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Pathways and patterns of cell loss in verified Alzheimer's disease: a factor and cluster analysis of clinico-pathological subgroups

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Thirty-seven patients with neuropathologically verified Alzheimer's disease (AD) have been studied prospectively. A principal components analysis of neuron numbers in cortical and subcortical areas revealed two variables: Variable I with high loadings for the hippocampo-parahippocampo-parietal neuron counts and Variable II with high loadings for coeruleo-frontal cell numbers. Both may reflect functional neuroanatomical connections which may act as pathways of neurodegeneration in AD. A cluster analysis based on these neuron numbers yielded three groups of patients: Cluster A with low hippocampo-parahippocampo-parietal cell counts, Cluster B with well-preserved neuron numbers, and Cluster C with low coeruleo-frontal neuron numbers. Differences in clinical features between these patient groups indicated the potential clinical relevance of these clusters.

Keywords: Alzheimer's disease, neurodegeneration, sub-groups, neuropathology

INTRODUCTION

Alzheimer's disease (AD) manifests a large variety of clinical symptoms and signs, course characteristics and neuroradiological or neuropathological changes (Burns *et al.*, 1990a). Several attempts have been made to characterize clinical "subtypes" of AD and to corroborate these distinctions by post-mortem evidence of different types of underlying pathology (Berrios, 1985; Jorm, 1985; Bondareff *et al.*, 1987). Because of the paucity of clinico-pathological studies, it is still unclear whether the clinical heterogeneity of AD is related to different patterns of neurodegeneration which may develop along different functional pathways (Pearson *et al.*, 1985; Hertz, 1989).

Clinical or neuropathological evidence for different subtypes of AD should be validated by external criteria (Jorm, 1985; Mohr *et al.*, 1990). We have therefore examined both neuropathological and clinical variables in patients with verified AD. The following questions were addressed using principal components and cluster analysis:

(1) Can the variation of neuron counts in different brain areas be explained by principal components related to functional neuroanatomical pathways?
(2) Can different patterns of neuronal loss be detected which distinguish different clusters of patients?
(3) Are these potential subtypes of AD associated with other characteristic clinical features?

METHODS

Demographic characteristics of the patient sample, details about the prospective clinical examination and the neuropathological work-up have been published in previous papers (Burns *et al.*, 1990a; Förstl *et al.*, 1992a). The clinical diagnosis of AD according to NINCDS-ADRDA criteria (McKhan 1984 etal) was verified neuropathologically in 56 of the first 65 patients from a prospective longitudinal study who came to post-mortem examination (Förstl *et al.*, 1992a). The clinical examination was last administered within 12 months before death. It included the Clinical Dementia Rating (CDR; Berg, 1984), the Cambridge Cognitive Examination (CAMCOG; Roth *et al.*, 1986), the Geriatric Mental State Schedule (GMSS; Copeland *et al.*, 1976; Gurland *et al.*, 1976) and a standardized neurological examination (Burns *et al.*, 1990b).

TABLE I. Principal components analysis of neuron counts in different brain areas of 37 patients with verified Alzheimer's disease. The Eigenvalues refer to the variances of the unrotated principal components, whereas the loadings refer to the rotated variables

	Factor	
	I	II
Eigenvalue	2.03	1.58
Percentage of variance explained	29%	23%
Parietal lobe (Area 7)	0.81	−0.06
Parahippocampal gyrus	0.80	0.17
Hippocampus (CA1)	0.73	−0.01
Frontal lobe (Area 32)	0.14	0.72
Locus coeruleus	−0.10	0.85
Dorsal raphe nucleus	0.16	−0.22
Substantia nigra	0.16	0.44

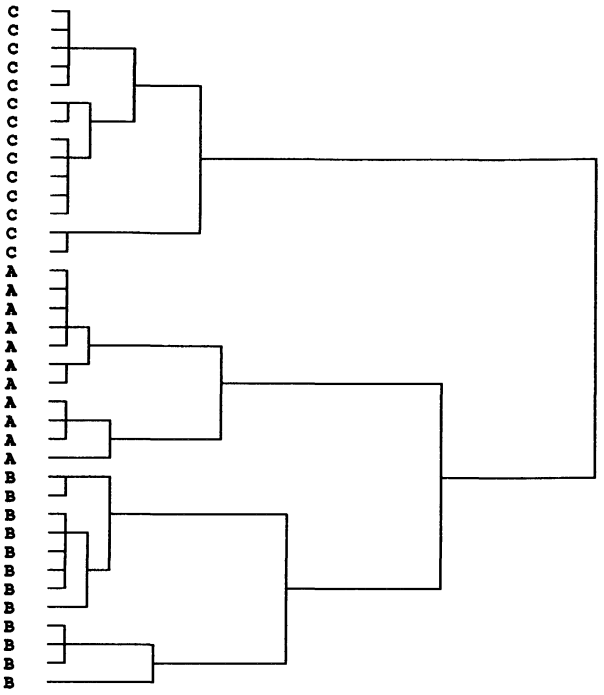

FIG. 1. Dendrogram demonstrating the hierarchical cluster structure based on cortical and subcortical neuron counts (A, B and C indicate cluster membership referring to Table II).

