A NEUROANATOMICAL ANALYSIS OF THE DISTRIBUTION OF
HISTOPATHOLOGIC LESIONS IN ALZHEIMER'S DISEASE

By

THOMAS GERALD BEACH

B.Sc., The University of Victoria, 1980
M.Sc., The University of British Columbia, 1984
M.D., The University of British Columbia, 1985

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES
(Neuroscience)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
June 19, 1991
copyright Thomas Gerald Beach, 1991
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Psychiatry

The University of British Columbia
Vancouver, Canada

Date September 11, 1991
ABSTRACT

The thesis was designed to address two major questions about the pathogenesis of Alzheimer's disease. These are: one, does Alzheimer's disease "spread" throughout the brain along neuroanatomical pathways? And two, can the histopathological lesions of Alzheimer's disease be the result of degeneration of a single neuronal system? The investigation began by comparing the pattern of cerebral gliosis in Alzheimer's disease (AD) with that seen in normal aging. This was done using an immunoperoxidase stain for glial fibrillary acidic protein (GFAP) to reveal astrocytic gliosis within whole-hemisphere sections. Gliosis was most severe, and most consistently seen, in the cerebral cortex and hippocampus. Subcortical nuclei were inconsistently affected. Within the cortex, the laminae which are most severely affected are II, III and V. Distinct bands of gliosis were present in these laminae in at least some cortical areas in every AD brain. The pattern of gliosis within area 17, the primary visual area, was anomalous in that there was the a thin band of gliosis at the border between layers IVc and V. This was matched by a line of senile plaques at the same site, revealed using an enhanced Bielschowsky stain. Such a precise disposition implies a neuronal origin for these plaques, but there is no known afferent system which is concentrated along this line. Specific afferent systems to layers IVc and V of area 17 were then examined histologically for evidence of morphological relationships to senile plaques. Only cholinergic elements were in obvious relation to senile plaques; in addition, only cholinergic elements showed evidence of depletion and morphological abnormality. In the last part of the thesis, the distribution of amyloid β-protein (AβP) in area 17 was studied using an immunoperoxidase method. This was found to differ substantially from the distribution of senile plaques, with obvious bands of dense staining in layers I and IVc, layers that did not possess significantly larger numbers of plaques as seen with
the enhanced Bielschowsky method. Acetylcholinesterase-positive fibres within area 17 were co-distributed with AβP to layers I and IVc, providing further evidence that amyloid may be formed at cholinergic fibres. The results of this thesis are most compatible with the hypothesis that Alzheimer’s disease represents a primary degeneration of the cholinergic system of the nucleus basalis of Meynert. A review of the literature suggests that current opinion relegating the cholinergic degeneration to a secondary role is not adequately substantiated.
# TABLE OF CONTENTS

Abstract .................................................................page ii

List of Tables ............................................................page v

List of Figures ............................................................page vi

Acknowledgement ..........................................................page viii

Thesis Introduction ........................................................page 1

Part One: The Distribution of Astrocytic Gliosis in Alzheimer's Disease and Normal Aging ................................................page 3

Part Two: Histopathology of Area 17 in Alzheimer's Disease .......................page 60

Part Three: An Investigation of Afferent Systems to Layers IVc and V in area 17 of Alzheimer's Disease Patients.............................page 87

Part Four: The Laminar Distribution of Amyloid Beta-Protein within Area 17 of Alzheimer's Disease .............................................page 114

Thesis Conclusion ..........................................................page 127

Bibliography .................................................................page 147
LIST OF TABLES

Table I (Part One): Clinical and pathological characteristics of subjects......page 7
Table II (Part One): Cerebro- and cardiovascular disease in subjects. ......page 8
Table III (Part Two): Clinical and pathological characteristics of subjects......page 63
Table IV (Part Two): Presence of visual disorders and histopathology of area 17 in subjects. ......page 65
Table V (Part Three): Patient characteristics and procedures used. ......page 93
Table VI (Part Three): Comparison of histopathology in the lateral geniculate nucleus and area 17. ......page 94
Table VII (Part Four): Amyloid β-protein immunoreactivity and acetylcholinesterase fibre density in area 17 of subjects. .....page 118
Table VIII (Concl'n): Results of studies describing the laminar distribution of cholinergic terminal indices in the neocortex. .....page 133
Table IX (Concl'n): Subregional density of senile plaques and cholinergic terminal indices in the hippocampal formation. .....page 135
LIST OF FIGURES

1. Pattern of GFAP immunoreactivity in whole-hemisphere section from a 41 year old normal female ..........page 29
2. Patterns of GFAP immunoreactivity in whole-hemisphere sections from a 60 year old male control patient and a 60 year old male Alzheimer’s patient ..........page 31
3. Patterns of GFAP immunoreactivity in whole-hemisphere sections from a 73 year old female control patient and a 75 year old female Alzheimer’s patient ..........page 33
4. Patterns of GFAP immunoreactivity in whole-hemisphere sections from an 83 year old female control patient and an 87 year old female Alzheimer’s patient ..........page 35
5. Pattern of GFAP immunoreactivity in posterior parietal cortex of Alzheimer’s patients ..........page 37
6. Pattern of GFAP immunoreactivity in primary visual cortex of Alzheimer’s patients ..........page 39
7. Pattern of GFAP immunoreactivity in young normal cortex ..........page 41
8. Pattern of GFAP immunoreactivity in normal aging cortex ..........page 43
9. Pattern of GFAP immunoreactivity in Alzheimer’s cortex ..........page 45
10. Pattern of plaque distribution and gliosis in Alzheimer’s cortex ..........page 47
11. Double-staining: amyloid and gliosis in Alzheimer’s cortex ..........page 49
12. Perivascular gliosis in the cerebral cortex ..........page 51
13. Patterns of periventricular gliosis ..........page 53
14. Patterns of gliosis in the deep cerebral white matter ..........page 55
15. Patterns of gliosis in the lenticular nucleus ..........page 57
16. Perivascular gliosis in the putamen ..........page 59
17. Diagram of linear plaque formation by “aberrant sprouting” ..........page 74
18. Pattern of gliosis in area 17  ..........page 77
19. Pattern of plaque distribution in area 17  ..........page 79
20. Sublaminar quantification of plaque density in area 17  ..........page 81
21. Neurofibrillary tangles in area 17  ..........page 83
22. Pattern of Alz-50 immunoreactivity in area 17  ..........page 85
23. Cytochrome oxidase staining of area 17 in Alzheimer's disease  ..........page 100
24. Cytochrome oxidase and senile plaques in area 17  ..........page 102
25. Acetylcholinesterase fibres in area 17  ..........page 104
26. Pattern of staining with di-I placed in layers II-III  ..........page 106
27. Di-I-filled fibres and senile plaques in area 17  ..........page 108
28. "  ..........page 110
29. "  ..........page 112
30. Amyloid β-protein and acetylcholinesterase in area 17  ..........page 123
31. "  ..........page 125
ACKNOWLEDGEMENT

I would like to thank all of those at the Kinsmen Laboratory who helped and encouraged me. First of all, Dr. Edith McGeer was unfailingly considerate and patient. One could not hope for a better supervisor. Joane Sunahara and Ron Walker provided good spirits and professional advice in equal measure. Dr. H. Tago was especially kind and helpful. Drs. S. Itagaki and H. Akiyama are thanked for their time and interest. I am grateful to Dr. H.D. Chung for providing much of the subject material from St. Louis. The financial support of the British Columbia Health Care Research Foundation was much appreciated. Thanks must also go to Dr. Pat McGeer for helpful discussion.
THESIS INTRODUCTION

Alzheimer's disease has been, and remains today, a disease defined by characteristic histopathological lesions. These are the neurofibrillary tangle and the senile plaque. Other microscopic phenomena, such as granulovacuolar degeneration, Hirano bodies, and amyloid angiopathy (Corsellis, 1983), have been described in association with Alzheimer's disease (AD), but only plaques and tangles are considered important enough to consider in the diagnosis (Khachaturian, 1985). The common occurrence of plaques and tangles in non-demented elderly individuals has led to speculation that AD is a form of accelerated aging of the brain, and has required that diagnostic criteria for AD specify that certain concentrations are present (Khachaturian, 1985). Current research in AD remains focussed on these enigmatic structures.

In recent years, anatomic studies of Alzheimer's disease (AD) cases have made available detailed descriptions of the distributions of senile plaques and neurofibrillary tangles (Hyman et al, 1984; Pearson et al, 1985; Rogers and Morrison, 1985; Duyckaerts et al, 1986; Lewis et al, 1987; Mann and Esiri, 1988). From this information, a general hypotheses of disease pathogenesis has been formulated. This hypothesis holds that AD "spreads" spatially and temporally throughout the brain from an initially circumscribed location, moving along nerve fibres. The region of original involvement has been suggested to lie within the parts of the brain associated with olfactory sensory input; in particular, the olfactory bulb, amygdala, hippocampus, and entorhinal cortex.

This hypothesis of disease advancement is worth examining for three reasons. The first is that, if AD does spread spatially and temporally within the brain, certain etiologies are implied, such as infectious or toxic. Research may then be directed toward testing these etiological hypotheses, with the ultimate objective being the prevention of disease initiation. The second reason is that study of the mechanism
of spread might lead to ideas for arresting its course. The third reason is that the hypothesis is testable, by yet more detailed anatomical studies.

