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Alzheimer's Disease and Senile Dementia: Loss of Neurons in the Basal Forebrain

Abstract. *Recent evidence indicates that the nucleus basalis of Meynert, a distinct population of basal forebrain neurons, is a major source of cholinergic innervation of the cerebral cortex. Postmortem studies have previously demonstrated profound reduction in the presynaptic markers for cholinergic neurons in the cortex of patients with Alzheimer's disease and senile dementia of the Alzheimer's type. The results of this study show that neurons of the nucleus basalis of Meynert undergo a profound (> 75 percent) and selective degeneration in these patients and provide a pathological substrate of the cholinergic deficiency in their brains. Demonstration of selective degeneration of such neurons represents the first documentation of a loss of a transmitter-specific neuronal population in a major disorder of higher cortical function and, as such, points to a critical subcortical lesion in Alzheimer's patients.*

Alzheimer's disease (AD) and senile dementia of the Alzheimer's type (SDAT) are associated with progressive deterioration of memory and cognitive function (*1*). These two disorders, differing in the age of onset and rate of progression, but similar in terms of pathology (*2*), are the most common causes of dementia in middle and late life. Perhaps 10 to 15 percent of the population over 65 years of age suffers from mild to severe dementia; 50 to 60 percent of these patients have SDAT, resulting in a prevalence of over a million affected individuals in the United States (*3*). Neurochemical investigations of patients with AD/SDAT have demonstrated a reduction in presynaptic markers for acetylcholine-utilizing neurons (*4*) in the hippocampus and cerebral cortex. The basis for this cholinergic abnormality is unclear, but recent evidence suggests that the cholinergic neurons in the nucleus basalis of Meynert (nbM) may selectively degenerate in AD/SDAT. The nbM (*5*), located in the substantia innominata (Fig. 1), contains clusters of neurons that can be recognized on the basis of their large size, abundant Nissl material, acetylcholinesterase (AChE) activity, and physiological properties (*5–8*). Choline acetyltransferase (CAT) activity, the best available marker for cholinergic neurons (*9*), is enriched in the substantia innominata, and the distribution of this enzyme parallels the topography of the large neurons in the nbM (*10*). These large neurons in the nbM project directly to the cerebral cortex (*11, 12*); similar neurons, located in the diagonal band of Broca and medial septum, project to the hippocampus (*13*). Moreover, excitotoxic lesions of the ventral pallidum, which, in the rat, contains cells homologous to the neurons of the primate nbM, cause a selective reduction in cholinergic presynaptic markers in cortex (*14*), similar to that described in patients with AD/SDAT (*4*).

These observations directed our attention to the basal forebrain in AD/SDAT, and we recently have shown a 90 percent loss of neurons in the nbM in a patient with a familial form of AD (*15*). We now describe a selective degeneration of neurons in the nbM in five patients with presumed sporadic AD/SDAT. We conclude that the loss of putative cholinergic neurons in the nbM is linked to the presynaptic neurochemical abnormalities in the cortex of patients with AD/SDAT.

The brains of demented patients, obtained from the collection of the Maryland State Medical Examiner's Office, were selected on the basis of three criteria: a history typical of AD/SDAT; the presence of the classical pathology of AD/SDAT including neurofibrillary tangles, senile plaques, and granulovacuolar degeneration; and the availability of paraffin-embedded, Nissl-stained histological sections (15 μm thick) containing the most extensive portion of the nbM, for example, the substantia innominata between the optic tract and anterior commissure. The brains of five adults without evidence of dementia were selected for study on the basis of comparable age and availability of matching histological sections of the ventral forebrain (Table 1). Cells were counted as nbM neurons on the basis of three criteria: cell size (larger than 30 μm); the presence of abundant Nissl substance; and a visible nucleolus. Cell counts (Table 1) were performed by two observers, blind to the diagnosis, using three different assessment methods. Cell loss was judged subjectively, according to a seven-point rating scale with 0 corresponding to complete loss of cells and 6 corresponding to normal cell number. The number of neurons contained within a zone measuring 600 μm by 600 μm was counted in the area of maximum cell density and was expressed as the average number of cells per grid (Table 1); and the total number of neurons within histological sections containing the major (midportion) of the nbM was counted directly. If the nbM's on both sides were available for counting, the average cell count on each side was used.