The following tissue blocks were taken from the brains, fixed in 10% formol saline and embedded in paraffin wax: frontal lobe (including Area 32), parietal lobe (Area 7), mediotemporal lobe (parahippocampal gyrus, hippocampus), mesencephalon (substantia nigra) and pons (including the largest diameter of the locus coeruleus and the dorsal raphe nucleus; Förstl *et al.*, 1992a). Fourteen μm sections were stained according to Klüver Barrera and impregnated with silver according to Glees and Marsland. Our earlier analyses had shown significant associations between the clinical features and neuronal change, therefore we decided to study neurons, and not plaques and tangles. Large neurons were defined as Nissl-positive, nucleolated cells with a maximum diameter of more than 20 μm in cortex (layer III), hippocampus (pyramidal cell layer of the CA1 field), substantia nigra and locus coeruleus, and of more than 25 μm in the dorsal raphe nucleus. All counts reported in this paper were carried out visually with an ocular grid at ×400 magnification. Numbers are given as counts per mm^2 for the cortical and hippocampal areas and as counts per nucleus per horizontal section for the brainstem nuclei. The examiner was blind to the clinical findings. A complete set of artefact-free slides and stains was available from 37 cases.

Variables accounting for the variance of cell numbers in different brain areas of the patients with verified AD were extracted with principal components analysis (Everitt and Dunn, 1983). The neuron counts were standardized using a z-transformation and the components were transformed orthogonally (Varimax rotation). Ward's method of cluster analysis was employed to differentiate the patterns of cell loss in the patient sample (Ward, 1963; Everitt, 1989). The differences of neuron numbers between the clusters were examined with a one-way analysis of variance and Scheffe's test for multiple comparisons (Maxwell and Delaney, 1990). The data analysis was carried out with SPSS/PC+ (Norusis, 1988).

RESULTS

A complete set of clinical and neuropathological data was available from 37 of 56 patients with neuropathologically verified AD. Principal components analysis based on the neuron counts in the frontal lobe (Area 32), parietal lobe (Area 7), the parahippocampal gyrus, the hippocampal CA1 field, the substantia nigra, locus coeruleus and dorsal raphe nucleus yielded two variables with Eigenvalues higher than 1 which accounted for 52% of the observed variance (Table I). Variable I accounted for 29% of the observed variance and showed high loadings for the cell counts in the hippocampus, parahippocampal gyrus and parietal lobe, indicating that the neuron numbers in these areas tended to vary together. Variable II had high loadings for the neuron numbers in the locus coeruleus and frontal lobe (Area 32).

The dendrogram of a cluster analysis based on these two variables is shown in Fig. 1. In a three cluster solution, 11 patients formed Group A