Aside from providing a test of the theory of neuroanatomical spread, detailed anatomical studies of the distribution of histopathological lesions in AD might also indicate whether any particular neurotransmitter-specific neuronal systems are selectively affected. Since the discovery of a cortical cholinergic deficit in AD in 1976 (Davies and Maloney, 1976; Bowen et al, 1977; White et al, 1977; Perry et al 1977) the cholinergic system arising from the nucleus basalis of Meynert has been scrutinized as a possible site of primary pathology. Other neurotransmitter systems, notably those using noradrenaline, serotonin, somatostatin, and glutamate have also been suggested to be affected (Perry and Perry, 1985; Mann and Yates, 1986; Rossor, 1987). A detailed map of lesions in AD could be compared with published distributions of these neurotransmitter-specific neuronal systems. If a particular system can be implicated as a site of early involvement, research efforts might then be focussed on that system. Replacement drug therapy could be designed with hopes of ameliorating symptoms or slowing progression of the disease.

The objective of this thesis is to examine the distribution of histopathological lesions in Alzheimer's disease and relate this distribution to that of neuroanatomical pathways and neurotransmitter-specific neuronal systems.
PART ONE

THE DISTRIBUTION OF ASTROCYTIC GLIOSIS
IN ALZHEIMER'S DISEASE
AND
COMPARISON WITH NORMAL AGING
The pattern of astrocytic gliosis in the AD brain was surveyed as an initial step in constructing a map of lesion distribution in Alzheimer's disease. The gliotic scar tissue formed by astrocytes is known to serve as a long-lasting marker at CNS sites where loss of neural elements has occurred; the study of astrocytic gliosis in Alzheimer's disease might therefore reveal a more complete picture of the distribution of neuronal degeneration than studies limited to plaque and tangle distribution. It has been implicitly assumed in the literature that the distribution of plaques and tangles is equivalent to the total distribution of neural degeneration in AD. It is possible, however, that plaques and tangles may not constitute the entire range of histopathology in AD; there may be changes in areas which do not possess these distinctive lesions. Secondary degeneration, which might be expected to occur in Alzheimer's disease, may or may not be associated with plaques and tangles. Thus the study of astrocytic gliosis might be expected to provide a more complete "map" of affected brain regions. Such a map would be over-inclusive, since gliosis is not AD-specific. Comparison of the pattern of gliosis in AD brains with that seen in normal aging should allow deductions as to which brain regions are consistently affected in AD.

The method of choice for illustrating astrocytic gliosis is immunohistochemical staining for glial fibrillary acidic protein (GFAP). GFAP is a primary constituent of glial intermediate filaments, and is found only in astrocytes (Bignami and Dahl, 1974). Not all astrocytes are stained by GFAP immunohistochemistry, however. Protoplasmic astrocytes are usually not stained; presumably their low intermediate filament content puts them below the threshold for immunohistochemical detection (Bignami et al, 1980; Duffy et al, 1980; Kitamura et al, 1987; Schechter et al, 1981). Astrocytes reacting to tissue injury contain increased numbers of intermediate filaments and stain strongly for GFAP (Bignami et al, 1980; Lindsay, 1986). These properties make GFAP immunohistochemistry very useful as a marker of astrocytic gliosis in the central nervous system; normal grey matter is relatively unstained so
that any grey matter immunoreactivity is immediately obvious and significant. Since, however, white matter astrocytes are normally GFAP-immunoreactive, sites of white matter pathology may be marked by both increases and decreases in staining intensity.

Previous descriptions of GFAP immunohistochemistry in Alzheimer’s disease and normal aging have established that there is an increase of GFAP-immunoreactive astrocytes in the cortex and hippocampus. Duffy (1980) was the first to report an increase in AD cortex and hippocampus; this was later confirmed and quantified by others. Schecter et al (1981) found a four-fold increase of cortical GFAP-immunoreactive astrocytes in AD; Mancardi et al (1983) reported even greater increases. Tobo (1984) and Hansen et al (1987) both reported that normal aging is also accompanied by an increase in numbers of GFAP-immunoreactive astrocytes. None of these groups, however, commented on the cortical laminar distribution of immunoreactive astrocytes, and there have been no published accounts of GFAP immunohistochemistry in subcortical areas in normal human aging or AD.

MATERIALS AND METHODS

Case selection

The institutions from which cases were obtained consisted of nursing homes, extended care hospitals and acute care hospitals located in the greater Vancouver region. Generally, consecutive brains received by the laboratory were accepted for inclusion in the study unless there was a clinical history of a neurological disease other than chronic unexplained dementia. One control case was accepted despite a history of hepatic encephalopathy because it was valuable as a relatively young age-matched control. One dementia case was accepted despite a clinical history of lacunar infarcts (seen on computed tomographic scan) because these had left no residual neurological deficits. Cases whose gross examination revealed extensive or multiple infarcts or softening or whose brains were received more than 24 hours
after death were excluded.

Clinical information was obtained entirely from chart review. In the cases in which death had occurred at hospitals affiliated with the University, charts were generally extensive and detailed. When death had occurred after a sudden illness in an individual who had had no prior hospital admissions, or in an individual who had been residing in a nursing home, clinical information was often very limited. Formal neuropsychological testing was not performed, with the exception of a single case.

Clinical summaries

Study subjects consisted of eight dementia cases and six non-dementia cases (Table I, next page). The non-dementia cases included five with no history of neurological disorders and one with a history of chronic hepatic encephalopathy. Seven of the dementia cases had no history of coexistent neurological disease; one had an additional diagnosis of multiple lacunar cerebral infarcts. Cerebrovascular and cardiovascular conditions present in the study subjects are listed in Table 1.2 (page after Table I). The dementia cases ranged in age from 63-98 (mean 79.7). The non-dementia cases ranged in age from 41 to 93 (mean 72.5).

Summary of neuropathologists' findings

Neuropathological examination resulted in a diagnosis of AD in seven of the dementia cases; the eighth case did not show distinctive histopathology and was therefore classified as an idiopathic dementia. One AD case also showed multiple lacunar infarcts of the lenticular nucleus and subcortical white matter, confirming the clinical history. An additional AD case showed severe cerebral arteriosclerosis (see Table II).
Table I. Relevant Clinical and Pathological Characteristics of Study Subjects. Duration of dementia, subjective density of plaques and tangles, average cortical choline acetyltransferase activity as percentage of control, brain weight, post-mortem interval.

<table>
<thead>
<tr>
<th>Code/Dx/Age/Sex</th>
<th>Dem</th>
<th>Pl/Ta</th>
<th>ChAT</th>
<th>BrWt</th>
<th>PMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal pre-elderly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc148: 41, F</td>
<td>-</td>
<td>-</td>
<td>/</td>
<td>1320</td>
<td>7h</td>
</tr>
<tr>
<td>Normal elderly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc166: 73, F</td>
<td>-</td>
<td>-</td>
<td>110</td>
<td>1150</td>
<td>6h</td>
</tr>
<tr>
<td>Mc168: 83, F</td>
<td>-</td>
<td>++</td>
<td>80</td>
<td>1500</td>
<td>6h</td>
</tr>
<tr>
<td>Mc146: 86, F</td>
<td>-</td>
<td>-</td>
<td>/</td>
<td>1210</td>
<td>8h</td>
</tr>
<tr>
<td>Mc152: 93, F</td>
<td>-</td>
<td>++</td>
<td>300</td>
<td>1150</td>
<td>5h</td>
</tr>
<tr>
<td>Hepatic encephalopathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc143: 60, M</td>
<td>-</td>
<td>-</td>
<td>90</td>
<td>1445</td>
<td>5h</td>
</tr>
<tr>
<td>Idiopathic dementia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc150: 69, M</td>
<td>5y</td>
<td>+</td>
<td>55</td>
<td>1300</td>
<td>5h</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc132: 63, M</td>
<td>5y</td>
<td>+++</td>
<td>25</td>
<td>1160</td>
<td>4h</td>
</tr>
<tr>
<td>Mc167: 73, F</td>
<td>3y</td>
<td>+++</td>
<td>30</td>
<td>1120</td>
<td>7h</td>
</tr>
<tr>
<td>Mc153: 75, F</td>
<td>9y</td>
<td>+++</td>
<td>16</td>
<td>880</td>
<td>7h</td>
</tr>
<tr>
<td>Mc159: 79, M</td>
<td>4y</td>
<td>+++</td>
<td>68</td>
<td>1090</td>
<td>4h</td>
</tr>
<tr>
<td>Mc174: 80, M</td>
<td>5y</td>
<td>+++</td>
<td>38</td>
<td>1240</td>
<td>6h</td>
</tr>
<tr>
<td>Mc183: 87, F</td>
<td>2y</td>
<td>+++</td>
<td>58</td>
<td>1220</td>
<td>5h</td>
</tr>
<tr>
<td>Mc151: 98, F</td>
<td>8y</td>
<td>+++</td>
<td>76</td>
<td>/</td>
<td>5h</td>
</tr>
</tbody>
</table>

