with higher lesion count)

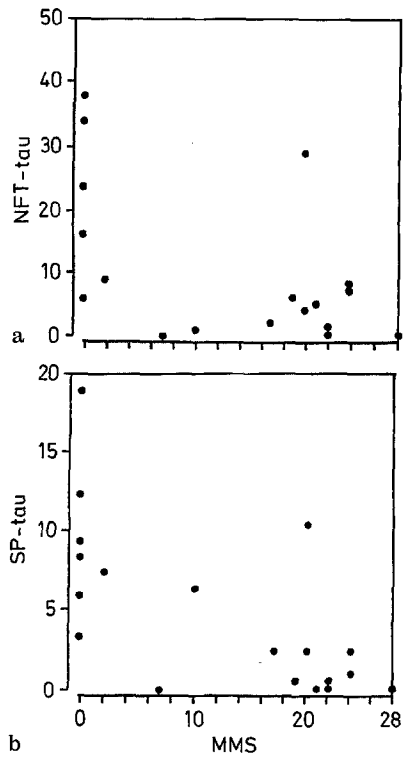

Fig. 1 a, b **a** Relationship between Mini-Mental State (MMS) score and the numbers of neurofibrillary tangles (NFT; seen with tau-1 immunostain) in inferior temporal isocortex. $r = -0.702$, $p < 0.01$. **b** Relationship between MMS score and the numbers of neuritic plaques (seen with tau-1 immunostain) in inferior temporal isocortex. $r = -0.610$, $p < 0.005$

The plot of the number of NP and NFT stained by anti-tau in the temporal isocortex against the MMS score is shown in Fig. 1. It is remarkable that the correlation is not linear and that high numbers of lesions are only found in severely demented patients. Indeed, the high significance of the correlation is due largely to the fact that those 6 patients with MMS 00 have very high numbers of lesions. If these 6 patients are excluded, all correlations between psychometric measures and quantitative neuropathology disappear (Table 1). In this case even a few correlations in the "wrong" direction emerge, i.e. less-demented patients have higher lesion counts in this remaining group of mildly and moderately demented patients (Fischer et al. 1991; Jellinger et al. 1992). These data are not inflated by the inclusion of severely demented patients with MMS scores of 00 and of normal controls with no or very few lesions. The inclusion of such patient groups may have helped to establish significant correlations between severity of dementia and histopathological changes, particularly SP, in previous studies (Blessed et al. 1968; Tomlinson and Henderson 1976; Wilcock and Esiri 1982).

The plots of the results of three other comparable clinicopathological studies (Delaere et al. 1989; Wettstein and Lang 1990; Dickson et al. 1995) show a similar hyperbolic distribution, confirming our observation on a small number of patients. Our results are further supported by two studies that have compared the numbers of plaques and tangles in biopsy and autopsy samples of the same patients, which were collected with a time lapse of several years. No increase of AD lesions could be observed between the two samples in those patients dying at a moderate stage of progression of AD (Mann et al. 1988; Bennett et al. 1993).

In summary, there is no correlation between severity of dementia and the number of NFT, NP and NT in a cohort of 13 mildly to moderately demented AD patients when normal controls and severely demented, nontestable patients are not taken into consideration. Inclusion of 6 severely demented patients at the end stage of the disease leads to the emergence of significant negative correlations due to a sharp increase of the number of neuritic lesions in these patients. These data suggest that large numbers of PHF-containing lesions (NFT, NP and NT) in the neocortex are found only in late stages of the dementing process. Thus, it appears unlikely that these lesions play an essential role in the pathogenesis of neuronal dysfunction. These data, together with the fact the NFT can be found in a variety of other unrelated central nervous system disorders, some of which have known aetiologies (Wisniewski et al. 1979), are in accordance with the notion that these lesions are epiphenomena of another primary underlying pathogenic process (Jellinger et al. 1994).