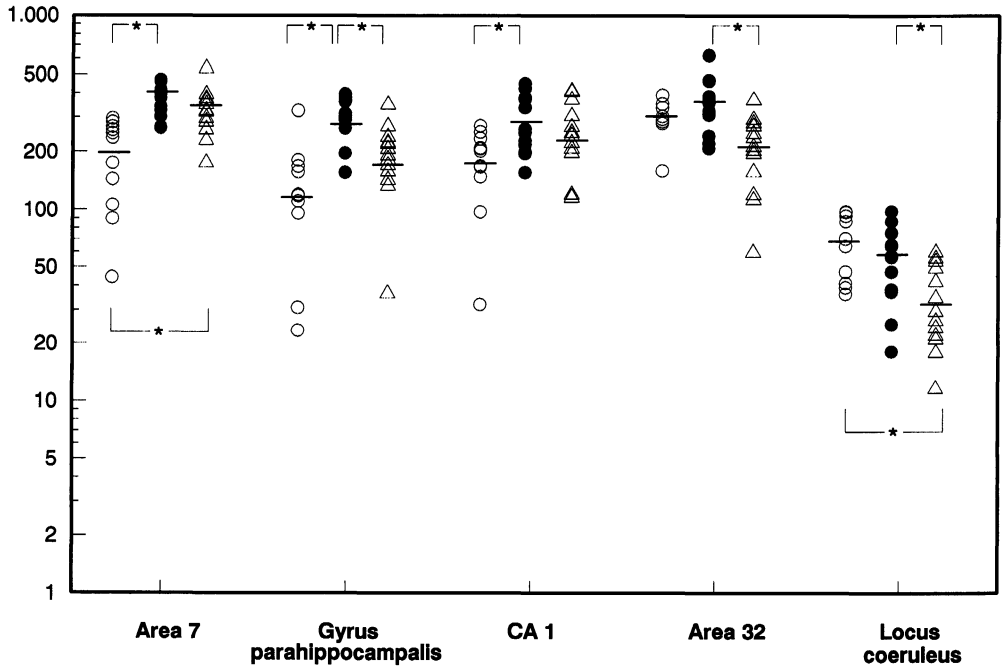

FIG. 2. Differences of neuron counts in the parietal lobe ($F = 9.0$, $p < 0.001$), parahippocampal gyrus ($F = 17.0$, $p < 0.001$), area CA1 of the hippocampus ($F = 3.8$, $p < 0.05$), frontal lobe ($F = 8.3$, $p < 0.01$) and locus coeruleus ($F = 9.3$, $p < 0.001$) between the patients in Cluster A (○), Cluster B (●) and Cluster C (△). *Significant between-cluster diffferences ($p < 0.05$) after application of Scheffe's test for multiple comparisons.

which was characterized by low hippocampo-parahippocampo-parietal neuron numbers (Fig. 2). Twelve patients formed Group B with well-preserved cortical and subcortical neuron numbers, but mildly decreased counts in the dorsal raphe nucleus. Group C consisted of 14 patients with predominant coeruleo-frontal cell loss. Solutions with larger cluster numbers will not be presented because of the small sample sizes.

The patients belonging to Cluster C had the earliest onset, the longest duration of illness, the highest rate of "frontal" release signs and the greatest density of intraneuronal neurofibrillary tangles (Table II). Depressive disturbances were least frequent in the group with the highest neuron counts in the locus coeruleus (Cluster A). Most delusions and hallucinations were observed in Cluster B. There were no significant differences between the groups regarding the clinical stage of dementia during the last year of life or performance on the last cognitive test administered.

TABLE II. Demographic and clinical features in three clusters of patients with verified Alzheimer's disease. None of the observed differences was statistically significant ($p > 0.05$)

	Cluster		
	A	B	C
n (♂/♀)	11 (3/8)	12 (5/7)	14 (2/12)
Age of onset (years)[1]	80 (64-88)	77 (60-87)	72 (58-90)
Duration (years)[1]	6 (2-11)	7 (4-15)	6 (2-20)
CAMCOG[1]	10 (0-57)	21 (0-46)	7 (0-57)
Family history	1	2	3
Clin. dementia rating (mild/moderate/severe)	1/2/8	0/2/10	0/2/12
Depression	1	4	4
Delusion/hallucinations	1/3	3/5	1/1
Myoclonus	1	4	2
Snout/grasp reflex	3/3	5/2	7/5

[1] Median (range).

DISCUSSION

The results can be summarized as follows:

(1) Principal components analysis revealed two variables underlying more than 50% of the observed variance of neuron numbers: a "hippocampo-parahippocampo-parietal" component and a "coeruleo-frontal" component.

(2) Three patterns of neuronal change emerged from a cluster analysis: hippocampo-parahippocampo-parietal cell loss (Cluster A), well-preserved neuron numbers (Cluster B), and coeruleo-frontal cell loss (Cluster C).

(3) The patient groups defined by these patterns tended to show different psychopathological, neurological and neuropathological features.

It has been shown that neurodegeneration in AD may extend along interconnected areas of the brain (Pearson *et al.*, 1985; Fewster *et al.*, 1991). The anatomical connection between the hippocampus, parahippocampus and parietal lobe is well established (Duvernoy, 1988; Cavada and Goldman-Rakic, 1989). In most cases of AD, changes in the hippocampus are severe and may precede less severe changes in other brain areas (Fewster *et al.*, 1991; Förstl *et al.*, 1993). A recent computed tomography and single photon emission tomography study demonstrated a close morphological and functional association between changes in the mediotemporal and parietal lobe occurring in the course of AD (Jobst *et al.*, 1992). Connections of similar functional importance may exist between the locus coeruleus and frontal lobe areas. Parallel changes of neuron numbers in the locus coeruleus and Area 32 may relate to neocortical noradrenergic projections which are most intense to the frontal lobe (Nagai *et al.*, 1981; Pearson *et al.*, 1990). Dopamine-β-hydroxylase, the noradrenaline synthesizing enzyme, is decreased in AD and this decrease is most severe in the frontal lobe (Adolfsson *et al.*, 1979; Cross *et al.*, 1981). The noradrenaline concentration in the frontal lobe is significantly correlated with the neuron numbers in the locus coeruleus (Ichimaya *et al.*, 1986). It has been hypothesized that neurodegeneration in AD may start in the aminergic brainstem nuclei (Hertz, 1989). A previous report indicated that this may be the case in patients with prominent affective disturbance early in the course of AD (Förstl *et al.*, 1992a). Regarding our first question, we hypothesize that the statistical relationship between neuron numbers in various areas of the brain may reflect anatomical connections shown in previous work and that these connections represent potential pathways of neurodegeneration in AD.

