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Research report

Differential damage in the frontal cortex with aging, sporadic and familial Alzheimer's disease

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ABSTRACT

In order to understand relationships between executive and structural deficits in the frontal cortex of patients within normal aging or Alzheimer's disease, we studied frontal pathological changes in young and old controls compared to cases with sporadic (AD) or familial Alzheimer's disease (FAD). We performed a semi-automatic computer assisted analysis of the distribution of β-amyloid (Aβ) deposits revealed by Aβ immunostaining as well as of neurofibrillary tangles (NFT) revealed by Gallyas silver staining in Brodman areas 10 (frontal polar), 12 (ventro-infero-median) and 24 (anterior cingular), using tissue samples from 5 FAD, 6 sporadic AD and 10 control brains. We also performed densitometric measurements of glial fibrillary acidic protein, principal compound of intermediate filaments of astrocytes, and of phosphorylated neurofilament H and M epitopes in areas 10 and 24. All regions studied seem almost completely spared in normal old controls, with only the oldest ones exhibiting a weak percentage of β-amyloid deposit and hardly any NFT. On the contrary, all AD and FAD cases were severely damaged as shown by statistically significant increased percentages of β-amyloid deposit, as well as by a high number of NFT. FAD cases (all from the same family) had statistically more β-amyloid and GFAP than sporadic AD cases in both areas 10 and 24 and statistically more NFT only in area 24. The correlation between the percentage of β-amyloid and the number of NFT was significant only for area 24. Altogether, these data suggest that the frontal cortex can be spared by AD type lesions in normal aging, but is severely damaged in sporadic and still more in familial Alzheimer's disease. The frontal regions appear to be differentially vulnerable, with area 12 having the less amyloid burden, area 24 the less NFT and area 10 having both more amyloid and more NFT. This pattern of damage in frontal regions may represent a strong neuroanatomical support for the deterioration of attention and cognitive capacities as well as for the presence of emotional and behavioral troubles in AD patients.

1. Introduction

A growing body of evidence demonstrates that several frontal cognitive functions decline with age – among others, inhibitory control of attention [47], working memory [19], speed of mental processing [20] – which may however, also affect long term declarative memory. Reduced executive function influences memory because remembering often relies on controlled processing such as strategic elaboration during memorization and guiding search at retrieval. A selective frontal decline has been proposed as a *unified frontal aging hypothesis* [4]. On the other hand, patients with Alzheimer's disease (AD) also show attention and executive deficits which may appear rather early – or in the middle of the evolution – and affect cognitive as well as daily living activities [34]. Executive cognitive dysfunction and frontally mediated neuropsychiatric symptoms may be strong predictors of functional status, even after accounting for dementia severity and depressive symptomatology [3]. Assessing and predicting functional impairment in AD is critical for improving both diagnosis and care, and an emerging role of frontal dysfunction is discussed by several authors [2,39].

Although possible neural substrates of attention and executive tasks include a great variety of structures, frontal cortex plays a predominant part and has been comparatively much less studied than other cortical regions. It is hypothesized that a severe neurofibrillary tangle and/or amyloid pathology in specific areas of the frontal lobe could contribute to attention, executive and

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behavioral deficits in AD patients and may possibly define different subgroups of patients with differential pathology, in particular when considering sporadic and familial (FAD) Alzheimer cases. We have therefore studied in 10 normal controls and 11 AD patients including 5 FAD cases, pathological hallmarks in 3 different frontal areas, areas 10 and 12 of Brodman, linked respectively to the frontal polar/dorsolateral prefrontal and ventro-infero-median orbitofrontal circuits, as well as area 24 in the anterior cingulate gyrus. The role of these regions in different aspects of working memory, decision, choice determination, goal achieving, emotional behavior, is now increasingly studied [5,16,30,32,40,41] and will be discussed with respect to AD lesions.

