Alzheimer’s Disease: Striatal Amyloid Deposits and Neurofibrillary Changes

HEIKO BRAAK, M.D., AND EVA BRAAK, PH.D.

Abstract. Sensitive silver methods were employed for the examination of extracellular amyloid and intraneuronal neurofibrillary changes in the striatum of Alzheimer’s disease patients. Numerous amyloid deposits were present in the striatum whereas neuritic (senile) plaques were only rarely encountered. Many large and a few medium-sized nerve cells had neurofibrillary tangles within their somata and according to morphological criteria corresponded to local circuit neurons. Numerous argyrophilic threads in the neuropil were scattered throughout the nuclear gray matter. The striatum of non-demented individuals was virtually devoid of amyloid and neurofibrillary changes.

Key Words: Alzheimer’s disease; Amyloid plaques; Neuritic (senile) plaques; Neurofibrillary tangles; Neuropil threads; Striatum.

INTRODUCTION

The brains of patients with Alzheimer’s disease (AD) are characterized by abnormal filaments deposited in both extracellular and intraneuronal locations. The extracellular material consists of amyloid. Neurofibrillary changes are responsible for the intraneuronal lesions and three major lesions occur as a result: neuritic (senile) plaques (NP), neurofibrillary tangles (NFT), and neuropil threads (NT) (1, 2).

The techniques generally employed for the demonstration of amyloid (congo red, thioflavine S) are relatively insensitive while methods used for neurofibrillary changes suffer from a lack of specificity (3, 4). This is also why the striatum analyzed with those techniques appears to be virtually devoid of Alzheimer-related lesions (5, 6).

There are, however, two silver techniques of high sensitivity and probably high specificity available for both the extracellular amyloid (7) and intraneuronal neurofibrillary changes (8). This study re-investigated the striatum using these techniques for the demonstration of Alzheimer-related changes.

MATERIALS AND METHODS

Twenty-four brains obtained at autopsy and fixed by immersion in a 4% aqueous solution of formaldehyde were examined. Ten brains were from individuals with Down’s syndrome. Eight brains were from patients with a history of presenile or senile dementia of the Alzheimer type (Table 1). Neuropathological examination of these brains revealed that 17 displayed a sufficient number of lesions (NP, NFT, and NT) in multiple allo- and isocortical regions to confirm the diagnosis of AD (9). Six brains were from non-demented individuals without known neurological disorder and served as controls. None of the brains showed ischemic lesions.

Two slabs of brain tissue of one hemisphere were cut in a coronal plane at a thickness of 2 centimeters. The rostral slab taken at the level of the anterior commissure contained the anterior striatum and large portions of the magnocellular nuclei of the basal forebrain (medial...
### TABLE 1
Sex, Age, and Neuropathology*

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*The striatal amyloid and neurofibrillary changes are graded: − no discernible change, + slight change, ++ moderate change, +++ severe change, ++++ very severe change. AD: Alzheimer's disease; Down: Down's syndrome; f = female; m = male.

The septal nucleus, nucleus of the diagonal band, and the basal nucleus of Meynert. The second slab included posterior striatum, pallidum, tuberomammillary nucleus and the mammillary body. The tissue was then embedded in polyethylene glycol (PEG 1000, Merck) (10) and sectioned at 100 μm.

Representative triplets of consecutive sections were processed according to the following three methods. The first section of each triplet was silver-stained for amyloid (7). The consecutive section was silver-stained for neurofibrillary changes (8, 11). The third section was stained for lipofuscin pigment and Nissl material (12–14). Sections stained for amyloid (7) also showed normal axons and lipofuscin pigment in astroglial cells of the striatum. Both the axons and pigment granules could easily be distinguished from amyloid deposits.