Alzheimer (1911) felt that the extent of neurofibrillary deposition may characterize subtypes of AD. It has been shown that patients with higher neurofibrillary tangle density have more severe clinical deficits and neuropathological changes (Terry *et al.*, 1987). This is in line with our findings in Cluster C, where patients had the highest intraneuronal tangle counts and the longest duration of illness. Our data indicate an association with "frontal" release signs and affective disturbance, the latter relating to neuron loss in the locus coeruleus (Förstl *et al.*, 1992a, b).

Bondareff (1982, 1987) distinguished mild senile AD1 from presenile AD2 with high genetic loading, rapid deterioration and severe neuropathological changes, typically a marked cell loss in the locus coeruleus. The patients in Cluster C did have the earliest onset and longest duration of illness, a positive family history in three cases and the worst cognitive performance, but none of these differences was statistically significant ($p > 0.10$). Berrios (1985) thought that a syndrome of "presbyophrenia" with predominant mnestic disturbance, confabulation and elated mood was related to neuronal loss in the locus coeruleus. This view cannot be supported by our data. They are in agreement with neuroimaging studies which described a subgroup of patients with clinically diagnosed AD and frontal hypometabolism (e.g. Haxby *et al.*, 1988). A recent investigation suggested a relationship between decreased frontal glucose metabolism or neuropsychological "frontal" lobe function and a faster progression of illness (Mann *et al.*, 1992).

Conversely, "parietal" lobe changes — sensory aphasia, visual agnosia, apraxia or even decreased radiodensity — have been related to rapid cognitive decline and increased mortality (McDonald, 1969; Naguib and Levy, 1982). These findings have not been replicated unequivocally (Gilleard *et al.*, 1987; O'Carroll *et al.*, 1991) and cannot be verified by our results. The patients in Cluster A with low hippocampo-parahippocampo-parietal neuron numbers had the shortest duration of illness, but this effect was not significant ($p > 0.10$).

The patient sample in our study was strongly biased towards senile dementia with a late onset of illness (Burns *et al.*, 1990a). The majority of the patients who came to post-mortem examination had reached a severe clinical stage of dementia according to CDR (Berg, 1984). Thus, neither large variations of demographic variables nor of cognitive test profiles were to be expected. These variables had contributed to the characterization of subtypes in earlier studies (Chen *et al.*, 1991; Chiu *et al.*, 1985; Mayeux *et al.*, 1985; Nyth *et al.*, 1991). Psychopathological, neurological and histopathological features however showed differences between Clusters A, B and C and these differences were in agreement with our previous analyses (Förstl *et al.*, 1992a, b).

It is still a matter of debate whether the clinical or neuropathological heterogeneity of manifestations can be subdivided into meaningful "subtypes" of AD (Jorm, 1985; Chui, 1987; Mohr *et al.*, 1990). The term "subtype" is open to different interpretations and the varying emphasis placed by previous authors may have contributed to the divergent and sometimes incompatible attempts at subclassification. Cluster analysis tends to yield results even if the data under investigation lack a clear structure (Everitt, 1989).

This may represent an additional source of conflicting results between cross-sectional studies based on different types of clinical information. In order to avoid a delineation of spurious or artefactual clusters we felt that the evidence for neuropathological subtypes should be strengthened by corresponding evidence of clinical differences between these clusters.

Our results suggest that subtypes of AD may be based on different patterns of neurodegeneration. It is unlikely that these subtypes are related to the admixture of other degenerative brain changes which had been allowed for by careful clinical and neuropathological examination. Patients with predominant coeruleo-frontal changes may benefit from different therapeutic strategies than those with predominant hippocampo-parahippocampo-parietal neurodegeneration. This and the stability of potential course characteristics will need further prospective investigation before the existence of subtypes of AD can be legitimately claimed (Jorm, 1985).

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