The nucleus basalis of Meynert in 20 definite cases of Creutzfeldt-Jakob disease

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SUMMARY The population of neurons and the neuronal size in the nucleus basalis of Meynert (nbM) were studied in 20 patients with definite Creutzfeldt-Jakob disease (CJD). When compared with a normal control group, the 20 CJD brains showed a significant loss of neurons and reduction of neuronal size, mainly in the middle level of the nbM and mostly affecting the right side. Since these findings show some parallelism with the amount of cortical damage and given the scarce gliosis and spongiosis found in only six of the 20 CJD brains, we postulate that the involvement of the nbM in CJD is a retrograde abnormality secondary to the damage of the neocortex.

After the demonstration that patients with Alzheimer's disease (AD) show a significantly decreased neocortical choline acetyltransferase activity associated with marked cell loss and degeneration of the nucleus basalis of Meynert (nbM),¹⁻⁴ an increasing interest concerning the histopathological study of this nucleus, considered as the main cholinergic input to the neocortex, has been focused on other neurological diseases with and without dementia.⁵⁻⁸ Since there are few reports describing the neuropathological changes of the nbM, and those were in a small number of cases of Creutzfeldt-Jakob disease (CJD),⁹⁻¹² a transmissible dementia showing a high prevalence in Chile,¹³ we present here the neuropathological findings in the nbM of 20 verified cases of CJD.

Materials and methods

We studied formalin-fixed brains from 20 cases of definite CJD that were preserved in the neuropathology laboratory. Ten were males and 10 were females, with a mean age at death of 58.5 years, ranging from 39 to 73 years and a mean total duration illness of 5.5 months, ranging from 1.5 to 9 months. In addition to the histologically proven diagnosis, four of the 20 cases had been experimentally transmitted to laboratory animals.¹⁴ A case control group consisted of brains from three males and three females who died of non neurological

diseases and without dementia, with a mean age at death of 61.8 years, ranging from 46 to 76 years.

In each case one block of 7 × 10 × 25 mm of the basal forebrain was obtained from both cerebral hemispheres by cutting behind the optic chiasm, in front of the mamillary bodies, at 10 mm from the subarachnoid space over the anterior commissure and 25 mm lateral to the third ventricle through the putamen, as described by Hedreen *et al.*¹⁵ Since some of CJD brains had been previously used in other investigations, we studied the parts of the blocks that were still available. The anterior, middle and posterior regions of the nbM were identified according to Gorry's description,¹⁶ while the anteromedial and anterolateral regions determined by Mesulam *et al.*¹⁷ in the rhesus monkey were often impossible to identify. Hence, the anterior region was considered as a whole.

Paraffin-embedded sections 15 µm thick were obtained, and at least 30 sections from each hemisphere including the three regions of the nbM were studied by the cresyl violet stain method. For the total neuronal count we selected two sections from the anterior region, four sections from the middle region and two sections from the posterior region. In each slide we took photographs of 2 × 3 mm area showing the highest cell density at × 40 magnification. The cell counting was conducted on a blind basis by two independent observers using photographs projected on a checkered screen. The counts performed by each observer were highly correlated ($r = 0.847$) ($p < 0.001$) and the slope for the straight line obtained was 0.965.

All neurons with a diameter of 30 µm or more were counted regardless of any morphological alteration and the results were separately compared for the right and left hemispheres by a nested analysis of variance.¹⁸ Since most of the projections to the neocortex arise from the middle region of the nbM, we selected a slide of the right or left middle region showing the maximum cell density and the greater

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Table 1 Cell counts in the right and left nucleus basalis of Meynert in controls and in Creutzfeldt-Jakob disease brains

	Right Anterior			Middle			Posterior		
	<i>n</i>	$\bar{X}$	<i>SD</i> *	<i>n</i>	$\bar{X}$	<i>SD</i> *	<i>n</i>	$\bar{X}$	<i>SD</i> *
controls	10	261.6	119.7	24	313.5	65.7	12	218.1	93.5
CJD	23	152.2	43.6	47	144.9	44.8	24	107.0	42.9
	Left Anterior			Middle			Posterior		
	<i>n</i>	$\bar{X}$	<i>SD</i> *	<i>n</i>	$\bar{X}$	<i>SD</i> *	<i>n</i>	$\bar{X}$	<i>SD</i> *
controls	12	267.3	55.3	24	294.5	62.0	12	214.3	63.0
CJD	24	160.0	45.5	38	178.5	38.2	15	155.8	50.0

n = number of sections
**p* < 0.001.