Correlations between MMS and histopathological AD stages

Braak and Braak (1991) have proposed a model for the progression of neurofibrillary pathology in human brain that is based on a topographical spreading of NFT and NT over different allo- and isocortical areas. In contrast to SP and amyloid deposits, this spreading pattern is highly hierarchical and predictable for NFT. This concept differs from the notion that increasing numerical lesion counts within a given brain area represent a reflection of the progression of the degenerative process. According to this concept, the earliest NFT are found in the transentorhinal cortex, a small allocortical area located at the junction between the entorhinal cortex and the adjacent inferior temporal isocortex, at a time when dementia is not yet clinically manifest, possibly decades before its onset (stage I; Ohm et al. 1995). From this region NFT spread into the superior pre-alpha layer of the entorhinal cortex. At this stage (stage II), the hippocampus and the isocortex are still free of NFT. Both "transentorhinal stages" are usually clinically silent. It is only at stage III, when additional neurofibrillary changes appear in the hippocampus, that memory impairment may become manifest. Stage IV is characterized by more severe involvement of the hippocampus, presence of additional NFT in the deep layers of the entorhinal cortex and patchy involvement of some neocortical areas. Stages III and IV together are termed "limbic stages". Stage V is characterized by the presence of moderate numbers of NFT in all isocortical association areas, whereas in stage VI there is severe involvement of the entire cortex, many subcortical nuclei and several

