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Neuropathological changes in the nucleus basalis correlate with clinical measures of dementia

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Abstract The present study correlates the severity of dementia in Alzheimer's disease with the degree of neuropathology present in the nucleus basalis of Meynert. We assessed neurofibrillary tangles, neuronal loss and morphometric changes in 21 patients with Alzheimer's disease who underwent extensive neuropsychological testing before death. We report a highly significant correlation between scores in the psychological tests and all of the neuropathological markers examined within the nucleus basalis of Meynert. The test that correlated most closely with these morphological measures was Folstein's Mini Mental State. Among the different neuropathological changes, the number of neurofibrillary tangles was strongly correlated with the degree of dementia. We also provide evidence for a differential involvement of the three subdivisions of the nucleus basalis in Alzheimer's disease neuropathology. The posterior subdivision, which provides a substantial cholinergic input to the parahippocampal gyrus, was the more profoundly affected. Taken together, these results point to an important participation of the nucleus basalis in dementia of the Alzheimer type. In addition, the strong correlation between neuropathological changes and neuropsychological scores indicates the reliability of these tests in assessing the progression of the disease.

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Introduction

Confirmation of Alzheimer's disease (AD) diagnosis requires histological examination of tissue samples from the brain and the observation of senile plaques and neurofibrillary tangles (NFT). NFT occur primarily in projection neurons of limbic and association cortices, and it is believed that their presence disrupts the flow of information within the medial temporal lobe [5, 6, 8, 18]. Several studies have demonstrated significant positive correlations between the presence of NFT or plaques and the degree of premortem dementia as measured by neuropsychological tests [6, 14, 76, 86, 98, 104], but NFT are reported to be more reliable predictors of dementia [6, 45, 47, 75, 87, 88]. Clarifying the relationship among loss of neurons, development of NFT and measures of dementia is a central goal in defining the pathophysiological basis of AD.

There is substantial evidence relating the neuropsychiatric changes in AD to cholinergic deficits (for a review see [30]). Patients with AD show prominent mnemonic dysfunction [27] and deficits in attention, even in the early stages of the disease [64]. These attentional deficits could be, at least in part, the result of damage to the basal forebrain cholinergic system, in particular the nucleus basalis of Meynert (nbM) [31, 100]. The nbM occupies a pivotal position in the transmitter circuitry of the brain, since it provides the major source of cholinergic innervation to the neocortex (for a review see [33]). The nbM not only participates in certain kinds of learning [7, 81] but also plays a more global role in cortical processing and mediates plastic changes [61].

We have previously reported cell loss and nuclear hypertrophy in the topographical subdivisions of the nbM in a small sample from AD and control brains [55]. In the present study we extend those observations and correlate the neuropathological data with clinical scores of dementia.

Table 1 Demographic data from the control group of patients. Brain weight is given in grams (*PMD* postmortem delay – in hours, *n/a* not available)

Case	Age/sex	Brain weight	PMD	Primary diagnosis	Cause of death
X	63/M	1 025	3	Pulmonary carcinoma	Respiratory failure
Ñ	69/F	1 450	14	Arteriosclerosis	Myocardial infarct
P	74/M	1 460	3	Pulmonary carcinoma	Respiratory failure
E	74/F	1 120	n/a	Ovarian carcinoma	Septic shock
IZ	74/M	1 300	n/a	Hodgkin's disease	Hodgkin's disease
T	75/F	1 100	3	Pancreatic adenoma	Respiratory failure
PP	77/M	1 400	12	Septic shock	Respiratory failure
TT	80/M	1 240	n/a	Multiple myeloma	Respiratory failure
BZ	80/M	1 500	12	Choledocal carcinoma	Hepatic failure
VV	80/F	1 110	23	Diabetes mellitus	Diabetes mellitus
Q	86/F	n/a	n/a	Pulmonary fibrosis	Bronchopneumonia
UU	89/M	1 250	15	Arteriosclerosis	Hypovolemic shock
HZ	106/F	1 200	n/a	Cardiorespiratory lesion	Cardioresp. insufficiency

Table 2 Demographic, clinical and neuropsychological data of the Alzheimer's disease group of patients (*PMD* postmortem delay – in hours, *MMS* Mini exam of mental state, *DID* test of deficiency, incapacity and dependence, *insuf.* insufficiency)