Fig 1 Middle region of the right nucleus basalis of Meynert in a normal control brain (a) and in a case of Creutzfeldt-Jakob disease (b) showing overall flattening of the nucleus and diminished number and size of neuron cells (cresyl violet). ($\times 40$)

Table 2 Individual cell count in the nucleus basalis of Meynert in controls and in Creutzfeldt-Jakob disease cases correlated with the cortical damage

Case	Sex-Age (yr)	Right			Left			Cortical damage
		anterior	middle	posterior	anterior	middle	posterior	
control 1	F 62	377	360	188	323	368	238	—
control 2	F 61	234	347	136	264	319	196	—
control 3	M 59	—	258	211	219	299	280	—
control 4	F 67	166	242	185	226	228	164	—
control 5	M 46	404	375	405	255	328	285	—
control 6	M 76	128	300	187	243	226	124	—
CJD ME	M 59	199	187	182	158	174	84	+
CJD PS	F 55	157	167	49	152	—	—	+
CJD GG	F 64	129	—	—	234	181	69	+
CJD RC	M 51	180	—	—	172	245	—	+
CJD RN	M 66	164	173	120	—	—	—	+
CJD RG	M 58	—	156	98	116	146	144	+
CJD AO	F 57	125	201	130	—	150	63	++
CJD DM	M 55	195	—	—	196	230	210	++
CJD OD	F 64	137	98	72	117	—	—	++
CJD AG	F 73	176	129	—	—	—	143	++
CJD VR	M 60	—	—	—	219	178	122	++
CJD LA	F 39	—	108	88	82	126	—	+++
CJD RS	F 56	—	—	81	221	203	96	+++
CJD GG	F 66	162	129	105	—	—	—	+++
CJD MC	M 54	—	160	144	130	—	—	+++
CJD JR	M 59	65	77	64	—	—	—	+++
CJD AE	F 55	—	—	162	—	186	—	+++
CJD EV	F 53	132	—	—	183	—	—	+++
CJD JR	M 60	—	185	162	—	—	—	+++
CJD RR	M 66	—	108	—	—	—	—	+++

diameters of all neurons were measured within an area of 1 mm² in the centre of the slide. The comparison between CJD cases and the control group was carried out by the Student's *t* test for independent samples. Additional stain methods used were haematoxylin-eosin, Bodian and Bodian-Nissl.

In 19 of the 20 CJD brains where the middle region of the nbM was available we evaluated the cortical damage of the frontal, parietal, temporal and occipital cortex according to the amount of spongiform change, neuron loss and gliosis, particularly taking into account the degree of the cytoarchitectural disturbance of the cortex. The cortical damage was defined as: (+) when all the cortical layers were clearly recognisable; (++) when the cortical layers were partially

recognisable, and (+++) when the cortical layers were no longer recognisable. The neuronal count and the neuronal size observed in the nbM were statistically compared with the degree of the cortical damage by means of the analysis of variance.

Results

Table 1 shows the resulting counts of neurons greater than 30 µm size in the right and left nbM in CJD cases and in the control group. There was a significant ($p < 0.001$) decreased number of those neurons in all the nbM regions of CJD cases when compared with the control group. The lower cells number displayed particularly by the middle regions in both cerebral hemispheres, but mainly in the right, is a noteworthy feature of CJD cases (fig 1). Although the amount of neuron loss in the middle region of the nbM is rather paralleled with the degree of cortical damage in CJD brains (table 2), this tendency is not statistically significant ($p > 0.10$).

The total of 2411 neurons measured in the 20 CJD brains gives an average of 126 neurons for each case, while in the six control brains the 825 measured neurons give an average of 138. The mean neuronal diameter in the nbM of CJD brains was 28.9 µm (SD 8.8), while in the control group brains the average was 35 µm (SD 7.1). As shown in fig 2, the percentage of neurons measuring less than 30 µm was 24.1% in the control group and 52.6% in CJD cases, while the percentage of neurons with a diameter over 30 µm was

Fig 2 Percentage of different neuron size in the middle region of the nucleus basalis of Meynert in controls and Creutzfeldt-Jakob disease brains.

Fig 3 Creutzfeldt-Jakob disease case. Spongiosis of the innominate substance without involvement of the nucleus basalis of Meynert. Gliosis and decreased size of neurons in the middle region (cresyl violet). ($\times 100$)

75.8% and 47.4%, respectively. In addition, there was a significant correlation ($p < 0.0001$) between the mean neuronal size in the middle region of the nbM and the amount of the cortical damage. Thus, the mean neuronal diameter in the nbM was $31.5\mu\text{m}$ (SD 9.8) in CJD brains with cortical damage +; $28.9\mu\text{m}$ (SD 8.4) in CJD brains with cortical damage ++ and $26.8\mu\text{m}$ (SD 8.1) in CJD brains with cortical damage +++. On the other hand, no relationship was observed between the decrease of number and size of neurons in the nbM and the total duration of CJD.