Additional tissue slabs were paraﬃn-embedded and sectioned at a thickness of 12 μm. The sections were stained with cresyl violet, congo red (15), and the above mentioned silver stains (7, 8). Further sections were immunostained with a) a polyclonal antibody (16) raised against synthetic A4 protein, b) a polyclonal antibody (17) raised against paired helical filaments (antibody 83e), and c) a polyclonal antibody (17) raised against bovine tau (antibody 92e). Immunocytochemistry was performed according to standard procedures using the avidin-biotin peroxidase complex to label bound antibody, and 4-chloro-1-naphthol was used as a chromogen. Following photographic documentation of some of the immunoreactive struc-
Fig. 1. Correlation of immunostaining and silver techniques. (a) Section of the striatum immunostained for demonstration of amyloid (A4 protein) as compared to (b) the same section decolorized and restained according to Campbell's silver technique. (c–f) Large tangle-bearing neurons of the striatum (c) immunostained with an antibody raised against bovine tau (92e) and (e) immunostained with an antibody raised against paired helical filaments (83e) compared to the same neurons (d and f) after decolorizing and restaining according to Gallyas' silver technique for neurofibrillary changes. The distribution pattern of abnormal material revealed in (a), (c), and (e) is virtually identical to that seen in (b), (d), and (f). 12 μm paraffin-embedded sections.

In order to confirm the immunohistochemical staining, the sections were decolorized in 70% ethanol and subsequently re-stained with the silver techniques of Campbell (7) and Gallyas (8) (Fig. 1).

RESULTS

The striatum of patients with AD revealed the presence of both amyloid and neurofibrillary changes. The distribution pattern of the immunoreactive material seen in sections stained for A4 protein (Fig. 1a) mirrored that seen in Campbell silver-stained sections (Fig. 1b). The distribution pattern of neurofibrillary changes demonstrated in immunostained sections (Fig. 1c, e) was virtually identical to that depicted in Gallyas silver-stained sections (Fig. 1d, f). The Campbell and the Gallyas silver techniques, therefore, were capable of detecting the presence of amyloid and neurofibrillary changes respectively.

Amyloid

The striatum of non-demented controls remained devoid of amyloid (Fig. 2a) or displayed only small amounts (Fig. 2c). In contrast to this finding, all patients with
Fig. 2. Development of amyloid deposits in the striatum. (a) Well-preserved striatum of a control (Case 23, age 74 years) compared to (c) a slightly changed striatum of a control (Case 24, age 75 years) and (e) the striatum of a patient with AD with densely packed amyloid plaques (Case 15, age 69 years). The right column displays a similar development in cases of Down's syndrome. (b) Striatum of a young individual devoid of amyloid (Case 1, age 17 years). (d) Slight changes in a 31-year-old (Case 3) and severe changes in a 55-year-old individual (Case 7). Campbell silver preparations. 100 μm. Bar in (f) is applicable to a–e.
AD had a striatum which was heavily infiltrated by amyloid deposits (Fig. 2e). Patients up to 30 years of age with Down’s syndrome had a normal striatum (Fig. 2b). Down’s syndrome patients older than 30 years had gradually increasing amounts of amyloid (Fig. 2d); those more than 40 years of age had severe involvement of the striatum (Fig. 2f). Control cases and middle-aged Down’s syndrome patients with few amyloid deposits did not show neurofibrillary changes (Table 1, Numbers 3, 20, 21, 24).

Globular deposits were more or less evenly distributed throughout the striatum of AD patients. The major subdivisions of the striatum (caudate nucleus, putamen, accumbens nucleus) did not show any site of predilection for amyloid. Cases with a less severely involved striatum showed only a patchy distribution of the deposits (Fig. 2c, d). Cases with severe involvement showed many tiny dots of amyloid admixed with medium-sized and large deposits. Most of the deposits remained devoid of a condensed core (Figs. 2c–f, 3a–c). Congo-red and Nissl preparations of the same material did not reveal the amyloid deposits. Accumulations of glial cells within or around the amyloid deposits were not encountered.