Case	Age/ sex	Brain weight	PMD	Primary diagnosis	Cause of death	Dura- tion	MMS	Crich- ton	DID	Stage
EAD	71/M	1 450	12	Chronic bronchitis	Cardiopulmonar insufficiency	2				III
VAD	75/F	1 050	2	Cardiac arrhythmia	Cardiac insufficiency	2				II
AAD	77/F	846	6	Osteoporosis	Sepsis	5				III
PAD	79/F	n/a	3	Hypertension	Cardiac failure	3	9	42	65	III
TAD	79/F	950	2	Hip fracture	Congestive heart failure	4	9	44	68	III
DAD	80/F	1010	2	Arteriosclerosis	Cardiac insufficiency	2	14	40	62	II
GAD	80/F	1200	12	Dementia	Hepatic failure	2	18	28	45	II
HAD	80/F	1150	15	Oligophrenic dementia	Sudden death	3	9	45	65	III
SAD	82/M	1110	2	Carcinoma	Cardiac arrest	1				I
XAD	83/F	940	2	Ischemic cardiomyopathy	Cardiac arrest	1				I
IAD	84/M	n/a	12	Ischemic cardiomyopathy	Cardiac failure	3	19	36	41	II
OAD	84/F	1400	12	Chronic renal insuf.	Acute renal failure	1				I
FAD	86/F	1320	15	Chronic bronchitis	Pneumonia	4	9	47	67	III
MAD	86/F	960	2	Ischemic cardiomyopathy	Hypovolemic shock	2	18	30	48	II
QAD	86/F	997	2	Osteoporosis	Cardiac failure	4	15	34	47	II
BAD	88/M	1100	2	Cardiorespiratory failure	Cardiac arrest	2	18	32	39	II
CAD	90/M	1350	12	Ischemic cardiomyopathy	Cardiac failure	3				II
JAD	92/F	1120	12	Hypertension	Cardiogenic shock	10	9	47	69	III
KAD	94/F	1070	12	Ischemic cardiomyopathy	Sudden death	5	9	43	67	III
RAD	94/F	875	4	Osteoporosis	Bronchoaspiration	10	9	43	67	III
LAD	95/F	1090	8	Degenerative arthropathy	Bronchopneumonia	2	17	36	62	II

Methods

Case materials

Tissue blocks containing the nbM were collected at autopsy from 13 patients with no history of neurological disease and 21 patients diagnosed with AD. Some of these cases were included in previous reports [34, 55]. Tables 1 and 2 summarize the relevant data for all cases. In the selection of cases for the control group, we excluded those that showed any neuropathological change upon histological examination. To avoid the difficulties of interpretation of studies that have used as controls patients with cerebrovascular disease [38, 68], with psychiatric disease [40] or with alcoholic syndrome [26], brains from those categories were also excluded.

Gross and microscopical examination of the brains was performed by a neuropathologist blinded to the participants' cognitive test scores. In all cases, an AD diagnosis was confirmed using Khachaturian's criteria [60]. The brains were also screened to eliminate other possible sources of dementia, indcluding: multiinfarcts, Pick's disease, Parkinson's disease, and neoplasias.

Of our 21 AD cases 15 had been under institutional care at the Casa de Misericordia of Pamplona, Spain, where patient records have been maintained since 1987, including a clinical protocol to study the evolution of the dementias. In the remaining 6 AD cases the neuropsychological assessment described in their clinical histories allowed us to include them in the appropriate evolutive groups as follows. The 3 cases with a definite neuropathological diagnosis of AD but without any overt clinical manifestations were included in stage I. These cases correspond to clinically silent stages of the disease [18] or transentorhinal stages I–II of Braak and Braak [19]. The remaining 3 cases had a detailed history of dementia, and were categorized according to the Global Deterioration Scale (GDS) of Reisberg [80].

Table 3 Criteria used to classify the group of patients with Alzheimer's disease into three different evolutive stages (*MMS* Mini exam of mental state, *DID* test of deficiency, incapacity and dependence, *GDS* Global deterioration scale)

	StageI	Stage II	Stage III
MMS	20–30	10–20	< 10
Crichton	< 22 Slight invalidity	22–35 Moderate invalidity	> 35 Severe invalidity
DID	< 25 Slight invalidity	25–45 Moderate invalidity	> 45 Severe invalidity
GDS	1–3	4–5	6–7

Neuropsychological assessment

The clinical histories included the following tests: Mini-Mental State Examination (MMS) of Folstein, test of appraisal of behavior of Crichton [84], and test of deficiency, incapacity and dependence (DID) [49], which allowed us to determine the evolutionary stage of the disease (Table 3). The Folstein MMS is a well-established, reliable and valid brief cognitive screening instrument [44, 48], which is also included in the CERAD test battery [72]. This test is used to evaluate in a simple way the spatio-temporal orientation of the patient, immediate and short-term memory, the ability of performing serial substractions, constructive capacity and use of language. Therefore, it is a useful test to detect cognitive alterations [39, 65]. A low score in this test indicates variable degrees of mental deterioration. All our cases had scores below 23. Crichton's scale offers a quantitative measure of behavior, useful to evaluate the mental, functional and animic status of elderly patients, and has been used in long-term care institutions because it is easy to apply. Highest scores correspond to the advance deterioration of self reliance [83].The DID test is a scale of invalidity and the highest scores occur in patients with a more profound impairment of their capacity of self care.