In each of the patients with AD/SDAT, all three methods of assessment showed severe loss of nbM neurons (Fig. 2 and Table 1). The maximum cell density of neurons was reduced by 73 percent, and the total number of neurons was reduced by 79 percent. In each independent rating scale, there was fourfold difference

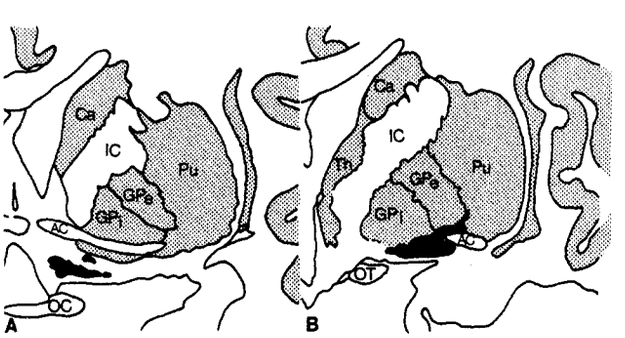

Fig. 1. Drawings of the human forebrain, adapted from (*6*), illustrating the anatomical relationships slightly rostral to and caudal to the forebrain sections available for morphological analysis. The region containing the nbM is shown in black. (A) Basal forebrain at the level of the optic chiasm. (B) Basal forebrain at the level of the infundibulum. Abbreviations: *AC*, anterior commissure; *Ca*, caudate nucleus; *GPi*, internal segment of the globus pallidus; *GPe*, external segment of the globus pallidus; *IC*, internal capsule; *OC*, optic chiasm; *OC*, optic chiasm; *OT*, optic tract; *Pu*, putamen; and *Th*, thalamus.

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between the control patients and those with AD/SDAT. Moreover, there was no overlap between the absolute cell counts in the nbM in the two groups. The correlation between two observers ranged from .92 to .95. Some neurons in the nbM of AD/SDAT patients showed neurofibrillary tangles. The AD/SDAT patients did not show significant clinical or pathological evidence of other types of chronic, progressive neurological disease.

Table 1. Quantitative analysis of cell numbers in patients with AD/SDAT and nondemented individuals. Summary data for each group are given as means ± standard errors of the mean.

Age	Rating	Density	Counts
	Alzheimer's patients		
58	0.5	0.8	55
60	1	1.4	51
63	3	1.9	69
65	1.5	1.5	123
73	2	2.3	64
63.8 ± 2.9	1.6 ± 0.5*	1.6 ± 0.3*	72.4 ± 14.6*
	Control patients		
50	5.5	8.0	537
50	5	3.7	255
54	5	6.2	399
59	5.5	6.9	323
66	5.5	4.6	209
55.8 ± 3.4	5.3 ± 0.1	5.9 ± 0.9	344.6 ± 64.6

*Significantly different from control patients, $P < .004$, Mann-Whitney U test.