A small zone close to the ependymal lining of the caudate nucleus consistently remained clear of amyloid deposits. Even in cases with a severely altered striatum such a zone extended from the ependyma through a meshwork of glial cells into the neuropil of the caudate nucleus (Fig. 3b: area between arrows).

The external pallidum was almost devoid of amyloid while the internal portion harbored some deposits (Fig. 3a). Frequently, amyloid was found in the adjoining white matter such as the external and internal capsule. These deposits appeared as agglomerations of condensed and particulate argyrophilic structures (Fig. 3d, e). Their features allowed a distinction from the deposits located in a nuclear gray matter, since the latter were characterized by a smoothly contoured outline (Fig. 3c). In general, the bundles of myelinated fibers traversing the striatum remained free of amyloid deposits (Fig. 3a, c).

Amyloid angiopathy represents an additional manifestation encountered in some cases of AD. However, in contrast to the numerous amyloid deposits, amyloid angiopathy was absent in the striatum.

**Neurofibrillary Changes**

There were no neurofibrillary changes in the striatum of non-demented controls (Fig. 4a, Table 1). However, numerous NFT and NT were observed in cases of AD. The striatum of Down’s syndrome patients up to 30 years of age remained well-preserved. However, beyond that age there was an increasing number of NFT and NT (Fig. 4b).

Neuritic (senile) plaques (NP) were small and occurred infrequently (Fig. 4c–e). The sections did not contain NP with a central amyloid core.

Voluminous NFT were recognized in large neurons scattered throughout the striatum (Fig. 4b). The cell bodies were almost entirely filled by the NFT which occasionally gave off a thin extension into the proximal portion of a dendrite (Fig. 4f–h). Neurofibrillary tangles (NFT) were also encountered in a few medium-sized nerve cells (Fig. 4i–k). Gallyas preparations counterstained for lipofuscin pigment and Nissl material revealed that NFT-bearing cells were characterized by an excenetric nucleus and peripheral basophilic material. Besides pathologically altered cells, there were also well-preserved large and medium-sized neurons of the same type in the striatum.

Neuropil threads (NT) were loosely scattered throughout the striatum (Fig. 4b) and consisted of nerve cell processes filled with argyrophilic material. Many NT
Fig. 3. Distribution of amyloid in the striatum. (a) Putamen and caudate nucleus are evenly filled with amyloid deposits. Note the severe insular cortical changes and amyloid deposits in the claustrum and internal portion of the pallidum. (b) Portion of the caudate nucleus demonstrating the distribution of amyloid deposits. Only a narrow zone close to the ependymal lining remains virtually devoid of amyloid. The right arrow points to the ependyma, ** indicates the subependymal glial layer, while * marks some neuropil of the caudate nucleus almost devoid of amyloid; left arrow points to the lower boundary of this zone. (c) Striatal...
followed an irregular course with kinks and sharp bends. Often, spindle-shaped formations developed out of the thread-like portions. Branching was rare (Fig. 4I–o). Close to the bundles of myelinated fibers NT became more and more regularly oriented and eventually followed the course of the bundles (Fig. 4b).

DISCUSSION

Severe involvement of the striatum is apparently frequent in AD. Patients with Down’s syndrome apparently inevitably develop AD (18, 19). Cases of Down’s syndrome, therefore, offer the possibility of studying the development of the pathological changes of AD.

Amyloid

The presence of an abundance of amyloid deposits characterizes the striatum of patients with AD, according to this study. Neurofibrillary changes, glial reactions, or distortions of the neuropil were not recognized within or around these deposits. Accordingly, these deposits do not correspond to and should carefully be distinguished from NP (4, 20, 21).

The fact that striatal amyloid deposits have so far virtually escaped recognition may be explained by the insensitivity of the previous techniques employed for their demonstration. Some authors have briefly noted the presence of Alzheimer-related plaques in the striatum. However, as far as can be gathered from their illustrations and descriptions, they most probably referred to NP (22–24). A more recent study of Rudelli et al (25) dealt with the occurrence of amyloid as seen in thioflavine S-stained material. Their preparations showed small and rather compact plaques loosely scattered throughout the gray matter. It appears questionable, therefore, whether and to what extent thioflavine S-positive plaques correspond to the amyloid deposits demonstrated in this study.