The clinical histories also provided the date on which the dementia was first diagnosed, and thus the duration of the disease was obtained.

Histological methods

Tissue blocks containing the nbM of the left hemisphere were fixed for 3 weeks in 10% buffered formalin, paraffin-embedded and cut on the coronal plane. Some cases were fixed by vascular perfusion, as previously described [33]. Paraffin blocks were cut at 7 μm, and two series of sections spaced 350 μm (1 : 50) were collected. One series was stained with cresyl violet for morphometric analysis. The second series was stained with an array of well-known standard techniques: Bielchowsky, periodic acid – Schiff (PAS), glial fibrillary acidic protein (GFAP) immunochemistry, and Thioflavin T.

Morphometric methods

The nbM can be divided into anterior (nbMa), intermediate (nbMi) and posterior (nbMp) subdivisions [34]. Cytoarchitectonic landmarks used in the present study were as previously described [34, 55]. Neurons were regarded as part of the nbM on the basis of their large size, abundant Nissl substance and prominent nucleolus. The total number of nucleolated neurons was counted in every 50th section throughout the entire length of the nbM. To avoid gaps or overlaps between the fields, the counting was performed using an ocular grid at a magnification of × 125. No correction factor for nucleolar bisection was used, since nucleoli usually do not split in 7 μm sections [63]. In each section, the nucleolated nuclei of ten randomly selected neurons were measured with the aid of a semi-automatic image analyzer (Inves PC) at a magnification of × 500; cross-sectional areas obtained from the respective perimeters were used as an indication of nuclear and cell size.

In AD cases, Bielchowsky-stained sections were used to count the number of NFT within the nbM, using the same method as described above. To avoid bias, the study was performed blind, having previously coded the slides to hide their identification.

Statistical analysis

Since neuronal counts and morphometry within each subject produced normally distributed data (Kolmogorov-Smirnov normality test, StatWorks software, Brain Power Inc.), parametric statistics were employed. The AD and control populations of neurons were compared using Student's unpaired *t*-test and ANOVA; linear and polynomial regression functions were used to evaluate correlations (Spearman coefficients) between the morphometric results and other parameters, and Fisher's r to z test was applied to calculate the significance (StatView Software, DataMetrics Inc.)

Results

Control (n = 13) and AD (n = 21) groups were comparable in terms of age (mean ± SEM, for controls 79 ± 2.9 years; for AD 84.1 ± 1.5 years) and postmortem delay (PMD; controls 10.62 ± 2.5 h; AD 7.45 ± 1.1 h). Statistical comparison (Kolmogorov-Smirnov test) showed no differences between the two groups with respect to age or PMD. AD patients had on average a 12% decrease in brain weight, which was not statistically significant.

Neuropsychological assessment

The results from the neuropsychological tests are given in Table 2. The nine patients included in stage III of the dementia showed a severe impairment of their cognitive functions, of their capacity to take care of themselves, and required extensive help for everyday life. The nine patients in stage II showed a moderate degree of dementia, characterized by an evident cognitive impairment, and moderate impairment in their selfcare and in their socio-familiar relationships. The three patients included in stage I did not show any evident signs of dementia or clinically detected cognitive impairment but fulfilled the neuropathological criteria of AD.

AD patients with longer duration of the disease were more severely demented (r = 0.611, P = 0.0042), but there was no statistical relationship between the age of the patient and the staging of the disease: patients with a variety of ages had a similar degree of severity of the disease (r = 0.032, $P > 0.05$). We compared the staging of the disease and the results of the neuropsychological tests, and found significant correlations in all three cases: r = 0.958, P = 0.0001 with Folstein's; r = 0.875, P = 0.0001 with Crichton's, and r = 0.82, P = 0.0003 with DID.