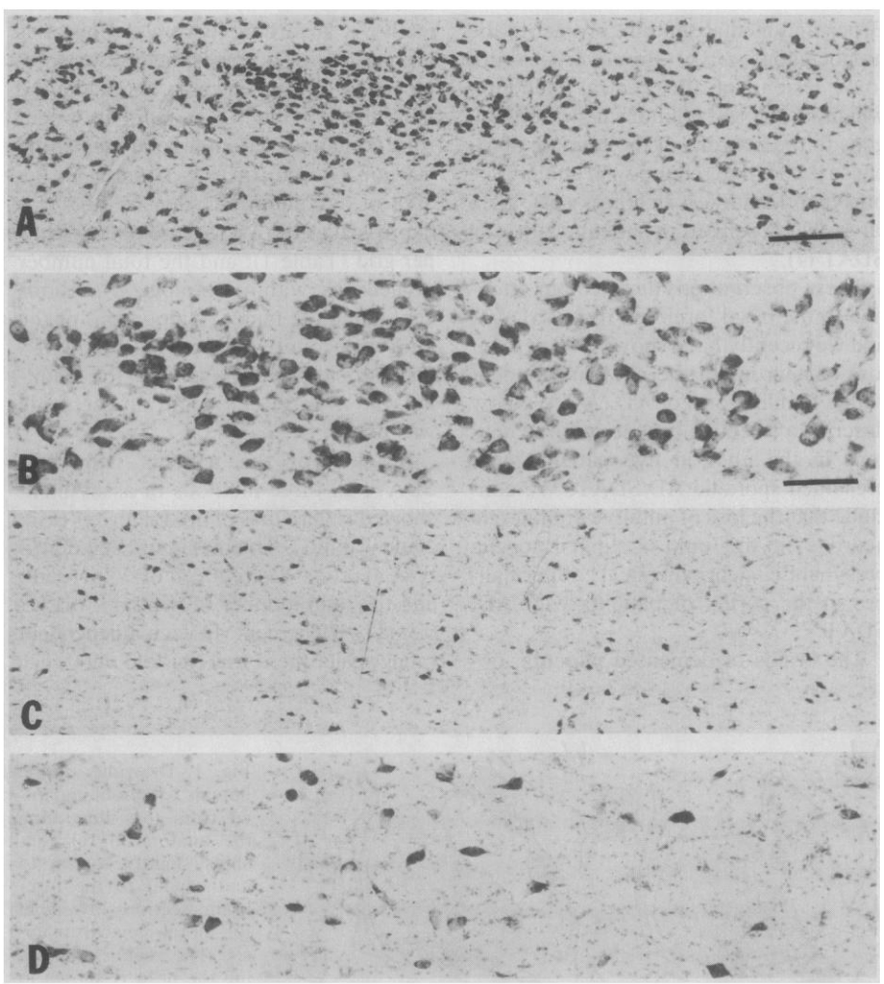

Fig. 2. Photomicrographs from the midportion of the nbM of an age-matched control (A and B) and a patient with AD (C and D). (A and C) Normal number and density of nbM neurons in the control and the profound loss of neurons in the nbM of the patient with AD. At higher magnification, the nbM neurons in the control (B) appear as large cells with densely stained Nissl substance. A few neurons are present in the nbM of the patient with AD (D). Magnifications: (A and C) ×10; (B and D) ×25. Scale bar is 200 μm in (A) and 100 μm in (B).

Since normal aging is associated with loss of neurons in the neocortex and hippocampal formation (*16*), it has been difficult to determine the significance of neuronal loss in the neocortex of patients developing AD/SDAT late in life (*1, 2, 17*). However, deficiencies in cholinergic presynaptic markers in the cerebral cortex of individuals with AD/SDAT are considerably greater than the described reduction in cortical neurons. This study demonstrates that the neurons of the nbM, a major source of extrinsic cholinergic innervation to the cortex, are decreased by as much as 80 percent in the brains of patients with AD/SDAT; the absolute decrement in the number of neurons in the nbM in our patients is congruent with the percentage reduction in concentrations of specific cholinergic presynaptic markers described in the cortex of patients with AD/SDAT (*4*). These results support the hypothesis that pathological changes in the nbM play an important part in the cholinergic synaptic abnormalities in AD/SDAT.