In most of CJD brains the nbM had lost its normal fusiform shape, appearing as a retracted, flat and less dense cellular band (see fig 1). Mild spongiform change affecting the nbM was observed in only two cases. In another four there was spongiosis of the innominate substance without evident involvement of the nbM (fig 3). Haematoxylin-eosin stains showed marked glial proliferation in the nbM of two of the 20 CJD brains, while moderate gliosis was observed in another four cases.

A remarkable neuropathological feature displayed by the nbM in all CJD brains was the rounded shape of most of the neurons, showing intracytoplasmic vacuoles with a tendency to aggregate, displacing the nucleus and conferring a foamy aspect on the affected neurons (fig 4). In addition, fine intra- and extra-vacuolar granules reminiscent of the hippocampal granulo-vacuolar degeneration were also present within the neurons, although showing different staining characteristics.

Staining by the Bodian method showed a diminished number of fibres and thickened and curled degenerated axons. Scant neurofibrillary tangles were

Fig 4 Creutzfeldt-Jakob disease case. Roundness and vacuolisation of neurons in the middle region of the right nucleus basalis of Meynert (cresyl violet). ($\times 400$)

demonstrated in the nbM of two CJD patients aged 60 and 66 years, respectively.

Discussion

The study of the nbM in our 20 CJD cases shows a substantially diminished number of those neurons with a diameter over $30\mu\text{m}$, which correspond to most of the cholinergic neurons,¹⁹ while a decreased size of all neurons was observed in an area studied in the middle region of the nbM, in sharp contrast to the scarce gliosis and spongiosis that characterise CJD.

The decreased number of neurons greater than $30\mu\text{m}$ found preferentially in the middle region of the nbM in our 20 CJD brains agrees in part with the observation of Roger *et al*⁹ who described an intermediate decreased neuronal population of the nbM in five CJD cases, although mostly affecting the anterior region. In addition, in the patient of Arendt *et al*¹⁰ a maximum loss of neurons was observed in those parts of the nbM projecting to the most severely damaged cortical areas. This is also in agreement with our findings, since the middle region of the nbM projects principally to the neocortex. On the contrary, the two CJD cases studied by Cutler *et al*¹¹ and by Clark *et al*¹² did not show neuron loss in either region of the nbM.

Although the prevalence of morphological changes in the right nbM of some of our CJD cases is difficult to interpret, it is tempting to consider this morphological asymmetry in the light of the controversial functional asymmetry of cholinergic activity that has been described in some normal and pathological brains.^{20,21}

The high percentage of neurons smaller than $30\mu\text{m}$ that was found in an area of the middle region of the nbM is remarkable in our CJD cases. Since the measurement of neurons was performed in an area with the largest cell density, the number of neurons measured in each CJD and control brains were alike. This and the flat and less dense aspect of the nbM in CJD brains shows that in addition to neuron loss, there is a consistent reduction in neurons size. However, it is not possible to prove whether the number of neurons smaller than $30\mu\text{m}$ size does not change.

The neuronal alterations described above in the present cases are comparable to similar findings reported by Pearson *et al*²² in monkeys and humans after cortical ablations and by Sofroniew *et al*²³ in rat brains following cortical injuries. This and the relationship observed between the reduced size of neurons in the nbM and the intensity of the cortical damage highly suggest that the neuronal alterations of the nbM in CJD cases are secondary to the cortical lesions. Thus, it is probable that in CJD the cortical synapses are not specifically affected through the primary damage in the regions of membrane

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apposition,²⁴ leading to a retrograde involvement of the nbM. Conversely, a specific and perhaps pathogenic involvement of presynaptic cholinergic cortical terminals has been hypothesised in patients with AD.^{4,25,26} An additional support for the hypothesis of a secondary damage of the nbM in CJD patients is the scarce gliosis and spongiosis displayed by this nucleus, in sharp contrast with that observed in the cerebral cortex of our 20 CJD cases.

The lack of relationship between the intensity of the histopathological changes of the nbM in CJD cases and the total duration of the disease does not rule out the hypothesis of a retrograde degeneration of the nbM, since the intensity of the cortical damage is related to the anatomoclinical form of the disease rather than to its total duration.²⁷

In accord with the hypothesis proposed by Clark *et al*,⁷ the dementing processes can be classified into two groups. First, those associated with a severe decrease of cortical cholinergic activity and a significant loss of nbM neurons, as occurs in AD patients. Second, those without impairment of the cortical cholinergic activity and with an unharmed nbM, as in Huntington's disease. Nevertheless, we believe that it is also important to determine if the changes in the nbM observed in some dementing diseases, such as CJD, is either primary or secondary to the cortical damage.

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