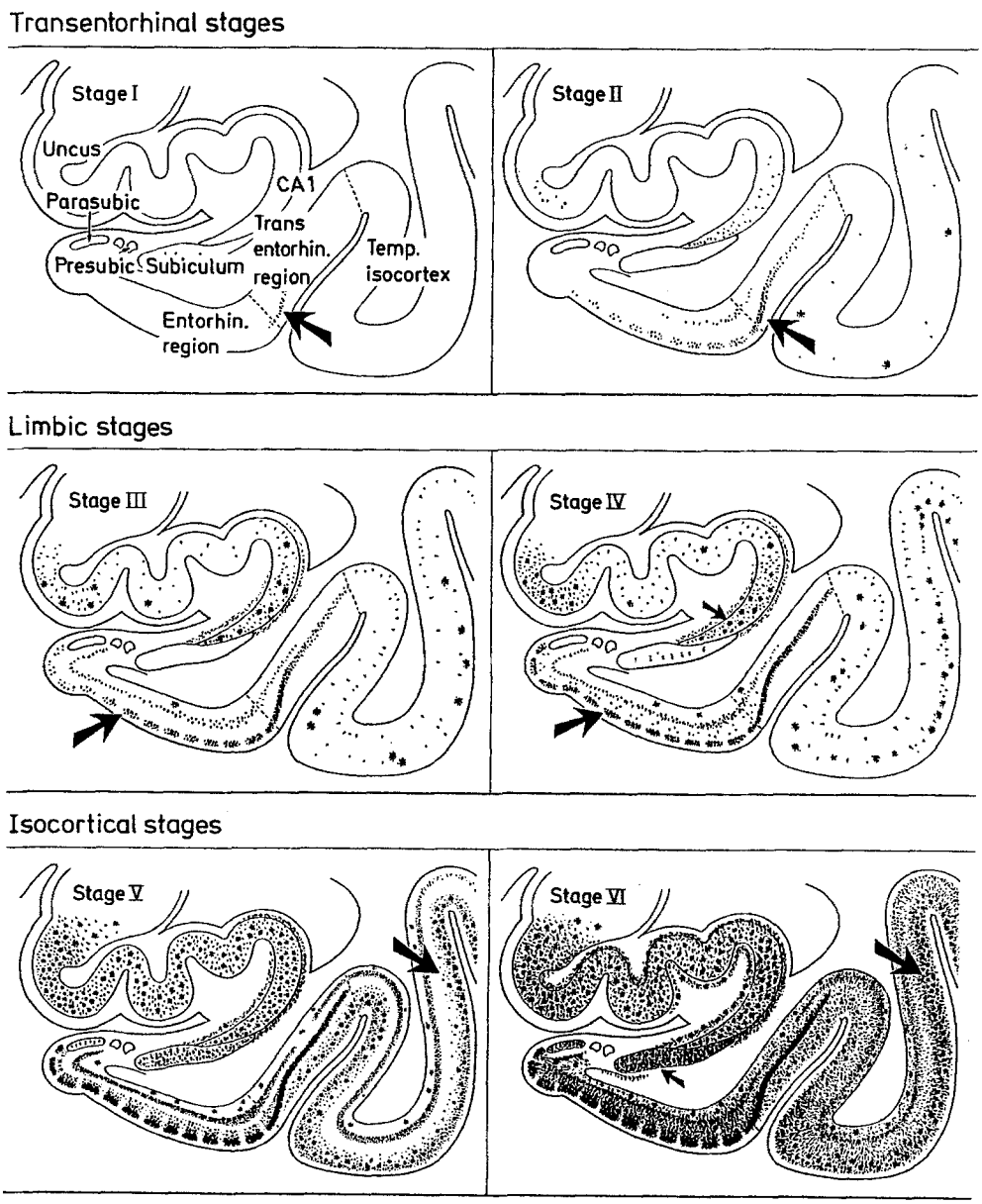

Fig. 2 Hierarchical spreading pattern of neurofibrillary changes seen in the hippocampal formation as well as in entorhinal and transentorhinal regions, and the adjoining temporal isocortex. Note the development of changes from stage I to stage VI. The *arrows* point to leading charcteristics. parasubic: parasubiculum; presubic: presubiculum. (Reprinted from Braak and Braak 1991)

structures that usually remain free of NFT at the earlier stages, e.g. the dentate gyrus. The isocortical stages V and VI correspond to late disease stages and are invariably associated with dementia (Fig. 2).

We have performed a clinicopathological correlation study on a total of 83 elderly patients without parkinsonian symptoms (mean age 79.0 years) and 41 patients with Parkinson's disease (mean age 79.3 years) relating the severity of cognitive impairment (MMS) to the histopathological stages according to Braak and Braak (Bancher et al. 1993). In both cohorts there is a linear relationship between MMS and the Braak stages (Fig. 3) which shows correlations with high significance levels ($r = -0.8788$, $p < 0.0001$ for the aged group; $r = -0.8203$, $p < 0.0001$ for the parkinsonian group). Again, it is remarkable that the isocortical stages V and VI are almost seen exclusively in severely demented patients, illustrated by the cluster of patients with end-stage dementia (MMS 00–02) at stages V and VI. These data confirm our previous results and demonstrate that large numbers of isocortical NFT are seen only in severely demented patients, and that these lesions can be considered as an end-stage marker of the Alzheimer degenerative process.

On the other hand, patients at the early (trans)entorhinal stages I and II show a wide range of cognition performance (MMS 17–30), although most of them show no or only very mild cognitive changes. A similar range of mental state (MMS 07–25) is seen in the "limbic" stages III–IV. These data suggest that the relevance of entorhinal AD changes for the development of dementia and the hippocampal disconnection hypothesis of AD (Hyman et al. 1986) warrants critical reconsideration, at least in the earlier stages of AD (Samuel et al. 1994a).

The highly significant linear correlation between MMS scores and the Braak stages suggests that the hierarchical spreading of neuritic AD lesions from the (trans)entorhi-