Table 4 Mean neuronal counts of the control and AD groups, expressed in mean number of neurons ± SEM. Unpaired Student's *t*-test was applied to compare the control and AD cell counts in each subdivision of the nucleus basalis of Meynert (*nbM*): anterior (*nbMa*), intermediate (*nbMi*) and posterior (*nbMp*) (*AD* Alzheimer's disease)

	nbMa	nbMi	nbMp	nbM
Control (n = 13)	335.3 ± 52.3	2229.6 ± 115.5	469.23 ± 69	2861.3 ± 180.9
AD (n = 21)	173.75 ± 20	1343.2 ± 128.3	204.15 ± 25.6	1721.1 ± 160.4
Significance	t = –3.319 P = 0.0023	t = –4.695 P < 0.0001	t = –4.159 P = 0.0002	t = –4.619 P < 0.0001
% Change	–48%	–40%	–56%	–40%

Table 5 Progressive loss of neurons (mean ± SEM) with the evolution of the disease. Stage III was significantly different (*) from stage I and II except in the nbMa, at 99% significance (see text)

Zone	Stage I	Stage II	Stage III
nbM	2466 ± 15	2125 ± 200.2	1151.67 ± 139.16*
nbMa	233 ± 15	185.9 ± 34	148.4 ± 27.4
nbMi	1870.5 ± 10.5	1681.3 ± 158	887.89 ± 118.2*
nbMp	362.5 ± 10.5	257.8 ± 31.5	115.3 ± 20.8*

Table 6 Correlation between the neuronal cell counts in the nucleus basalis and its subdivisions, and the scores obtained by each patient in the three neuropsychological tests

	nbMa	nbMi	nbMp	nbM
Folstein	r = 0.04 P > 0.05	r = 0.721 P = 0.0025	r = 0.826 P < 0.0001	r = 0.716 P = 0.0029
Crichton	r = 0.02 P > 0.05	r = –0.565 P = 0.033	r = –0.792 P = 0.0004	r =–0.587 P = 0.025
DID	r = 0.042 P > 0.05	r = –0.492 P > 0.05	r = –0.67 P = 0.0071	r = –0.505 P > 0.5

Neuropathological data

Cell counts

The number of neurons in the nbM was not affected by age, sex or PMD in either the AD or the control group. The AD group showed a highly significant cell loss (Table 4). Individual cases within the AD group showed large variations in the overall neuronal loss in the nbM, ranging from 11% to 87%. In addition, the extent of neuronal loss in the nbM of the AD cases was markedly different in the three subdivisions.

This loss of neurons paralleled the progression of the disease, as shown in Table 5. There was a 53.3% loss of neurons in the nbM between stages I and III, which was not homogeneous: with slight loss between stages I and II (13.82%) and very important between stages II and III (45.8%). These differences were statistically significant (ANOVA $F_{(2,17)}$ = 12.813, P = 0.0009). The nbMa showed an overall 36.3% loss of neurons between stages I and III, but these differences were not significant (ANOVA $F_{(2,17)}$ = 0.872, P > 0.05). The nbMi showed a slight loss of neurons between stages I and II (10%) that increased between stages II and III (47.2%). These differences were also highly significant (ANOVA $F_{(2,17)}$ = 10.451, P = 0.0011). The nbMp showed more pronounced cell loss from stage I to III (68.2%), distributed unevenly: 28.88% loss between stages I and II, and 55.27% between II and III. These differences were also statistically significant (ANOVA $F_{(2,17)}$ = 12.146, P = 0.0005).

The duration of disease had no influence in the number of neurons in the nbMa (r = 0.035, P > 0.05), but had a significant impact on the other subdivisions, such that with increasing time, the neuronal loss was correlatively increased (nbMi: r = 0.577, P = 0.0066; nbMp: r = 0.625, P = 0.0025; nbM total: r = 0.557, P = 0.0095).

The three neuropsychological tests correlated closely with the nbM cell counts in the AD cases, with Folstein's test giving the best correlation. When studied by subdivisions we found no correlation between nbMa cell numbers and any of the neuropsychological tests. The correlations for the other subdivisions of the nbM are given in Table 6.

Morphometry

We studied cell and nuclear size as an indicator of reactive changes in the surviving population of neurons in the nbM. There was no influence of sex, age, PMD nor fixation method on any of the morphometric parameters. We found a hypertrophic reaction in the nucleus of nbM neurons of AD cases, compared with controls (Table 7). The small (3%) increase in cytoplasmic size in the AD group was not statistically significant. Interestingly, AD cases conserved the graded differences in size among subdivisions that are present in the controls (see Table 7). These differences among subdivisions were significant for cell and nuclear size in both the control (ANOVA $F_{(2,2405)}$ = 37.301, P = 0.0001 for nuclear; $F_{(2,1987)}$ = 17.029, P = 0.0001 for cell size) and in the AD group (ANOVA $F_{(2,4744)}$ = 47.397, P = 0.0001 for nuclear; $F_{(2,4382)}$ = 85.827, P = 0.0001 for cell size).