Dem: Duration dementia
Pl/Ta: Plaques and tangles
ChAT: Choline acetyltransferase activity
BrWt: Brain weight
PMI: Post-mortem interval

- condition not present
+ condition present or plaques and tangles present in hippocampus only
++ plaques and tangles in hippocampus; plaques numerous but tangles rare or absent in neocortex
+++ plaques and tangles numerous in hippocampus and neocortex
/ parameter not evaluated
<table>
<thead>
<tr>
<th>Patient Code</th>
<th>Cerebro/Cardiovascular Disease Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mc166</td>
<td>Atrial fibrillation, atherosclerotic heart disease</td>
</tr>
<tr>
<td>Mc168</td>
<td>Rheumatic heart disease, prosthetic mitral valve, tricuspid regurgitation, atrial fibrillation, congestive heart failure</td>
</tr>
<tr>
<td>Mc146</td>
<td>Abdominal aortic aneurysm, remote MI, severe coronary atherosclerosis, renal arteriolosclerosis, nephrosclerosis, left ventricular myocardial hypertrophy</td>
</tr>
<tr>
<td>Mc152</td>
<td>Acute, subacute and remote MI, angina, congestive heart failure, nephrosclerosis</td>
</tr>
<tr>
<td>Mc150</td>
<td>Congestive heart failure, generalized atherosclerosis</td>
</tr>
<tr>
<td>Mc167</td>
<td>Hypertension, remote MI</td>
</tr>
<tr>
<td>Mc153</td>
<td>Severe cerebral arteriosclerosis</td>
</tr>
<tr>
<td>Mc159</td>
<td>Remote MI, TIA, Stokes-Adams attacks, atherosclerotic aortic and mitral valve disease, permanent pacemaker</td>
</tr>
<tr>
<td>Mc174</td>
<td>Moderate generalized atherosclerosis</td>
</tr>
<tr>
<td>Mc183</td>
<td>Numerous lacunar infarcts, lenticular nucleus</td>
</tr>
</tbody>
</table>
Tissue handling

After gross inspection and weighing of the brain by the pathology resident, samples were dissected for diagnostic histopathology and choline acetyltransferase (ChAT) assay (methodology is described in McGeer and McGeer, 1976; McGeer et al, 1984; tissue chemistry was not performed by the candidate but the data are included for general interest). Samples were taken from varying areas, but certain areas were always sampled and average ChAT values given in Table I are derived from these: frontal pole, Broca's area, precentral gyrus, post-central gyrus, temporal pole, middle temporal gyrus, and occipital pole. Following this, the cerebri were divided sagittally. The left hemisphere was cut into 1 cm coronal slices and fixed by immersion in 4% paraformaldehyde with 1% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for approximately one year. The slices were then embedded in 7% gelatin with 30% sucrose (two days of infiltration at 4°C followed by one day of hardening at 4°C and one week of fixation in 4% paraformaldehyde; gelatin embedding was done as part of a quantitative study of cortical volume in AD and this procedure was found not to adversely affect GFAP immunostaining). A series of whole-hemisphere 25 μm sections were taken from each 1 cm slice on a standard sledge-type freezing microtome (American Optical) fitted with a custom-made freezing platform.

Sections from each series were stained by several methods, including cresyl violet, Luxol Fast Blue, haematoxylin and eosin, and an immunoperoxidase method for GFAP. Immunohistochemistry was done on free-floating sections; all wash steps and dilutions were done with 0.1M phosphate-buffered saline (PBS) with 0.05% Triton X-100 on an oscillating platform except where otherwise noted. The sections were first incubated with a monoclonal antibody to GFAP (Lee et al, 1984) diluted 1:1000 (the most effective dilution as determined by test dilutions ranging from 1:100 to 1:10,000), overnight at 4°C. After washing (three changes of solution over 30 minutes between each of the steps described below), the sections were incubated similarly with biotinylated anti-mouse IgG (Vector) diluted 1:500. The sections were
then incubated for one hour at room temperature with an avidin-biotin-peroxidase complex ("ABC", Vector), diluted 1:400, and, in a final step, reacted with 3,3'-diaminobenzidine tetrahydrochloride (0.02% in 0.05M Tris buffer, pH 7.4, with 1% nickel ammonium sulphate). Stained sections were mounted onto slides coated with chrome-alum gelatin and coverslipped when dry with a cold-setting polyester resin. Control sections were subjected to the same procedure, with omission of the primary antibody.

Selected portions of whole-hemisphere sections were cut out and mounted on standard-size slides; these were then stained by a modified Bielschowsky technique for plaques and tangles. Areas evaluated in this manner included temporal and parietal neocortex as well as hippocampus. From these sections, subjective evaluations of plaque and tangle density were derived (see Table I).

Cortical laminae referred to throughout the text are meant to correspond to those described by Brodmann (1909).

RESULTS

Clinical data are given in Tables I and II, along with plaque and tangle density and distribution, choline acetyltransferase results, and brain weight.

Photographic prints of whole-hemisphere sections from seven different cases are shown to provide an impression of the range of different staining patterns seen (Figures 1-5). Detailed discussion of the staining results will be given by region.

In the text, the term "normal aging" will be used and is defined here as meaning individuals aged 60 or over who had no history of neurological disorders and were judged by chart review to have been mentally competent.

Cerebral cortex

The staining pattern of the pre-elderly, healthy cortex is represented by that seen in the case of a 41 year old woman who died of breast cancer (Figure 1;
This woman had no history of neurological impairment and was free of brain metastases. There was a thick network of GFAP-immunoreactive fibres in the molecular layer, particularly the outer half, and occasional fibres extended up from the white matter into layer VI, but layers II to V were almost entirely devoid of immunoreactive structures (Figure 7). Blood vessels were ensheathed with immunoreactive fibres as they passed through layers I and II, but were unstained in lower layers (Figure 7a,b). Occasional isolated, fine fibres extended down to layer II.

The brains of elderly, mentally competent individuals all displayed considerable cortical gliosis (Figure 3a). Immunoreactivity tended to be concentrated in the upper three cortical laminae, and often took the form of clusters of large astrocytes with numerous processes (Figures 5c,d, 8a). Upon correlation with Bielschowsky-stained sections, it was apparent that the large, bushy clusters of astrocytes occurred only in cases with cortical plaques. Cases without senile plaques showed diffusely distributed, moderate populations of small immunoreactive astrocytes occurring mainly in layers I-III. Ensheathment of blood vessels with immunoreactive fibres was more extensive than that seen in the young brain; vessels running through areas with parenchymal gliosis were especially affected (Figure 8a and 8b). A band of hypertrophied astrocytes at the interface of grey and white matter was seen inconstantly. Regional differences were not prominent, but it was apparent that the most heavily affected areas were superior and middle frontal gyrus and posterior parietal cortex.

The pattern of cortical gliosis in Alzheimer's disease was very distinctive. All cases displayed a specific laminar staining pattern in at least some areas, with heavy bands of gliosis in layers II-III and V (Figure 2b, 3b, 5e,f,g). Occasionally a third band of staining at the interface of grey and white matter was visible (Figure 2a, 3a). In some cases, this typical laminar pattern could be seen in all cortical areas (Figure 2b, 3b) while in others it had a restricted distribution. The superior and middle frontal gyrus, cingulate gyrus, and posterior parietal cortex displayed the
typical pattern most consistently. Area 17, the primary visual cortical area, displayed a different, but also highly selective, laminar pattern. Staining was distributed preferentially to layers II-IVa and often formed a thin line along the interface of layers IVc and V (Figure 6c-g). The primary auditory, somatic sensory and motor areas generally were not as heavily stained, with significant immunoreactivity often being restricted to the supragranular layers.

At the microscopic level it was seen that the bands of staining in AD cortex consisted of aggregations of dense astrocytic clusters (Figure 9a,c). These resembled those seen in cases of normal aging with cortical plaques (Figure 8a) except that, in the AD cases, there were large numbers of them in layers II-III and V, whereas they were mostly confined to layers II and III in the normal aging cases (compare Figure 8a with Figure 9a). The clusters often were in the shape of rings or "haloes", with large hypertrophied astrocytes encircling a central, unstained region. The band of staining at the grey matter-white matter interface (Figure 9b) consisted of 3-5 rows of hypertrophied astrocytes (Figure 9d).

Comparison with nearby sections stained with a modified Bielschowsky's method revealed that the bands of heavy GFAP immunoreactivity were sometimes matched by bands of concentrated senile plaques (Figure 10a,b). Double-staining of selected sections for GFAP and for amyloid (by thioflavin S) showed that the centres of many of the plaque-like astrocytic clusters often contained amyloid (Figure 11a-d). The "halo" configuration of many of the astrocytic clusters was thus seen to be due to the encircling of amyloid plaque cores.

Cortical blood vessels were often heavily ensheathed by GFAP-immunoreactive fibres. Often the ensheathment was segmental in nature (Figure 12a), but some vessels were ensheathed through the full depth of cortex (Figure 12b).