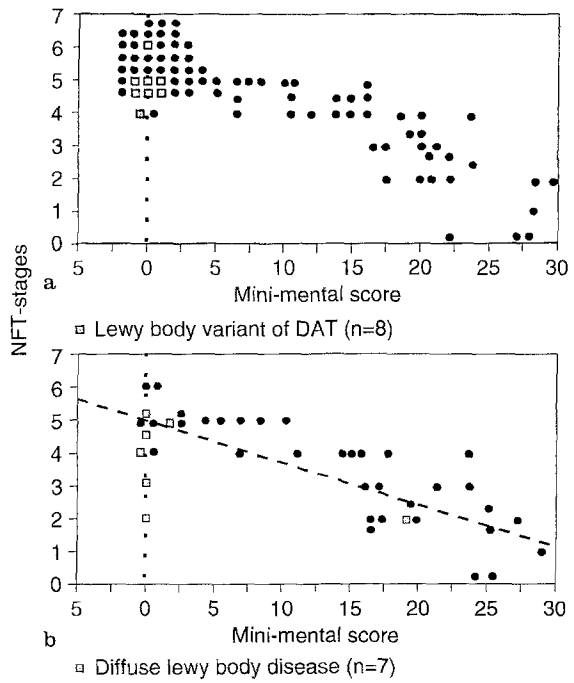

Fig. 3 a, b **a** Correlation between MMS socre and neuritic Alzheimer's disease (AD) stages in 83 demented and nondemented individuals without parkinsonian symptoms. $r = -0.8788$, $p < 0.0001$. **b** Correlation between MMS score and neuritic AD stages in 41 patients with Parkinson's disease. $r = -0.8203$, $p < 0.0001$

nal region via the hippocampus into isocortical association areas represents a better correlate of the progression of the degenerative process than purely numerical lesion counts within given brain areas (Bancher et al. 1993; Bierer et al. 1995). Therefore, it appears desirable that the histopathological staging of neuritic AD lesions be considered in future autopsy criteria for the diagnosis of AD.

Correlations between synaptic pathology and mental state in AD

Other clinicopathological correlation studies have shown that among a variety of investigated parameters typical for the AD brain, the one showing the closest correlation to the degree of dementia was the loss of cortical synapses revealed by the loss of anti-synaptophysin immunoreactivity (Terry et al. 1991; Terry and Masliah 1994). We have performed an immunochemical study on several markers for small synaptic vesicles (synaptophysin, p65 and SV2) and large dense-core vesicles (chromogranin A, secretogranin II) in AD brains and normal controls. While all three small vesicle markers and secretogranin II are lowered in AD temporal cortex, the levels of chromogranin A are increased. This yields a significant increase in the ratios of chromogranin A to synaptophysin, p65, SV2 and secretogranin II (Table 2).

There is a significant negative correlation between the Global Deterioration Scale (GDS) and the levels of the small vesicle markers and secretogranin II in the temporal cortex in 14 patients. A significant positive correlation was found between the GDS score and the level of chromogranin A (Table 2). In the frontal cortex correlations do not reach statistical significance, although the trends are similar. The ratios of chromogranin A to the other markers investigated are correlated with the GDS with a high level of significance. All significances disappear after the exclusion of controls (four brains) indicating a qualitative, rather than a quantitative, relation between synaptic proteins and dementia. The correlations between the levels of these proteins and the numbers of SP and NFT were also significant (Lassmann et al. 1992).

Table 2 Correlation between GDS socre and levels of synaptic proteins in 14 AD patients. r nonparametric Spearman correlation coefficient; p significance level; ChA chromogranin A; SgII secretogranin II; Syn synaptophysin; ChA/other marker: ratio of Chromogranin A to other marker. (From Lassmann et al. 1992)

	r	p
ChA	0.556	0.019
SgII	-0.473	0.044
Syn	-0.644	0.006
SV2	-0.537	0.024
p65	-0.674	0.004
ChA/SgII	0.624	0.009
ChA/Syn	0.695	0.003
ChA/SV2	0.674	0.004
ChA/p65	0.715	0.002

Relationship of plaques and tangles to dying neurons

Using a newly developed molecular biological technique to visualize dying cells by labelling nuclear DNA fragmentation in the tissue (in situ tailing; Iseki 1986; Gavrieli et al. 1992; Gold et al. 1993, 1994), we have identified the cells undergoing degeneration in 18 AD brains and 15 age-matched controls. Not only neurons, but also large numbers of oligodendrocytes and microglial cells, undergo nuclear DNA fragmentation. The vast majority of the cells, however, do not show the classical morphological features of apoptosis. On average, there are 23 times more glial cells and 55 times more labelled neurons in AD temporal cortex than in controls.