These morphometric changes were a good index of the progression of the disease, and advanced stages showed a higher increment in size both of the nucleus and the cytoplasm (Table 8). The small increase in cytoplasmic size was statistically significant only for the nbMi.

Cell size was also significantly related to the duration of the disease (Table 9), and the longer the disease, the greater the cellular and nuclear hypertrophy in the nbM. Nonetheless, there was no correlation between cellular or nuclear size and the scores of the neuropsychological tests.

Table 7 Karyometric values (cell and nuclear size) from 2408 nbM neurons in the control and 4385 in the AD group (mean ± SEM μm²). Statistical comparisons were obtained using unpaired Student's *t*-test. Note the graded changes in size in the rostro-caudal extension of the nucleus basalis, with the anterior subdivision containing larger neurons than the intermediate, and this one larger than the posterior subdivision (*n.s.* not significant)

Zone	Control	AD	% change	Significance
Nuclear size				
nbMa	103.31 ± 4.4	117.91 ± 2.76	+14.1%	$t = 2.960$, $P = 0.0062$
nbMi	99.76 ± 5.9	113.61 ± 2.19	+13.88%	$t = 2.535$, $P = 0.0165$
nbMp	90.34 ± 4.32	106.51 ± 3.02	+17.89%	$t = 3.168$, $P = 0.0035$
nbM	97.58 ± 4.37	113.85 ± 2.06	+16.67	$t = 3.746$, $P = 0.0007$
Cell size				
nbMa	470.82 ± 19.89	463.02 ± 14.46		n.s.
nbMi	463.80 ± 16.2	476.481 ± 12.81		n.s.
nbMp	412.94 ± 18.23	400.24 ± 17.68		n.s.
nbM	447.60 ± 13.57	460.66 ± 13.86	+3%	n.s.

Table 8 Hypertrophy of nucleus basalis neurons in AD. Modifications in cellular and nuclear size (mean ± SEM μm²) correlate with the stage of the disease. Statistical significance was obtained applying ANOVA, as indicated

	Nuclear size	Cell size
nbM		
Stage I	105.23 ± 1.43	424.18 ± 6.67
Stage II	112.98 ± 0.65	465.124 ± 3.5
Stage III	116.08 ± 0.77	459.863 ± 3.7
Significance	$F_{(2,4575)}$: 18.34, $P = 0.0001$	$F_{(2,4212)}$: 10.669, $P = 0.0001$
nbMa		
Stage I	117.19 ± 3.03	459.1 ± 13.6
Stage II	116.78 ± 1.46	470.65 ± 7.32
Stage III	121.93 ± 1.63	474.61 ± 7.8
Significance	$F_{(2,1094)}$: 2.915, $P > 0.05$	$F_{(2,1050)}$: 0.417, $P > 0.05$
nbMi		
Stage I	100.72 ± 1.64	417.03 ± 8.63
Stage II	115.29 ± 0.87	488.35 ± 4.5
Stage III	115.53 ± 0.98	472.63 ± 4.83
Significance	$F_{(2,2643)}$: 20.656, $P = 0.0001$	$F_{(2,2397)}$: 19.462, $P = 0.0001$
nbMp		
Stage I	95.36 ± 2.63	365.65 ± 10.85
Stage II	102.22 ± 1.16	387.27 ± 6.8
Stage III	110.18 ± 1.94	402.1 ± 7.8
Significance	$F_{(2,832)}$: 9.637, $P = 0.0001$	$F_{(2,759)}$: 1.97, $P > 0.05$

Table 9 Karyometric values (μm² ± SEM) of AD patients, according to the duration of the disease, in years from diagnosis to death. Analysis of variance (ANOVA) within zones of the nbM indicated highly significant differences among the four groups

Zone	< 2 years	2–4 years	4–6 years	> 6 years	Significance
Nuclear area					
nbM	105.23 ± 1.43	113.17 ± 0.6	116.2 ± 1.04	118.2 ± 1.83	$F_{(3,4574)}$: 13.101; $P = 0.0001$
nbMa	117.2 ± 3	118.02 ± 1.33	120.97 ± 2.7	121.4 ± 2.67	$F_{(3,1093)}$: 0.709; $P > 0.05$
nbMi	100.72 ± 1.6	115.17 ± 0.78	116.24 ± 1.3	113.92 ± 2.77	$F_{(3,2642)}$: 14.057; $P = 0.0001$
nbMp	95.36 ± 2.6	100.72 ± 1.07	112.9 ± 2.32	121.73 ± 5.6	$F_{(3,831)}$: 16.394; $P = 0.0001$
Cellular area					
nbM	424.18 ± 6.67	450.21 ± 3.12	486.1 ± 4.7	477.02 ± 10	$F_{(3,4211)}$: 21.338; $P = 0.0001$
nbMa	459.1 ± 13.6	464.54 ± 6.44	492.25 ± 12.3	480.74 ± 14.6	$F_{(3,1049)}$: 1.821; $P > 0.05$
nbMi	417.03 ± 8.36	472.32 ± 4	494.82 ± 6.1	496.41 ± 16.9	$F_{(3,2396)}$: 14.853; $P > 0.05$
nbMp	365.65 ± 10.8	360.94 ± 6.2	454.75 ± 8.8	417.29 ± 19.1	$F_{(3,758)}$: 27.527; $P = 0.0001$