A case of dementia showing both Alzheimer's changes and multiple lacunar infarcts (Mc183; Figure 4b) showed less cortical gliosis than other "pure" AD cases. This case was an 87 year old woman who had a clinical history of stroke resulting in
mild motor dysfunction, a CT scan diagnosis of bilateral basal ganglia infarcts, and a neuropathological diagnosis of multiple lacunar infarcts of the lenticular nucleus and deep white matter, along with moderate to severe Alzheimer's changes. There were scattered plaque-like clusters of immunoreactive astrocytes in the cortex, but they were too sparsely distributed to form laminar bands of staining (Figure 5h, 6h), except in small areas of posterior parietal and superior frontal cortex.

A 60 year old man with a six month preterminal history of hepatic encephalopathy and an autopsy diagnosis of micronodular hepatic cirrhosis (Mc143) displayed a grossly normal staining pattern (Figure 2a). The only unusual features were especially fibrous, GFAP-immunoreactive astrocytes at the the boundary of cortex and white matter, occasional large, "hairy" astrocytes scattered across all laminae and increased perivascular gliosis of blood vessels in layers I-III (Figure 13c).

A 69 year old man with a five year history of dementia without neocortical plaques and tangles or signs of cerebrovascular disease was classified as an idiopathic dementia (Mc150). In this case there was heavy, confluent gliosis of all laminae throughout much of the cortex, but, in some areas where gliosis was lighter, it was apparent that there were especially dense aggregations of immunoreactive astrocytes in layers II and III.

White matter

The deep cerebral white matter (substantia alba hemisphéri or centrum semiovale) of the pre-elderly, healthy brain (Mc148), was diffusely and strongly stained for GFAP (Figure 1). The case of hepatic encephalopathy (60 years old) was very similar in appearance. Microscopically the staining in both cases was found to be due to evenly distributed populations of fibrous astrocytes. The staining was not completely uniform; there was a mottled quality which was caused by elliptical areas
of lighter staining. At higher magnification these were seen to consist of zones of decreased astrocytic density around blood vessels (Figure 14c,d). The blood vessels themselves were generally ensheathed by a thin coat of immunoreactive fibres. The white matter surrounding the extremities of the horns of the lateral ventricle and that surrounding the superolateral angle of the body of the lateral ventricle were stained more intensely than other white matter areas (Figure 1, 13a). This was due to an increased density of small immunoreactive astrocytes and a dense network of fine immunoreactive fibres. The white matter of the tapetum was outlined by astrocytes and fibres aligned along the large fibre bundles which make up this structure.

White matter staining in the normal elderly brains had a patchy quality due to areas of both relatively increased and relatively decreased staining (Figure 3a). Overall, there appeared to be decreased staining as compared to younger cases and foci of intense staining were prominent against this decreased background (Figure 3a). Some of these foci of increased staining were associated with blood vessels, consisting of wide zones of relatively increased densities of astrocytes and fibres, but others had no such vascular association. Astrocytic ensheathment of blood vessels was generally more pronounced in the white matter of elderly brains. Intensely stained periventricular white matter areas were also more pronounced and extensive in elderly brains (Figure 3a, 5a, 13b,c).

Alzheimer cases did not show a distinctive pattern of white matter staining. Like non-demented elderly cases, elderly AD cases had a patchy quality instead of the myelin-stain appearance of the young brain. A "young" AD case (Mc 132; age 63) did not have a patchy appearance, but had a pronounced mottling pattern, which, as in non-demented cases, was seen to consist of perivascular zones of decreased staining (Figure 2b, compare with Figure 1 and Figure 14c,d). As in the non-demented elderly, elderly AD cases appeared to have an overall loss of white matter staining intensity relative to pre-elderly brains, although one AD case with
severe cerebral atrophy (Mc 153) displayed very intense staining of the white matter (Figure 2b, 5f, 6e). Hypertrophy of astrocytes at grey matter-white matter interface zones was more common and widespread in AD cases than in non-AD cases (Figure 9b, 15a,b). Periventricular white matter staining was often enhanced (Figure 13d,e,f) but did not differ appreciably from that seen in non-demented elderly cases.

The case of Alzheimer's disease with multiple lacunar infarcts (Mcl83) was notable for pronounced perivascular gliosis, intense periventricular staining, and numerous foci of intense staining in the centrum semiovale (Figure 4b). Blood vessels showing degenerative changes, such as tortuosity, increased perivascular spaces with hemosiderin-bearing macrophages and perivascular corpora amylaceae, were surrounded by profuse networks of GFAP-immunoreactive fibres. The white matter bordering on lacunar infarcts was also intensely immunoreactive.

The case of idiopathic dementia (Mcl50) had patchily-stained white matter as in other aged brains. There were no unusual or unique features.

Neostriatum and claustrum

The putamen and caudate nucleus of the pre-elderly healthy brain (Mcl48) appeared to be relatively unstained when viewed with the naked eye (Figure 1). Microscopically, the only immunoreactive elements seen were those associated with blood vessels. These consisted of thin sheaths of immunoreactive fibres outlining the vessel wall, and small numbers of immunoreactive astrocytes in contact with or in the immediate vicinity of the vessel wall (Figure 16a).

Normal elderly individuals differed from younger cases in that they always displayed larger and denser perivascular astrocytic reactions, and in some cases also showed moderate densities of parenchymal astrocytes. Perivascular staining of larger blood vessels was often so intense that it could be easily seen with the naked eye. Under the microscope, such areas were seen to consist of dense fibre networks and numerous perivascular astrocytes (Figure 16b,c). Vessels often appeared to be
abnormally dilated and tortuous, with enlarged perivascular spaces.

In cases of Alzheimer's disease neostriatal staining generally did not differ appreciably from that seen in non-demented cases. As in non-demented normal aging cases, AD cases showed an increase in perivascular GFAP immunoreactivity, and, as with the non-demented elderly cases, some displayed enlarged, ragged perivascular spaces (Figure 15f). In the most heavily gliosed AD brain, plaque-like clusters of immunoreactive astrocytes, similar to those associated with senile plaques in the cortex, were seen in the caudate and putamen (Figure 16d). In another AD case, plaque-like clusters were seen in the nucleus accumbens but not in the caudate or putamen proper. Plaque-like clusters or halo-shaped aggregations were also observed in two cases in the claustrum. No such clusters were observed in the neostriatum or claustrum of non-demented cases.

The AD case with multiple lacunar infarcts (Mc183) displayed very heavy perivascular gliosis in the caudate and putamen (Figure 4b, 15e). The putamen was, in fact, the region most severely affected by lacunae, which were prominently outlined by immunoreactivity (Figure 4b). Perivascular spaces were generally enlarged, contained hemosiderin-bearing macrophages, and had a ragged outline which was bordered by intense fibrous gliosis (Figure 16e).

The case of hepatic encephalopathy (Mc143) showed some increased perivascular gliosis but was otherwise free of immunoreactivity (Figure 2a). The case of idiopathic dementia (Mc150) showed moderate densities of immunoreactive astrocytes in the parenchyma and increased perivascular gliosis.

**Globus pallidus and thalamus**

The globus pallidus and thalamus were exceptions to the general observation that, in the pre-elderly healthy brain, grey matter was relatively free of immunoreactivity. Light staining was seen macroscopically (Figure 1); microscopically this consisted of light to moderate populations of small, delicate
astrocytes.

These two nuclei were heavily gliosed in some of the healthy elderly individuals (Figure 3a). A large proportion of the immunoreactivity was obviously associated with the white matter bundles passing through these nuclei, immunoreactive astrocytes being aligned along the edges of the bundles. Perivascular gliosis, however, was less prominent than in the neighbouring caudate and putamen.

Cases of Alzheimer's disease displayed a range of thalamic and pallidal staining which was similar to that seen in elderly non-demented cases (Figure 3b, 15c,e,f).

The case of idiopathic dementia (Mc150) displayed moderate populations of immunoreactive parenchymal astrocytes in globus pallidus and thalamus; perivascular gliosis was minimal. Staining of pallidum and thalamus was not increased over the non-elderly control level in the case of hepatic encephalopathy (Mc 143; Figure 3a).

Hippocampus

Hippocampal grey matter of the pre-elderly, healthy brain was free of GFAP-immunoreactive elements except for the subpial glia limitans, and the polymorphic and granular layers of the dentate gyrus, which possessed moderate populations of immunoreactive astrocytes.

Healthy elderly cases always showed some degree of hippocampal gliosis. The subiculum appeared to be most affected. The entorhinal cortex was also frequently gliosed, particularly the large stellate cell clusters of layer II.

Alzheimer cases generally showed heavier hippocampal gliosis than non-demented elderly cases. In some cases, gliosis and atrophy were extremely marked (Figure 3b). The distribution of gliosis tended to parallel that of plaques and tangles (ie CA1/subiculum > CA2-CA4), but, in severe cases, the entire structure was involved. The large stellate cell clusters of layer II of the entorhinal cortex were often outlined. Neurofibrillary tangles were occasionally GFAP-immunoreactive.
The case with multiple lacunar infarcts (Mc183) showed less hippocampal gliosis than other AD cases (Figure 4b).

The case of hepatic encephalopathy (Mc143) showed a patch of intense gliosis in CA1 and a band of heavy staining in CA2-4. The case of idiopathic dementia (Mc150) displayed heavy gliosis of CA1 and the entorhinal cortex.

Other areas

The substantia innominata was moderately stained in the pre-elderly brain and intensely stained, with marked perivascular gliosis, in the normal aging cases (Figure 3a, 4a). Alzheimer cases were also heavily gliosed in this region.