Several specimens in this study were infiltrated with amyloid but lacked neurofibrillary changes. On the other hand, we did not encounter cases rich in neurofibrillary changes and poor in amyloid (Table 1). This fact suggests that the development of amyloid may precede that of neurofibrillary changes.

A zone consistently devoid of amyloid is located subjacent the ependymal lining of the caudate nucleus, an area that unequivocally includes portions of the neuropil, suggesting that partially soluble precursors of amyloid may escape from this narrow zone into the cerebrospinal fluid.

Neurofibrillary Changes

The statement of Rudelli et al (25) that the striatal plaques (as seen in thioflavine S preparations) belong to the class of NP is not confirmed by the present study. Our observations demonstrate only occasional NP as a meshwork of changed neurites devoid of an amyloid core.

The presence of NFT within large nerve cells of the striatum has been briefly noted by several authors (26–28). The morphological characteristics of the large and the medium-sized NFT-bearing neurons in the striatum correspond to those of the aspiny type IV (large) and type V (medium-sized) local circuit neurons as disclosed

→ amyloid deposits. Note the considerable variation in size. A myelinated fiber bundle indicated by arrows is free of amyloid deposits. (d, e) Portions of the internal capsule with several “white matter amyloid deposits.” Case 9. Campbell silver preparation. 100 μm.
Fig. 4. Distribution of neurofibrillary changes in the striatum. (a) Well-preserved striatum of a control (Case 20, age 65 years) compared to (b) altered striatum of a patient with AD with many NFT and NT (Case 8, age 56 years). Tiny dots in the control brain represent lipofuscin pigment in astrocytes. This argyrophilic pigment is easily distinguished from NFT within nerve cell somata. (c–e) Show typical NP. (f–k) Show NFT in large somata (f–h) and medium-sized cell bodies (i–k). Note the thin extensions into the proximal dendrites. (l–o) Show NT with a kinked course. The diameter of the threads changes considerably. Bar in o is applicable to c–n. Case 8. Gallyas silver preparation. 100 μm.
in Golgi studies (14). Type IV cells are most probably cholinergic neurons (29–31). The large cholinergic neurons in the magnocellular nuclei of the basal forebrain are particularly prone to develop NFT in AD (32). Therefore, it is not surprising that the large cholinergic neurons in the striatum are similarly changed. The level of the striatal choline acetyltransferase, however, does not decrease much during the course of AD (33). Parent et al. (34) even stress the preservation of the large neurons in AD. Large neurons may in fact be capable of bearing a tangle for a relatively long period of time and some of them remain devoid of NFT even in severe cases of AD. Nevertheless, there should be a cell loss of NFT-bearing nerve cells in the striatum. Our finding of the severe affection of the large neurons is consistent with quantitative investigations carried out on Nissl-stained material, revealing a significant numerical decrease of large striatal neurons in AD (35).

Neuropil threads (NT) represent a substantial part of the total amount of the striatal neurofibrillary changes. Cortical NT occur within distal portions of dendrites of NFT-bearing isocortical pyramidal cells (36). When this observation is applied to the striatum, differences become apparent. The large type IV neurons in the striatum have only a small dendritic domain with thin dendrites bending around the parent soma and giving off many fine branches at short intervals (14). However, a dense meshwork of NT surrounding the NFT-bearing type IV cells cannot be found and branching of the NT is also missing. Therefore, it seems unlikely that striatal NT are exclusively located in the dendrites of the type IV neurons.

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REFERENCES


22. Bouman L, Bok ST. Senile plaques in the corpus striatum. Ztschr f d ges Neurol u Psychiat (Berl) 1923;85:164–9

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