Neuropathological lesions

We found NFT in the nbM of all AD cases, and in 3 of the 13 controls examined. In these 3 control cases, the number of NFT was less than 4% of the cell number. In the AD cases this percentage was greatly increased.

The majority of the NFT were globose tangles, and only a few showed a flame-like morphology, of the type found in cortical pyramidal cells. NFT were present in magnocellular and also in smaller neurons, filling the cytoplasm and displacing the nucleus to the periphery. There were also some extracellular tangles.

Table 10 Counts of NFT in the nucleus basalis of AD cases, in each stage of the disease (mean ± SEM) (*NFT* neurofibrillary tangles)

%NFT/neurons				
Zone	Stage I	Stage II	Stage III	Significance
nbMa	6.43 ± 3.5	21.85 ± 5.7	45.53 ± 7.8	$F_{(2,15)}$: 5.094, P = 0.02
nbMi	14.8 ± 1.3	16.74 ± 3.2	47.74 ± 6	$F_{(2,17)}$: 12.212, P = 0.0005
nbMp	13.74 ± 0.5	27.75 ± 5.4	54.68 ± 6.9	$F_{(2,17)}$: 6.959, P = 0.0062
nbM	13.13 ± 2.4	18.05 ± 3.5	43.86 ± 4.5	$F_{(2,15)}$: 12.975, P = 0.0005

Table 11 Correlation between NFT counts in the nucleus basalis and the scores obtained by each patient in the three neuropsychological tests. Statistical significance was obtained applying Fisher's r to z test

	Folstein	Crichton	DID
% NFT/neuron	r = –0.797	r = 0.694	r = 0.681
	P = 0.0006	P = 0.0068	P = 0.0087

In every case, Bielchowsky-stained sections through the nbM were searched for senile plaques (see Methods). Only 2 control cases (both of very old age) showed senile plaques within the nbM. In 4 AD cases we found small neuritic plaques in the neuropil of the nbM. The remaining cases showed no signs of neuritic plaques in the nbM, in clear contrast with the high number of senile plaques in cortical areas included in the same sections.

We counted NFT and calculated the percentage of NFT-containing neurons with respect to the number of neurons in each case. Overall, one third of the neurons of the nbM showed NFT, and were present in highest percentage in the nbMp (40%), with 26% of NFT in nbMa and nbMi.

The number of NFT increased with the progression of the dementia (Table 10), at a higher rate than neurons were lost. Overall, stage II neuronal counts were 13.8% lower than stage I, and stage III neuronal counts were 45.8% lower than stage II. By contrast, the amount of NFT greatly exceeded the number of neurons dying. NFT counts in stage II were 137.5% of stage I, and those in stage III were 243% of stage II. This discrepancy between the evolution of cell loss and that of NFT numbers was reflected also in the lack of correlation between cell and NFT counts.

To examine if these lesions were an accurate morphological correlate of dementia, we compared the percentage of NFT with the clinical manifestations of the disease and with the scores from the neuropathological tests. Higher mental deterioration correlated significantly with the amount of NFT in the nbM (Table 11).

Discussion

The most significant finding in this study is that the NFT content of the basal forebrain correlates strongly with the degree of dementia, as measured by three different neuropsychological tests. Although a correspondence between nucleus basalis NFT number and dementia cannot be assumed to be causal, it strongly suggests a link between dementia and basal forebrain pathology in AD. We also describe a dramatic loss of neurons in the nbM, most pronounced within those areas that are believed to project to limbic cortex. In addition, our findings include evidence for a plastic response in the surviving population of nbM neurons.

There are many ways of staging AD dementia. We have employed the one proposed by the Geriatric Unit at the Casa de Misericordia, which has been used in other work [49], similar although not identical to the CDR scale [11, 22] and to the scale in DMS-III-R. In this staging, neuropsychological assessment is a necessary instrument to determine the cognitive and behavioral impairment of demented patients, and we have employed the MMS of Folstein, test of appraisal of behavior of Crichton [84], and DID test [49].