The subthalamic nucleus was lightly to moderately stained in the pre-elderly brain. Some normal aging cases showed intense gliosis of this nucleus, usually in conjunction with heavy thalamic and nigral gliosis (Figure 3a). In AD cases the subthalamic nucleus was usually only lightly to moderately stained but in some cases was heavily gliosed; again, this occurred in conjunction with thalamic and nigral gliosis.

The substantia nigra of the pre-elderly brain was free of GFAP immunoreactivity. As with the subthalamic nucleus, considerable gliosis occurred in some cases of both AD and non-demented elderly (Figure 3a), while other cases were relatively free of immunoreactivity.

The amygdala and hypothalamus were not often well represented, due to unfavourable planes of section, but they appeared to follow the general pattern of grey matter areas in being unstained in the pre-elderly, with variable gliosis in the elderly and in Alzheimer's disease. Plaque-like astrocytic clusters were seen in the amygdala in two AD cases.
DISCUSSION

Although this study was not quantitative in nature and did not examine large numbers of cases, some of the findings were consistent enough to permit a limited discussion of the distribution of GFAP immunoreactivity in aging and Alzheimer's cerebrum.

The healthy, pre-elderly human cerebrum is immunoreactive for GFAP only in white matter areas. The only immunoreactive elements in grey matter are those of the glia limitans. Some nuclei, such as the thalamus and globus pallidus, contain moderate populations of immunoreactive astrocytes but these are probably white matter astrocytes associated with the myelinated fibre bundles running through these areas. The grey matter of the polymorphic and granular layers of the dentate gyrus appears to be exceptional in the possession of GFAP-immunoreactive astrocytes in the absence of intermixed white matter. In general, the presence of GFAP-immunoreactive structures in grey matter is unusual and likely indicative of pathological change. White matter is normally stained to a macroscopically uniform degree; a disruption of this uniformity would suggest a pathological process. These conclusions are supported by animal studies and previous studies in humans (Bignami et al, 1980; Duffy et al, 1980; Kitamura et al, 1987; Schechter et al, 1981).

Normal aging is accompanied by the appearance and increasing density of GFAP-immunoreactive structures in grey matter. A large proportion of these elements are associated with blood vessels as sheaths which grow in thickness and density with age. Parenchymal gliosis also occurs but is less consistently seen. The white matter has a patchy appearance macroscopically, due to areas of both increased and decreased immunoreactivity. There is a tendency of astrocytes at grey-white matter interfaces to hypertrophy.

In Alzheimer's disease the changes associated with normal aging are combined with some that do not appear in normal aging. Laminar gliosis of the cortex, in particular, is a dramatic feature which appears to be a consistent finding in
Alzheimer's disease but is not seen in normal aging. Bands of staining in layers II-III and V (II-IVa and IVc/V) in area 17) are visible to the naked eye. Much of the cortical immunoreactivity appears to consist of clusters of astrocytes which are associated with senile plaques.

The altered distribution of GFAP immunoreactivity in the aging brain could be viewed, like other aging changes, as a developmental pattern or as a pathological process. Whatever the categorization, many changes in the aging brain are degenerative in nature: neurons die, vasculature undergoes atherosclerotic and arteriolosclerotic change, and there is atrophy of both grey and white matter. These degenerative changes are therefore the most likely cause for the increased numbers of GFAP-immunoreactive astrocytes in aging grey matter.

Neuronal loss, which is thought to occur in aging cerebral cortex, substantia nigra, nucleus basalis of Meynert, and hippocampus (Coleman and Flood, 1987), undoubtedly accounts for some of the age-related cerebral gliosis. Numerous experimental studies document hypertrophy and increased numbers of GFAP-immunoreactive astrocytes at sites of neuronal loss (Bjorklund et al, 1986; Graeber and Kreutzberg, 1986; McLoon, 1986; Reier, 1986; Stromberg et al, 1986; Tetzlaff et al, 1988). It is not known whether this is due to migration of immunoreactive astrocytes to the injury site or an induction of increased GFAP production in nearby astrocytes. There is some experimental evidence that astrocytes are capable of travelling between brain regions (Goldberg and Bernstein, 1988) and can migrate into damaged areas (Rose et al, 1976). Tissue culture studies have demonstrated that various brain extracts promote GFAP production (Lim et al, 1977; Nieto-Sampedro et al, 1982; Sensenbrenner et al, 1982); degeneration of neurons may release stimulatory compounds into the extracellular fluid. Alternatively, the increased tissue movement resulting from loss of tissue components may be stimulatory in itself (Mathewson and Berry, 1985).
Cerebrovascular disease is most probably the cause of age-related perivascular gliosis. Most of the patients in this study had well-documented cardiovascular disease (see Table 2). Only one had a clinical history of stroke, but several were noted to have small infarcts upon examination of the whole-hemisphere sections. The arterial vessels of the putamen and caudate nucleus are known to undergo the greatest degree of senile change and this is where the greatest degree of perivascular gliosis was observed. The pathogenesis of this vascular degeneration, manifest by dilatation and tortuosity of the vessels, along with hyalinization of vessel walls, is thought to be related to mechanical "wear and tear" on these vessels, exerted by hemodynamic forces. Support for this concept comes from the observation that similar changes of greater severity are seen in people who have had a history of arterial hypertension (Gautier, 1983; Stehbens, 1972; Toole, 1984). Extreme degeneration of the penetrating arteries which feed the caudate, putamen, periventricular white matter and parts of the diencephalic nuclei results in the conditions known as état criblé and état lacunaire, in which perivascular spaces and perivascular infarcts, respectively, become very numerous. A recent study has confirmed our impression that GFAP-immunoreactive gliosis is especially profuse around vessels in these conditions (Mancardi et al, 1988). In the present study, only one case possessed vascular change that was so extensive as to justify the diagnosis of état lacunaire (Mc183), but several other cases, both AD and normal aging, displayed similar changes of lesser degree. Although cerebrovascular disease appears to be the cause of perivascular gliosis, the mechanism by which this occurs is is not completely explained at the present time.

Thickening of the vessel wall, which is known to occur in aging and particularly in hypertensive conditions, may cause perivascular gliosis by physically impairing exchange of oxygen and metabolites (Miquel et al, 1983). There is, in fact, a perivascular capillary-free space around larger arterioles and venules which is thought to be dependent on direct diffusion through the walls of these larger vessels.
Although capillaries sometimes appeared to show perivascular gliosis, it was the larger vessels which displayed this most prominently. Impairment of exchange of choline (Mooradian, 1987) and peptides (Banks and Kastin, 1985) across cerebral vessels has been reported to occur in normal aging. It has been suggested that the perivascular gliosis of aging is therefore a direct result of vessel wall thickening (Miquel et al, 1983). There is some experimental evidence to support this. Tissue culture studies have shown that hypoxia stimulates astrocytes to produce more GFAP (Goldberg et al, 1987). There is also the well known tendency for astrocytic gliosis to occur in ischemic zones around infarcts. Thickening of the vessel wall may therefore create a perivascular zone of hypoxia which stimulates astrocytic production of GFAP.

The altered distribution of GFAP immunoreactivity seen in Alzheimer's disease is probably due to normal aging factors combined with factors specific to Alzheimer's disease. In the cortex, many of the astrocytic clusters are associated with thioflavin-S or PAS-positive material, consistent with senile plaque cores. The occurrence of reactive astrocytes at the margins of senile plaques has been known since at least 1898 (Fuller, 1912a, 1912b) but the significance of this relationship is still uncertain. Most investigators assume that the astrocytes are simply reacting to the presence of the plaque, but the possibility that astrocytes may actively participate in plaque formation has also been suggested.

The laminar pattern of cortical immunoreactivity in AD, forming bands of heavy staining in laminae II-III and V, matches that which has been reported for neurofibrillary tangles (Pearson et al, 1985). Senile plaques do not have such a well-defined distribution, but most studies have reported that the greatest numbers occur in layers II and III, with some reports also indicating concentrations within layer V (Duyckaerts et al, 1986; Rogers and Morrison, 1985; Pearson et al, 1985; Rafalowska et al, 1988). Since tangles seldom were accompanied by a GFAP-immunoreactive response, it is not likely that the laminar distribution of gliosis in AD merely reflects...
the location of tangles. Conversely, most plaques exhibited vigorous immunoreactivity which extended well out into the neuropil around the plaque. A large fraction of the immunoreactive staining was in the form of large, roughly spherical clusters, which were shown to frequently be organized around an amyloid core. Therefore it seems most likely that the laminar gliosis observed in AD cases indicates the underlying distribution of senile plaques. The large astrocytic aggregations around plaques may become confluent more readily than the plaques themselves, making the pattern of their distribution more readily apparent. Some of the gliosis may also reflect underlying neuronal loss. The laminar distribution of cortical neuronal loss in Alzheimer's disease has not yet been described, but reports to date of preferential loss of the largest neurons suggest that it is laminae III and V that are most affected (Terry et al, 1981).