Because we are interested in learning more on the relationship between NFT and SP and the degenerating neurons, we performed a double labelling study for DNA fragmentation and immunohistochemical markers for SP and NFT. In 7 AD brains there is no correlation between the area covered by amyloid deposits and the number of degenerating cells. There is a weak, but significant, positive correlation between the number of dying neurons and that of NFT. In situ tailing and immunohistochemical counterstain for β-A4 (4G8) reveals that the somas of most degenerating cells (73%) are not located within or in contact with amyloid deposits. However, cell bodies located within an amyloid deposit are at a 5.7 times higher risk to

degenerate than those outside. Similarly, in situ tailing and immunohistochemical counterstain for PHF/ubiquitin (3–39) shows that most dying neurons do not bear a NFT, but NFT-bearing neurons are at a 3 times higher risk to degenerate than those without NFT (Lassmann et al. 1995).

The fact that cells whose somas are located within amyloid deposits have a higher risk to degenerate is intriguing and may reflect the cytotoxic action of fibrillized β-A4, which has been documented in a number of in vitro studies (Yankner et al. 1990; Yankner and Mesulam 1991). Because typical morphological hallmarks of apoptosis are rare, we believe that most neurons in AD die due to necrosis or some form of programmed cell death other than classical apoptosis. This is in line with the fact that β-A4 induces necrosis rather than apoptosis in tissue culture systems (Behl et al. 1994). However, because most degenerating cell bodies are not in contact with amyloid deposits and do not carry NFT, these lesions do not appear to be the only cause of cell death in AD.

Conclusions

A number of clinicopathological studies, including our work, have demonstrated significant correlations between psychometric measures of dementia and quantitative data concerning both the severity of classical AD pathology (SP and NFT) and various biochemical parameters (Blessed et al. 1968; Tomlinson and Henderson 1976; Wilcock and Esiri 1982; Crystal et al. 1988; Terry et al. 1991; Price et al. 1991; McKee et al. 1991; Arriagada et al. 1992; DeKosky et al. 1992; Jellinger et al. 1992; Berg et al. 1993; Braak et al. 1993; Bancher et al. 1993; Samuel et al. 1994a, b; Bierer et al. 1995; Dickson et al. 1995; Nagy et al. 1995). Our own data show significant correlations for the number of NP, NFT and NT, the histopathological AD stages according to Braak and Braak (1991), and the levels of several synaptic marker proteins. Although some discrepancies between individual studies warn against oversimplified interpretations of these results, at least two points appear to emerge clearly:

1. Large numbers of neuritic AD lesions in the neocortex are found only at late disease stages and, conversely, all patients with neocortical NFT are demented. These results are in line with data on brains of patients with Down's syndrome who invariably develop severe AD histopathology after age 40 years (Mann et al. 1986; Rumble et al. 1989). Studies that have examined brains of patients with Down's syndrome who have died at different ages show that the formation of PHF is preceded by the deposition of β-A4 in this condition (Mann and Esiri 1989); clinicopathological studies on AD patients have confirmed this result (Duyckaerts et al. 1988). This concept, together with our results, can also be reconciliated with the "amyloid cascade hypothesis", which states that the deposition of β-A4 amyloid is a primary pathogenic factor in AD (Selkoe 1994). In this model as well, PHF-containing lesions appear at a late stage in the pathogenic cascade leading to the manifestation of AD.

2. There is only a weak correlation between the amount of β-A4 deposits and the degree or even the presence of dementia (Delaere et al. 1993). In fact, nondemented or questionably demented aged individuals may have large numbers of amyloid-only SP (Crystal et al. 1988; Katzmann et al. 1988; Morris et al. 1991), a condition that has been defined as "pathological aging", possibly a preclinical stage of AD (Dickson et al. 1991). Moreover, in the neocortex, only SP with dystrophic neurites containing tau have been shown to be specific for AD (Shin et al. 1989) and recent criteria for the histopathological diagnosis of AD actually disregard plaques lacking a neuritic component (diffuse plaques) (Mirra et al. 1993).