Earlier investigations have reported limitations to the MMS as a cognitive screen. Documented weaknesses have included the instrument's insensitivity of specific brain lesions, amnesia, and mild cognitive deficits (discussed in [48]), but it remains the recommended screen of cognitive function for AD and related disorders [91]. The results obtained with MMS in the present work show a clear difference among the different stages of the disease and, therefore, further validate its discriminative capacity, and its utility in these investigations [39, 44, 65]. The diminution in the scores parallels the progression of the dementia, in accordance with similar results from Mayeux et al. [69] and Sulkawa et al. [92].

Crichton's behavioral scale is also a useful tool to evaluate the degree of dementia, and it is indispensable to assess the impairment of patients that are unable to respond to neuropsychological tests [107]. We have found a clear correlation between the stage of the dementia and the scores in these two tests. By contrast, there is a weaker correlation with the results from DID, which may be due to the fact that DID provides mainly functional and motor assessment. The value of these neuropsychological tests is reinforced by the finding, as discussed below, of a close correlation between the results from the tests and the morphometric changes described in the nbM of these patients. It is particularly interesting to note the tighter correlation shown with the morphometry of the posterior subdivision.

Studies on neuronal loss in different neurodegenerative diseases and aging offer contradictory results. The diminution in the number of neurons is a fact that many authors consider characteristic of physiological aging in the CNS [21, 35, 52]. Yet more recent investigations have raised serious doubts regarding the extension, and even

the existence, of a neuronal loss in aging [50, 51, 97]. Results from different morphological studies on the nbM have also shown controversial results, with some reporting a continuous cell loss [55, 66, 68, 70] and others a stability with aging [13, 26, 71]. In AD, the loss of neurons is considered one of the defining facts of the disease, present both in cortical [14, 47, 76, 104] and subcortical structures [67, 70]. Yet, it is still unclear how the loss of neurons, the development of NFT and that of senile plaques each contribute to the development of dementia and to define the pathological basis of AD.

We report a high variability in the amount of cell loss in the nbM, ranging from 11% to 87% of the control group. This is consistent with previous work [29, 55]. Such extreme cases, if studied individually, could give rise to the disparity reflected in the studies by Pearson et al. [77] and Whitehouse et al. [l02] who reported, respectively, a non-significant cell loss in the nbM in the first case, or an almost complete loss of neurons in the nbM, in the second case.

The subdivisions of the nbM participate in the degeneration of the nucleus in AD in different degrees. The posterior subnucleus shows the most severe degeneration, consistent with the reports from others [3, 105]. The intermediate subdivision, the area of the nbM most frequently used as representative of the whole nucleus, also exhibits an important cell loss in AD, but interestingly the variability in cell numbers in this subdivision is very high, both in AD and control cases.

Assuming that individuals that ultimately develop AD start with a similar number of neurons as those who do not, our analysis of neuronal counts in the nbM of AD brains discloses that 40% of the original population of the nbM is lost during the course of the disease. When the subdivisions of the nbM are considered independently, the posterior cell group exhibits a 56% cell loss, which could be related to neuronal losses reported in specific cell populations of the limbic cortex in AD [47, 54, 101]. In agreement with our data, a strong correlation between the duration of the disease and basal forebrain cell loss has also been noted in past studies [62], although this could not be supported by others [29].

These data document a strong correlation between nbM cell numbers and the neuropsychological scores. Cullen et al. [29] did not find a correlation between nbM cell numbers and the clinical measures of dementia, but, as they acknowledge in their discussion, this discrepancy could be due to the advanced stage of the disease in the AD patients included in their study.

In our cases, the duration of the disease varied from 1 to 10 years, with an average slightly over 3 years. There was a linear correlation between age of the patients and the length of the disease, indicating that dementia evolves slower in the older patients. Obviously, there was also a correlation between the length of the disease and the severity of the dementia, as has been pointed out by Diesfeld et al. [36] and Becker et al. [10] among others. As the disease progresses, the morphological parameters also evolve, particularly the neuronal loss in the nbM, as has been also noted by others [3, 28, 57].

There is a close relationship between variations in cellular, nuclear and nucleolar size, and the functional status of a neuron [41, 59, 67], and an increase in cellular activity is followed by an increase in nuclear size [23, 37]. A number of authors have also employed morphometric methods to study changes in the nbM and other nuclei, in aging and AD [41, 67, 68, 99]. Very detailed morphological studies in the nbM with Golgi stains have shown that there are at least two populations of neurons that react differently in AD: while multipolar neurons exhibit evident signs of atrophy, reticular neurons show signs of regenerative reaction. Other authors have reported an increase in the size of small neurons and a decrease in the size of big neurons [82, 99], but we have found a homogeneity in morphometric changes, no matter what the size of the neurons. In non-diseased brains, the three subdivisions of the nbM differ in the size of the neurons [34], suggesting that each subdivision constitutes a well-defined subpopulation. The degree of nuclear hypertrophy that we describe in the nbM in AD is also different for each subdivision, as we have reported earlier [55].