2. Methods

2.1. Cases

A total of 21 brains were examined, 6 sporadic AD cases without known familial history, going from 67 to 87 years (mean age: 77.7 ± 8.2 years), 5 cases of the same family with early onset AD (FAD) going from 55 to 80 years (mean age: 67.2 ± 9.6 years) and 10 normal control cases divided in 5 young controls (mean age: 50.0 ± 12.8 years) and 5 old controls (mean age: 79.4 ± 9.3 years). All AD and FAD cases have been hospitalized in the Service of Old Age Psychiatry in Lausanne, and diagnosed following the DSM-IIIR criteria. Clinical diagnosis was confirmed by neuropathological examination in the Department of Pathology. All controls have been without neurological disorder and old controls were age-matched with sporadic AD cases. The mean age of FAD cases was lower, due to younger cases in the family (see Table 1).

2.2. Histology and immunohistochemistry

The brains were removed with a postmortem delay of no more than 24 h (except for one case) and stored in buffered 10% formaldehyde until sampling. Samples of cortex were then removed in three frontal regions: Brodman area (BA) 10 was removed from the dorsolateral part of the frontal lobe in the middle frontal gyrus, BA12 was removed from the ventro-infero-median part in the inferior frontal gyrus and BA24 was removed from the first anterior tier of the cingulate gyrus.

Blocks of approximately 5 mm × 5 mm were cut into 50 μm thick frozen sections and stained with the method of Gallyas [11] to reveal neurofibrillary tangles (NFT) degenerating neurons and with β-amyloid immunostaining to reveal amyloid deposits/senile plaques (antibody against Aβ 40, Sigma, 1:100) [23].

Parts of each block were also kept for paraffin embedding, then 20 μm thick sections were generated for immunohistostaining and densitometric measurement. Immunohistochemical staining was performed against glial fibrillary acidic protein, principal compound of intermediate filaments of astrocytes (polyclonal rabbit anti-human GFAP, Dako Z0334, 1:1500) and against phosphorylated neurofilament H and M epitopes (mouse monoclonal SMI-31, Sternberger, 1:5000); it has to be noted that this antibody reacts not only against phosphorylated neurofilaments [10] but also against phosphorylated Tau [25] and MAP1b [50]. After pretreatment for 10 min in methanol:H_2O_2 (97:3), 2 rinsing for 5 min in H_2O millipore, 2 rinsing for 10 min in PBS and 1 h incubation in normal serum (rabbit or swine), sections were then incubated overnight at 4 °C with the specific primary antibody. After 2 rinsing for 10 min in PBS, sections were incubated in secondary antibodies for 2 h at room temperature (swine anti-rabbit immunoglobulin, Dako E0353, 1:300) for GFAP staining and rabbit anti-mouse (Dako E0413, 1:300) for SMI-31, then again rinsed twice for 10 min in PBS and incubated for 1 h in ABC Complex/HRP (Dako K 0355). After rinsing twice in PBS, staining was revealed for 10 min in 0.03% 3,3′-diaminobenzidine (DAB; Sigma D 5637) and 0.015% H_2O_2/PBS. For each batch of sections, control consisted of omitting primary antibody. All sections were mounted with pertex.

2.3. Quantification of β-amyloid deposits and neurofibrillary tangles

Quantification of β-amyloid deposits and neurofibrillary tangles (NFT) was performed in the three areas, BA10, 12 and 24. This was performed using the Zeiss/Kontron image analysis system, having a stage with stepping motors for the three axes and a video camera for image capture (512 × 512 pixels). The histological section was moved under program control in order to scan the whole cortical depth along columns going perpendicularly from pia to white matter. For the best acuity, each individual field was viewed at a magnification of 10× for Aβ or 20× for NFT, which made the counting window respectively 568 or 284 μm wide and 408 or 204 μm height. In each brain, four cortical columns in two to three different sections were screened in all three areas. Taking into account the surface of the cortical column and the number and surface of β-amyloid deposits in the column – corresponding to senile plaques both diffuse and compact – we then calculated for each frontal region, the cross-sectional area covered by β-amyloid, which was expressed as a percentage of the measured cortical area. For NFT measurements, taking into account the mean number counted in the cortical column and the mean surface of the column (number of reticules between pia and white matter × surface of the reticule), we calculated the mean number of NFT per square millimeter of cortical surface in each area. Individual and mean data for each case and each group are given in Table 2 and also expressed in boxplots for comparison with densitometric data (see further) in Fig. 1.