In the primary visual cortex of AD brains, there is a broad band of relatively heavy GFAP immunoreactivity in layers II-IVa and a narrow band at the interface of layers IVc and V. Senile plaques have been reported to favour lamina IV; in one study 46% of all area 17 plaques occurred there (Lewis et al, 1987). Neurofibrillary tangles favour lamina II, III, and V (Braak et al, 1989a). Therefore the pattern of gliosis does not completely match literature descriptions of either plaques or tangles in this cortical area.

The subcortical grey matter, as in normal aging, showed variable gliosis in AD cases, so the role of the AD process in its causation is uncertain. Retrograde degeneration of the thalamus has been reported to occur in AD (McMenemey, 1963), and heavy thalamic gliosis was found in this study in some, but not all, AD cases. Some normal aging cases, however, also showed heavy thalamic gliosis. The same situation was found in other subcortical nuclei. It is therefore not possible to comment on whether or not the subcortical gliosis seen in AD cases represents degeneration that is specific, or even secondary to, the Alzheimer's disease process. Since AD patients, like normal aging patients, often had clinically documented
cardiovascular disease, and AD brains, like normal aging brains, showed considerable perivascular gliosis in subcortical areas, it is likely that an age-related vascular degeneration may be responsible for at least part of the subcortical gliosis in Alzheimer's disease. The halo-shaped gliotic formations seen in the striatum, nucleus accumbens, and claustrum of some of the AD cases, however, are highly suggestive of the presence of senile plaques (Thioflavin S counterstaining was not done in these areas), which have been reported to be consistently present in these areas in AD (Rudelli et al, 1984).

The substantia innominata (SI) showed marked gliosis in all the normal aging and AD cases. This is not surprising as there are numerous reports of pathology in this area, both in normal aging and AD. Plaques, tangles, and substantial neuronal loss occur within this region in AD (Whitehouse et al, 1982; Arendt et al, 1983; Candy et al, 1983; Tagliavini et al, 1983; Wilcock et al, 1983; Mann et al, 1984; McGeer et al, 1984; Jacobs et al, 1985; Rasool et al, 1986; Rogers et al, 1985; Rudelli et al, 1984). Normal aging is also associated with neuronal loss in the SI (Mann et al, 1984; McGeer et al, 1984). In this study, the SI of both aging and AD cases showed severe perivascular changes, consisting of enlargement of the perivascular spaces with a marked gliotic response.

The altered white matter distribution of GFAP immunoreactivity seen with aging in both Alzheimer's disease and control brains (see Figures 2-6) is interesting in view of other recent reports of age-related white matter changes. White matter lucencies on computed tomographic (CT) scans (George et al, 1986a; Gupta et al, 1988; Hachinski et al, 1987; Naeser et al, 1980; Steingart et al, 1987; Valentine et al, 1980), and hyperintensities on magnetic resonance imaging (MRI) scans (Bradley et al, 1984; Crooks et al, 1982; George et al, 1986b; Kertesz et al, 1988) have been suggested to represent loosening or thinning of myelin (Englund et al, 1987; Grant et al, 1987; Kirkpatrick and Hayman, 1987), ischemia at arterial border zones (Roman, 1987), incomplete infarction (Brun and Englund, 1986; Englund et al, 1987), or état
criblé (Awad et al, 1986). Based on the results of the present study, it is suggested that astrocytic gliosis may be the structural basis of these imaging anomalies. Reactive gliosis has been reported to be clearly visualized as an increased MRI signal, presumably due to the increased water content of gliotic areas (Barnes et al, 1988). In addition, the patterns formed by white-matter gliosis closely resemble those reported from imaging studies. For example, intense GFAP-immunoreactive gliosis was observed in this study to occur in formations resembling periventricular "rims", "caps" (Figure 6), and "unidentified bright objects" (Figure 2a, 3a,b), as described by MRI (Kertesz et al, 1988; Zimmerman et al, 1986).

A peculiar finding of this study were the hypertrophied lines of reactive astrocytes at grey matter-white matter boundaries. This has also been reported in a study of aging mice (Mandybur et al, 1989). The reason for this is unknown, but it is interesting to note that interface areas are affected preferentially in other conditions as well. Transient increases in intracranial pressure produce lines of haemorrhages at the corticomedullary boundary (Heck et al, 1974), and blunt trauma to the skull also may produce such haemorrhages (Moritz, 1954). In severe cases of hepatic encephalopathy, spongy necrosis has been reported at junctions between grey and white matter throughout the brain (Malamud and Hirano, 1974; Victor et al, 1965). The common cause of pathology at grey-white junctions may be the vulnerability to physical stress inherent at interfaces between dissimilar substances.

The lack of increased grey matter GFAP immunoreactivity in the hepatic encephalopathy case agrees with previous reports (Kimura and Budka, 1986). Presumably the studied cases, like the one described here, lacked the spongy necrosis seen in advanced cases (Malamud and Hirano, 1974), as it is difficult to accept that necrotic change can occur without the subsequent appearance of GFAP-immunoreactive astrocytes. The encephalopathy of hepatic failure is thought to impair function primarily by interfering with metabolic processes, without necessarily producing structural changes. The lack of a GFAP-immunoreactive astrocytic
reaction in the case studied here supports that concept. The type II astrocytes
described by Alzheimer in cases of hepatic encephalopathy have sparse cytoplasm
and enlarged, pale nuclei. They thus differ morphologically from the common
"reactive" astrocyte, which exhibits increased nuclear chromaticity and prominent,
eosinophilic cytoplasmic processes. Type II astrocytes also differ from common
reactive astrocytes by lacking immunoreactivity for GFAP (Kimura and Budka, 1986).

This study of the distribution of astrocytic gliosis in Alzheimer's and normal
aging cerebrum has shown that gliosis is consistently present and is widespread in
both conditions. In the cortex of normal-aging cases, gliosis is mainly restricted to
the supragranular layers while Alzheimer's cases show a laminar gliosis of layers II-
III and V in association cortex, and layers II-IVa and IVc/V in primary visual cortex.
Subcortically, with the exception of the substantia innominata, Alzheimer's and
normal aging cases show considerable gliosis in patterns which suggest that vascular
disease is the primary cause. In the substantia innominata, both neuronal loss and
vascular disease are likely contributory to the consistent gliosis seen in both normal
aging and AD.

These results reinforce those derived from studies of neurofibrillary tangle
distribution and neuronal counts which indicate that Alzheimer's disease
preferentially affects specific neuronal populations, particularly those of the
entorhinal cortex, hippocampus, and laminae II-III and V of the neocortex.

The results of this study are also supportive of the concept that Alzheimer's
disease spreads along neuroanatomical pathways from an initially circumscribed
location (Pearson et al, 1985). The consistently heavy gliotic scarring of the
hippocampus and entorhinal cortex is compatible with the hypothesis that the
disease process begins in that locale (Mann and Esiri, 1988). The preferential gliosis
of laminae II-III and V supports the concept that AD spreads throughout the
neocortex along corticocortical association pathways, since these pathways largely
originate within, and are distributed to, these cortical laminae (Jones, 1981).
The observed pattern of histopathology is consistent with the suggestion that glutamatergic neurons are the primary neuronal type affected. Glutamate, and/or aspartate, are believed to be the major neurotransmitters used by neocortical pyramidal cells, the principal neuronal type of layers II, III and V (Emson and Lindvall, 1986; McGeer et al, 1989). Because these amino acids are also present in all cells as integral metabolites, indirect methods must be used to provide estimates of the fraction which is associated with neurotransmission. The validity of these methods is still controversial, however, and there have been reports both denying (Ellison et al, 1986; Procter et al, 1988) and supporting (Hardy et al, 1987; Cowburn et al, 1988; McGeer et al, 1989) significant decrements in the relevant brain areas in AD.