According to Hinds and McNelly [53] and Flood and colleagues [42, 43], cell loss can be accompanied by a reactive cellular hypertrophy. The increase in cytoplasmic size could be explained by the abnormal accumulation of NFT, but it is more difficult to explain the nuclear hypertrophy in AD. The increase in nuclear size described in this report could be a plastic response of the nbM neurons, or it could be understood as a pathological process [24]. Yet, the fact that we were unable to correlate this morphometric change with any of the clinical measurements of dementia leads us to suggest that this phenomenon could be a plastic reaction to injury and, therefore, only indirectly related to the disease. Of all three, the posterior subdivision of the nbM shows the greatest hypertrophy and also the highest neuronal loss, followed by the intermediate subdivision. By contrast, none of the parameters in the anterior subdivision exhibit any relation with the disease stage. This is a very interesting observation considering that the cell loss in this subdivision is highly variable and does not correlate either with the evolution of the disease.

With regard to the neuropathological changes, different groups have described that cases at the threshold for clinical detection of AD have already very substantiated neuropathological lesions [46, 78]. Therefore, it is clear that the disease process must begin before any cognitive change can be detected clinically or neuropsychologically. The relatively few non-demented cases with many NFT in the nbM provide an example for such preclinical cases. Furthermore, since neurofibrillary changes of the Alzheimer type do not inevitably occur in the brain of aged individuals, NFT must be considered abnormal [20].

NFT appear in numerous neurons of the cerebral cortex and the hippocampus in AD. Although the presence of NFT has been traditionally considered as a sign of degenerative disease, the relation between the formation of NFT and the degeneration of the neuron remains unclear, and the functional viability of NFT-laden cells is not known. NFT in the nbM are morphologically different from corti-

cal NFT, and they are also more frequent than senile plaques [79, 89, 103]. Ishii [56] described them as "globose tangles", similar to the ones found in the locus ceruleus, raphe nucleus and other nuclei in the brain stem [32, 56, 106]. In addition to the morphological differences, these globose tangles have been shown to be made up mainly of 15– to 20-nm straight microfilaments and a variable number of paired helical filaments [9].

To perform counts of NFT we have employed 7-μm sections stained according to Bielchowsky's method, and we have compared those counts with neuronal counts in adjacent sections stained with cresyl violet. This comparative quantification is considered a tedious method [74] and one that involves a lot of effort [16, l7, 25], but it seems to be more reliable than the estimation of density performed by other authors [2, 73, 93], which has been highly criticized [104] because of its difficult interpretation. Previous studies have also shown that the number of neurons undergoing neurofibrillary degeneration in the cortex correlates with the degree of dementia, and increases with the progression of the disease [6, 12, 88]. The nbM also shows extensive neurofibrillary degeneration in AD, both in cases with mild and with severe cell loss.

Several authors have described neuritic plaques, NFT and perivascular deposits of amyloid in the nbM in AD [4, 15, 17, 90, 96]. A few reports have indicated lack of NFT in the nbM in AD [1, 58], but our results coincide with many others that have reported the presence of NFT in nbM in AD [70, 85, 90, 95, 102] and quantified them [15, 17, 25, 79]. In our cases, over one third of the neurons contained NFT. Among the subdivisions, the nbMp is the one that contains a greater number of NFT, which is not surprising since it is also the subdivision with higher neuronal loss. It has been suggested that the neurons with NFT would be the magnocellular, cholinergic neurons [15, 68, 85]. In our cases, NFT were present in neurons of all sizes, in accordance with the studies of Rasool et al. [79] and Svendsen et al. [94], showing that both cholinergic and non-cholinergic neurons contain NFT. Samuel et al. [87] found that, while the nbM had a lower NFT count than did cortical sites, counts in that region were strongly correlated with the degree of premortem dementia on three neuropsychological tests, whereas NFT counts in motor and visual cortices and in the subiculum were not significantly related to test scores. In our AD patients, the number of NFT shows no correlation with the age of the patient at the onset of the disease, nor with the age at death nor the duration of the disease. There is, in contrast, a strong correlation with the stage of the dementia, and with the cognitive impairment as determined by the three neuropsychological tests employed.

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