The question now arises of whether a significant correlation between a certain type of change and the degree of dementia allows to draw conclusions as to the pathogenic relevance of the lesions in question. In principle, the lack of a correlation does not rule out that the change observed plays a pathogenic role. This is the case for the deposition of β-A4 which is found in both demented and nondemented individuals. Because neuronal circuits have a large amount of plasticity and the brain has a certain reserve capacity, it is conceivable that the clinical manifestation of dementia may be a threshold phenomenon, this threshold being set at different levels in different individuals. In this case, even large amounts of β-A4 deposits may be accommodated by the neuronal network without major impact on synaptic morphology (Masliah et al. 1990), cerebral glucose metabolism (DeCarli et al. 1992) or clinically evident loss of function. This possibility is in line with the fact that dementia is less prevalent in individuals with higher levels of education and increased occupational attainment (Evans et al. 1993; Stern et al. 1994), whose brains may have larger numbers of synapses and thus better reserve capacity delaying the onset of clinical manifestations. Retrospective autopsy series on the prevalence of AD lesions in this group and correlations with the prevalence of dementia may help to give additional information on this issue. Taken together, the points mentioned above suggest that the lack of correlation between presence or degree of dementia and the intensity of β-A4 deposition does not rule out a central role of this change.

On the other hand, the strong correlation between mental state and the number of neuritic AD lesions containing PHF (NFT, NP and NT) does not necessarily imply a causative role of these changes. Indeed, there is increasing evidence that a major part of these correlations is due to the presence of large numbers of neocortical NFT only at late disease stages when dementia has long been present. These data indicate that the formation of PHF in neurons cannot be an essential factor in the pathogenesis of dementia and suggest that neocortical neuritic AD lesions are epiphenomena of another underlying primary disease process. The significance of the early presence of NFT in certain allocortical areas (Braak et al. 1994) remains to be

elucidated and their role in possible transneuronal spreading of the degenerative process is under study.

Taken together, the points mentioned above put a question mark on the relevance of the classical AD lesions, deposition of β-A4 amyloid and accumulation of PHF in neurons, for the pathogenesis of cortical neuronal function loss in AD. Several immunochemical and ultrastructural studies on cortical synapses have shown a decrease in the number of synapses in AD brains; clinicopathological studies have reported highly significant linear correlations between this decrease and the severity of dementia (Terry et al. 1991, 1994). These data may indicate that a pathogenic process at the synaptic level may be the major correlate of the loss of higher cortical functions in AD, a notion which is supported by the synaptic localization of the amyloid precursor protein (Schubert et al. 1991; Shimokawa et al. 1993) undergoing aberrant processing in AD. In fact, studies of familial AD cases with mutations in the APP gene on chromosome 21 suggest that a mismetabolism of this protein due to certain amino acid substitutions in the APP sequence may be the cause of dementia in these cases (Haass et al. 1994; Felsenstein et al. 1994). It is plausible that this aberrant processing may play an important role in the pathogenesis of AD, whereas the resulting deposition of fibrillized β-A4 can be considered an epiphenomenon of this process. Interestingly, recent studies on the relationship between SP, NFT and dystrophic processes in AD and experimental data showing that injection of PHF-tau into rat brain induces β-A4 deposition suggests that the formation of NP could involve interactions between β-A4 and neuronal cytoskeletal proteins released into the extracellular space (Trojanowski et al. 1995; Masliah 1995).

In conclusion, our clinicopathological studies suggest that none of the classical AD lesions, plaques and tangles, are reasonable candidates for being at the origin of the dementing process in AD. The causal role of both amyloid and PHF is further questioned by our molecular genetic study, which shows that the cell bodies of most degenerating neurons in AD are not necessarily located in physical contact with amyloid deposits and do not carry NFT (Lassmann et al. 1995). Whereas neuritic AD pathology (NFT, NP and NT) is a useful histopathological marker for the diagnosis and the staging of the disease, the major correlate of cognitive deficit in AD is the loss of corticocortical and corticosubcortical connections reflected by the depletion of synapses in neocortical association areas, the pathogenesis of which remains unknown.

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