2.4. Densitometry

Densitometric measurements for GFAP and SMI-31 were performed only in two areas, BA10 and 24, due to a limited number of sections in BA12. In each case, colour photographs were taken with the help of the KS400 image analysis system at a low magnification (1×) allowing to see the whole cortical plate and at the same illumination thanks to controlled voltage (6.45 V). Using the computer ImageJ analysis system (Java source code, free internet download at http://www.rsbweb.nih.gov/ij/), 10 densitometric measurements were performed on the colour files, along radial lines going perpendicularly from pia to white matter on each photograph. Measurements were obtained in arbitrary units, taking into account that 255 represents complete opacity (dark) and 0 complete transparency (white). A mean value was calculated for each case and then for each group. Background was measured on each slide just outside the section, to take into account the light going through the mounting medium, and was subtracted from the densitometric measurements before calculating a mean density value for each staining in each area.

2.5. Statistical analysis

For statistical analysis, we operated a rank transformation, before using a two-way analysis of variance to compare the amyloid percentage and the density of NFT between the four groups (young and old controls, sporadic and familial Alzheimer cases) and the three cortical regions (BA10, 12 and 24), or the mean densitometric value between the four groups and only two regions (BA10 and 24). A correlation analysis between parameters was also performed. We used the statistical analysis package SAS [42].

3. Results

3.1. Quantification of β-amyloid deposits and neurofibrillary tangles (Table 2 and Fig. 2)

Quantification was performed using the Zeiss/Kontron image analysis system scans of the whole cortical depth along columns going perpendicularly from pia to white matter. Young control cases had neither β-amyloid deposits nor NFT. Among old controls, only two had less than 1.0% of β-amyloid deposit per square millimeter of cortex, one control of 78 years (case no. 8) in areas 10 and 24, and another one of 93 years (case no. 10) in areas 10, 12 and 24.

All Alzheimer cases (AD) exhibited, except one case (no. 13), higher percentages of β-amyloid deposit in all three areas and the five familial cases (FAD) demonstrated still higher percentages. A two-way analysis of variance indicated that both the difference between groups ($p < 0.001$) and between areas ($p < 0.02$) were significant. In addition, the Tukey's test indicated significant differences not only between controls and Alzheimer cases, but also between sporadic AD cases and familial AD cases in all three areas.

Old control cases had absolutely no NFT in none of the three areas. This was also true for the 93 years old case exhibiting a small percentage of β-amyloid deposit (case no. 10). On the contrary, all Alzheimer cases, both AD and FAD cases revealed a rather high number of NFT/mm^2, the mean FAD percentage being higher than the AD one, although some individual cases revealed comparable numbers. This was particularly true for AD case no. 12, which had a heavy pathology similar to that in FAD cases. On the whole the inter-individual variability was high and the two-way analysis of variance indicated no significant difference between areas, but a significant difference between groups ($p < 0.001$). However, the Tukey's test indicated that the difference between AD and FAD groups was significant only for area 24. Taking into account all AD and FAD cases, a correlation analysis between the mean percentage of β-amyloid and the mean number of NFT per mm^2 was statistically significant only in cingular area 24 ($R = 0.67$; $p = 0.03$). Considering the distribution across the layers, the difference between AD and FAD cases was related to the amount of lesions. While in AD cases, NFT lesions were mainly localized in pyramidal layers 3 and 5, in FAD cases they were more broadly spread in the whole cortical plate, with