The laminar pattern of gliosis in the primary visual cortex, area 17, differs from that seen in the rest of the neocortex. There was appreciable staining in laminae II and III, but the band of gliosis in layer V was lacking. In its place was a thin line of gliosis at the border of layer IVc and V. This obvious deference to laminar architecture suggests a dependence upon specific neuronal factors and thus deserves further investigation.
Figure 1. Whole-hemisphere frozen section (25 μm) from the brain of a 41 year old female who died of breast cancer (Mc148). The section has been stained with an immunoperoxidase method for GFAP. In (a) the section is depicted as photographed conventionally with background illumination: immunoreactive areas appear dark. In (b) the same section is shown as a negative image, achieved by placing the section in a photographic enlarger and printing directly: immunoreactive areas appear light. The direct print has greater resolution and contrast, and is less time-consuming than conventional photography. This section displays the distribution of GFAP immunoreactivity in the pre-elderly healthy brain. Note that it is essentially equivalent to a myelin-stained section, with prominent staining of the white matter and very little staining of grey matter. A thin line of intense immunoreactivity outlines the outer surface of the cortex (a: arrowheads); this represents the astrocytes of the sub-pial glia limitans. Calibration bar equals 0.5 cm, and serves for both (a) and (b).
Figure 2. Whole hemisphere frozen sections stained immunohistochemically for GFAP. (a) is a section from a 60 year old male who died as a result of hepatic failure with encephalopathy due to micronodular cirrhosis (Mc143). In addition, this patient had a three year history of treated hypertension. (b) is a section from a 63 year old male who had a five year preterminal history of Alzheimer's disease, confirmed by neuropathological analysis (Mc132). The staining pattern in (a) is essentially that of a young, healthy brain, with very little staining of grey matter and prominent, uniform staining of white matter. Only the amygdala (am) displays increased parenchymal immunoreactivity. Intense perivascular immunoreactivity is seen in the caudate and lenticular nuclei (arrows). In (b) note the two intense bands of immunoreactivity in the cortex (arrows); the superficial band is located in laminae I-III and the deep band is in lamina V. A third, less intense band is visible in some areas (arrowhead); this is located at the interface of the grey and white matter. There is light to moderate immunoreactivity in the caudate (Cd) and putamen (Pu) and there is some increased perivascular gliosis. The white matter of the centrum semiovale has a mottled appearance, due to zones of decreased immunoreactivity surrounding blood vessels. Calibration bar equals 0.5 cm, and serves for both (a) and (b).
Figure 3. Whole-hemisphere frozen sections stained immunohistochemically for GFAP. (a) is a section from a 73 year old woman with no history of psychiatric or neurological disease who died of pancreatic cancer (Mc166). The patient also had a history of atrial fibrillation. The hippocampus was dissected out for diagnostic purposes. (b) is a section from a 75 year old woman with a nine year history of dementia and autopsy diagnoses of severe Alzheimer's disease and cerebral arteriosclerosis (Mc153). In (a) note that there is a speckling of immunoreactivity in the cortex, mainly in the superficial laminae, and heavy gliosis of the thalamus (th), globus pallidus (gp), substantia innominata (si), subthalamic nucleus and substantia nigra (arrowheads). Note also the intense immunoreactivity of the white matter in the periventricular area (pv) and the scattered foci of intense staining elsewhere in the white matter (arrows). In (b), note that the entire section is strongly immunoreactive. More specifically, note that there is laminar gliosis of the cortex (arrowheads) and intense staining of the thalamus (th) and globus pallidus (gp). The temporal lobe shows extreme atrophy with intense staining of the white matter, and the hippocampal formation (hi) is severely gliosed. Calibration bar equals 0.5 cm and serves for both (a) and (b).
Figure 4. Whole-hemisphere frozen sections stained immunohistochemically for GFAP. (a) is a section from an 83 year old female who died of congestive heart failure secondary to rheumatic valvular disease (Mc168). (b) is a section from an 87 year old woman with a two year history of dementia (Mc183). The autopsy of (b) revealed multiple small infarcts of the lenticular nucleus and white matter of the parietal and occipital lobes. Moderate numbers of plaques and tangles were seen in the hippocampus; in the cortex moderate numbers of plaques were seen but tangles were rare. In (a) note that there is intense perivascular gliosis (white arrows) in the putamen (pu) and substantia innominata (si). Also note the foci of intense staining in the white matter of the centrum semiovale (black arrows). In the amygdala (am) there is intense immunoreactivity adjacent to the temporal horn of the lateral ventricle (white arrow). In (b) note the light speckling of the cortex, the patchy staining of the white matter and the perivascular gliosis of putaminal (pu) and thalamic (th) vessels. There is a lacunar infarct in the caudate nucleus (white arrow). The white matter shows foci of intense immunoreactivity, often around blood vessels (black arrowheads). The claustrum (black asterisk) and capsular white matter are strongly immunoreactive. The hippocampus (hi) is only lightly stained. Bar in (a) = 0.5 cm (for a and b).
Figure 5. Negative prints of sections through posterior parietal cortex stained immunohistochemically for GFAP. (a) to (d) are from different individuals (Mc148, Mc143, Mc166, Mc152, respectively). All were free of neurological symptoms except (b), who had a history of hepatic encephalopathy. Note that the grey matter is free of GFAP immunoreactivity in the younger individuals (Mc148:41yrs; Mc143:60yrs), except for a thin line adjacent to the pia mater, representing the glia limitans. The older individuals (Mc166:73yrs; Mc152:93yrs) display a speckled pattern of immunoreactivity which is concentrated in the superficial laminae. (e), (f), and (g) are from different individuals (Mc132, Mc153, Mc151, respectively) who had a history of dementia and a neuropathological diagnosis of Alzheimer’s disease. Note that all have heavy staining of the cortex, concentrated in two bands (arrowheads) corresponding to laminae II-III and V. (h) is from an individual with a history of dementia and neuropathological diagnoses of multiple lacunar infarcts combined with mild Alzheimer’s changes (Mc183). Note that the cortex displays only light immunoreactivity. (i) is from an individual with a five year history of dementia of unknown etiology (Mc150); plaques and tangles were moderately numerous in the hippocampus but were not seen in the neocortex. Bar (a) = 4 mm (for a - i).
Figure 6. Negative prints of sections through primary visual cortex (area 17) stained immunohistochemically for GFAP. (a) and (b) are from individuals with no history of neurological symptoms (Mc148 and Mc152 respectively). Note that the light staining of the cortex in (a) and heavy staining in (b) is mainly distributed in the upper layers; microscopically this was seen to correspond to layers IVc and above. (c) - (g) are from individuals with neuropathologically-confirmed Alzheimer's disease (Mc132, Mc167, Mc153, Mc159, and Mc151, respectively). Note that all have considerable gliosis, which is dispersed evenly throughout layers II-IVa and concentrated in prominent bands in layer IVc (arrows). Microscopically, the layer IVc staining was often found to be situated at the border of layers IVc and V. (e) and (g) have two thin bands of staining in layer IVc, one at the lower edge of IVc and one at the upper edge of IVc. (h) is from an individual with a history of dementia due to multiple lacunar infarcts and mild Alzheimer's changes (Mc183). There are no prominent bands of gliosis. (i) is from an individual with a five year history of dementia of unknown etiology (Mc150). Note the confluent, heavy gliosis of all layers, with a relative sparing of layers III and IVc (arrowheads). Calibration bar in (a) equals 4 mm and serves for (a) - (i).
Figure 7. Photomicrographs of cortex from the collateral sulcus (temporal lobe) of a 41 year old woman who died of breast cancer (Mc148). (a) is a low magnification view of the entire depth of cortex, illustrating that GFAP-immunoreactive structures in the young, healthy cortex are found only in a narrow subpial zone (spz), roughly corresponding to the molecular layer, and in the interface zone (ifz), where immunoreactive fibres from the white matter sometimes extend up into layer VI. Calibration bar equals 250 μm. (b) is a higher magnification of the subpial zone, showing immunoreactive astrocytes and fibres lying in the neuropil and associated with blood vessels. Calibration bar equals 100 μm and serves for (b) and (c). (c) is a higher magnification of the interface zone between layer VI of cortex and the underlying white matter.
Figure 8. Photomicrographs of cortex from: (a) aged normal individual with numerous cortical plaques; (b) aged normal individual without cortical plaques. (a) is from the middle temporal gyrus of a 73 year old woman who died of pancreatic cancer (Mc 166), and shows a low magnification view of the entire depth of cortex. Note the large, "bushy" clusters of immunoreactive astrocytes in layers II and III (asterisks). Note also the GFAP-immunoreactive investment of large and small blood vessels (arrowheads). Calibration bar equals 400 μm and serves for both (a) and (b). (b) is from the precentral gyrus of an 86 year old woman who died of a ruptured abdominal aortic aneurysm (Mc 146). The whole depth of cortex is displayed, at the same scale as (a). Note the absence of the large, bushy clusters of immunoreactive astrocytes seen in (a). There are moderate densities of immunoreactive astrocytes in the superficial layers (I-III) and deep layers (V and VI) while the middle layers (III-IV) are relatively spared. A blood vessel (small arrowheads) is ensheathed with immunoreactive processes. Occasional small clusters of astrocytes are seen (large arrowhead).
Figure 9. Photomicrographs of cortex from the inferior parietal lobule of a 63 year old man with a five year history of dementia and a neuropathological diagnosis of severe Alzheimer's disease (Mc132). (a) is a low magnification montage of the entire depth of cortex, showing bushy clusters of astrocytes in layers II-III and V. Layers IV and VI are spared. Calibration bar equals 250 μm. (b) is a low-magnification view of another site in the inferior parietal lobule of the same patient, illustrating the same laminar pattern of gliosis with the addition of a thin line of immunoreactive astrocytes at the interface zone of grey and white matter (ifz). Calibration bar equals 1 mm. (c) is a high magnification view showing a plaque-like cluster of immunoreactive astrocytes in layer V. Calibration bar equals 100 μm. (d) is a high magnification view of the interface zone (ifz), showing the hypertrophy and close packing of immunoreactive astrocytes located there. Layer VI of cortex is above the zone, subcortical white matter is below. Calibration bar equals 100 μm.
Figure 10. Low magnification photomicrographs of cortex from the superior parietal lobule of a 63 year old man with severe Alzheimer's disease (Mc132). (a) is stained immunohistochemically for GFAP, illustrating the heavy gliosis of layers II-III and V (heavy arrows). (b) is a nearby section stained with a modified Bielschowsky's method, showing that senile plaques are concentrated (heavy arrows) in the same laminae which exhibit heavy gliosis. Calibration bar in (a) equals 1 mm.
Figure 11. Photomicrographs of cortex from the frontal pole of a 63 year old man with severe Alzheimer's disease (Mc132). The section is double stained: immunohistochemically for GFAP (a,c) and with Thioflavin S for plaque amyloid (b,d). Note that the plaque-like astrocytic clusters, which often appear to form rings or haloes (a,c), are wrapped around the periphery of plaque cores (b,d). Calibration bar in (a) equals 50 µm and serves for (a) - (d).
Figure 12. Photomicrographs illustrating perivascular GFAP-immunoreactive structures in cerebral cortex. (a) is a section from the frontal pole of a 63 year old man with severe Alzheimer's disease (Mc132). Note that GFAP-immunoreactive fibres are seen only on a short segment of this blood vessel (arrowheads). Perivascular GFAP-immunoreactivity was often found to be limited to segments of blood vessels running through areas of parenchymal gliosis. The section has been counterstained with cresyl violet. Calibration bar equals 100 µm. (b) is a section from the cortex of a 69 year old man with dementia of unknown etiology (Mc150). Three blood vessels with thin perivascular sheaths of GFAP-immunoreactive fibres are seen. Calibration bar equals 200 µm. (c) is a section from the frontal pole of a 60 year old man with hepatic encephalopathy (Mc143), showing a vessel in cross section with a thick, "hairy" perivascular sheath of GFAP-immunoreactive fibres. Calibration bar equals 50 µm.
Figure 13. Negative prints of sections through periventricular regions, stained immunohistochemically for GFAP. Ventricles are marked by "v". (a) shows the superolateral angle of the lateral ventricle near the caudate nucleus (cd) in a 41 year old woman who died of breast cancer (Mc148). A small zone of increased immunoreactivity is seen. Calibration bar equals 4 mm and serves for (a) - (f). (b) is a section through an equivalent area in a 73 year old woman who died of pancreatic cancer (Mc166). There is a larger zone of more intense immunoreactivity, which is even more marked in a section farther anterior (c). (d) shows an intensely immunoreactive area farther caudal, but still at the superolateral angle of the lateral ventricle, in a 79 year old man with Alzheimer's disease (Mc159). (e) is from the same case and shows an intensely immunoreactive area at the inferolateral corner of the lateral ventricle, in the temporal lobe. (f) shows intense immunoreactivity bordering the occipital horn of the lateral ventricle in a 98 year old woman with Alzheimer's disease (Mc151).
Figure 14. Photomicrographs illustrating aspects of GFAP immunoreactivity in the cerebral white matter. (a) shows the frontal lobe corticomedullary junction and (b) shows a white matter bundle in the putamen; in both there are hypertrophied astrocytes at the interface between grey and white matter. Both (a) and (b) are from a 73 year old woman with severe Alzheimer's disease (Mc167). Bar (in a) = 200 μm (for a,b,d, f). (c) is a low-magnification negative print of a section of parietal lobe from a 60 year old man with hepatic encephalopathy (Mc143). Note the mottled appearance of staining in the white matter. This mottled appearance was also seen in Mc148, the young, normal case. Small patches of decreased staining (arrow) are seen microscopically to be due to zones of decreased immunoreactivity around blood vessels. The area identified by the arrow is depicted at higher magnification in (d). Calibration bar in (c) equals 4 mm. (e) is from the same case as (a) and (b) and shows GFAP-immunoreactive structures at the edges of enlarged perivascular spaces in frontal lobe white matter. The ring-shaped structures (arrowheads) are astrocytic processes around corpora amylaceae. Calibration bar equals 100 μm. (f) shows a concentration of GFAP-immunoreactive fibres around an enlarged perivascular space in the frontal pole white matter of a 63 year old man with severe Alzheimer's disease (Mc132).
Figure 15. Negative prints illustrating patterns of gliosis in the lenticular nucleus. (a) is from an 83 year old woman with congestive heart failure due to rheumatic heart disease (Mc168). Note the very intense immunoreactivity surrounding large blood vessels (arrows) in the putamen (pu) and substantia innominata (si). The globus pallidus (gp), in contrast, is relatively unstained. Bar (a) = 4 mm (for a - f). (b) is from a 93 year old woman who died of an acute myocardial infarction (Mc152). Again note the perivascular gliosis of the substantia innominata and putamen. (c) is from a 73 year old woman with Alzheimer’s disease (Mc167). Note the perivascular gliosis in the putamen. (d) is from an 87 year old woman with multiple lacunar infarcts and moderate Alzheimer’s changes (Mc183). Perivascular gliosis and lacunes (arrows) are present, involving the entire lenticular nucleus. (e) is from a 79 year old man with Alzheimer’s disease (Mc159). There is a markedly enlarged perivascular space and intense perivascular gliosis around a blood vessel in the putamen (arrows). The globus pallidus is diffusely gliosed. (f) is from an 80 year old man with Alzheimer’s disease (Mc174), also showing markedly enlarged perivascular spaces (asterisk) and intense perivascular gliosis (arrows). The globus pallidus is diffusely gliosed.
Figure 16. Photomicrographs of sections through the putamen, stained immunohistochemically for GFAP. (a) is from a 41 year old woman who died of breast cancer (Mc148). The only immunoreactivity in the putamen of this patient was that associated with blood vessels. The vessel wall is outlined by immunoreactivity and a fine network of fibres is located in the neuropil around the vessel, along with a few astrocyte cell bodies. Bar (a) = 125 μm (for a, b, d). (b) is from a 73 year old woman who died of pancreatic cancer (Mc166). Again, the parenchyma of the putamen was generally free of immunoreactive elements, except near blood vessels. Here a blood vessel is surrounded by a vigorous glial reaction. (c) is from a 93 year old woman who died of an acute myocardial infarction (Mc152). The parenchyma contained moderate populations of immunoreactive astrocytes and blood vessels were heavily ensheathed with an exuberant fibrous astrocytic reaction. Bar = 250 μm (for c and e). (d) is from a 98 year old woman with Alzheimer’s disease (Mc151). The parenchyma was moderately to heavily gliosed, as illustrated. (e) is from an 87 year old woman with moderate Alzheimer’s changes and multiple lacunar infarcts (Mc183). Note the enlarged, irregularly shaped perivascular spaces (asterisks) and the fibrous astrocytic reaction in the surrounding neuropil.
PART TWO