Recent anatomical investigations of the connectivity of the basalis neurons in nonhuman species have suggested that these cells receive afferents from a variety of sources, including the amygdala, hypothalamus, peripeduncular nucleus, midbrain, and other brainstem nuclei (*12, 18*) and that, in turn, nbM neurons project to the amygdala, thalamus, hypothalamus, brainstem, olfactory bulbs, hippocampus, entorhinal cortex, and neocortex (*11–13, 19*). Hence, the nbM is in a critical position to integrate information from a variety of subcortical sources and to directly influence the cerebral cortex. The roles of the nbM in behavior and cognition remain to be defined. Psychopharmacological studies and experimental lesions suggest that the basal forebrain cholinergic pathways, particularly those projecting to the hippocampal formation (*13*), play an important role in memory processes (*20*). Clinical observations have suggested that neurons in the nbM may be of special importance in modulating the speed of motor and cognitive activity (*21*). Extracellular recordings in the nbM in awake performing monkeys indicate that these neurons exhibit characteristic firing patterns, and these firing rates are modulated during the delivery of a reward (*8*). Destruction of these neurons in the basal forebrain may be responsible for some of the cognitive disturbances occuring in these individuals with AD.

Our demonstration of the selective degeneration of nbM neurons provides

what we believe to be the first example of loss of a transmitter-specific cell population in a major disorder of higher cortical function and, as such, represents a significant step in the understanding of the pathophysiology of this neurological disorder.

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Heterogeneity in Epidermal Basal Keratinocytes: Morphological and Functional Correlations

Abstract. *Two structurally distinct populations of basal keratinocytes, nonserrated and serrated, were observed in cynomolgus monkey and human palm epidermis. Anatomical location, fine structural features, and kinetic properties suggest that nonserrated cells represent a stem cell population and that serrated cells help anchor the epidermis to the dermis.*

Although relatively little is known about epidermal stem cells, studies of other renewing cell populations, such as blood cells and seminiferous and intestinal epithelia, suggest that stem cells possess the following properties: (i) ultrastructurally, their cytoplasm is primitive and contains few, if any, differentiation products; (ii) they have very low mitotic activity; (iii) they give rise to transient amplifying cells that undergo several rounds of division before terminal differentiation; and (iv) they can be induced to proliferate by tissue demand or specific stimulation (*1*).

While studying epidermal differentiation in cynomolgus monkeys and humans, we noted that palmar epidermis has two morphologically distinct, spatially segregated populations of basal keratinocytes. We report here that one population has features typical of stem cells and that the other may anchor the epidermis to the dermis. The two populations are present in epidermis from different regions of the body and can easily be identified with the light microscope.

Cynomolgus monkey palm epidermis consists of alternating deep and shallow downgrowths (rete "ridges"). The shallow ridges interface with troughs formed between the apices of bifurcated dermal papillae (Fig. 1). The surface hyperkeratotic layer reflects the alternating pattern of ridges and sulci. This is visible as dermatoglyphics. Basal cells in the tips of the deep rete ridges are more heavily pigmented than those in the shallow ridges, resulting in a dark and light pattern in the epidermis. These regional differences have been noted in the palms and soles of other primates (*2*); however, it has not been appreciated that the architecture of the dermal-epidermal junction is very different in the deep and shallow ridges. In 1-μm plastic sections (*3*), basal keratinocytes at the tips of deep ridges are flattened and slightly convoluted at the dermal-epidermal junction (Fig. 2A). In contrast, keratinocytes in shallow ridges display well-developed cellular projections extending deep into the papillary dermis, resulting in a highly convoluted dermal-epidermal junction (Fig. 2B). Accordingly, we call the two cell types nonserrated and serrated basal keratinocytes.

Nonserrated cells are small and cuboidal and have a large ratio of nuclear to cytoplasmic matter (Fig. 2A). The cell periphery has numerous microvilli, which occasionally are punctuated by desmosomes, and the cytoplasm is filled with free ribosomes, mitochondria, and melanosomes (Fig. 3A). Frequently the melanosomes are concentrated around the apical end of the nucleus. In addition, the cells show a paucity of keratin filaments and a diffuse distribution of nuclear chromatin. These characteristics suggest a relatively primitive cell (*4*). In contrast, serrated basal cells contain copious bundles of keratin filaments extending all the way to the tips of the

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