HISTOPATHOLOGY OF THE PRIMARY VISUAL CORTEX

IN ALZHEIMER'S DISEASE
The findings of Part One of this thesis, regarding the anomalous distribution of astrocytic gliosis within the primary visual cortex, prompted a more detailed analysis of the histopathology of this area in Alzheimer's disease.

The primary visual cortex is generally regarded as being spared by the Alzheimer's disease process and thus research on AD has left this area relatively neglected. Recent quantitative studies, however, have shown that this sparing is far from complete. Neurofibrillary tangles are relatively scarce, but senile plaques are as numerous as in adjacent visual association areas (Lewis et al, 1987; Braak et al, 1989a). Since more is known about the structure and function of the primary visual cortex than any other cortical region, and because it is relatively easy to assess its function clinically, studies of the visual system in Alzheimer's disease could contribute greatly to an understanding of the pathogenesis of this disease.

In this section of the thesis, the distribution of histopathology in Alzheimer's disease visual cortex was further examined, and an attempt has been made to correlate the laminar patterns of pathology with literature descriptions of laminar distributions of cortical afferents, vascularity, and neurotransmitter-specific markers. The primary visual cortex is especially well suited for such correlations, as the lamination is well demarcated and the anatomy well characterized.

MATERIALS AND METHODS

Case selection. Case selection criteria were as detailed in Part One of this thesis. Of those included in the study (see Table III, next page), eleven were non-neurological control cases (mean age 69.9), nine were miscellaneous neurological control cases (mean age 58.2), and sixteen were AD cases (mean age 73.5). The presence or absence of a clinical history of a visual disorder was noted (see Table IV, following Table III).
Tissue processing. Brains were weighed in the fresh state and standard regional samples dissected out for neuropathological diagnosis and choline acetyltransferase assays (see Methods, Part One). The right hemisphere was frozen for unrelated studies; the left was sliced into 1 cm coronal slices and fixed by immersion in buffered 4% paraformaldehyde (PFA) at 4°C for two days. Following fixation, the slices were stored at 4°C in phosphate buffered saline (PBS) with 15% sucrose for periods ranging from 3 months to 3 years. Some brains were fixed and stored in buffered 4% PFA with 1% glutaraldehyde at 4°C for one to two years, followed by embedding in a gelatin/sucrose mixture prior to sectioning (brains used in Part One). Brain slices were sectioned on a freezing microtome and the sections stored in 0.1 M phosphate buffer at 4°C.

Staining procedures. Sections were stained with cresyl violet, haematoxylin and eosin, thioflavin S, two different modifications of Bielschowsky's method, and with immunohistochemical methods for GFAP and Alz-50. The two Bielschowsky methods are briefly described as follows: the first, which is derived from a standard method used to demonstrate plaques and tangles, utilizes three successive incubations in 20% silver nitrate, with ammonium hydroxide added in the second incubation and both ammonium hydroxide and a developer (acidified formalin) in the third. The second method, which is designed to demonstrate plaques selectively (method of H. Tago, given in Akiyama et al, 1990) is identical except for the use of 1% silver nitrate in the first incubation. These methods were performed on free-floating sections.

The immunohistochemical methods were also performed on free-floating sections, with an avidin-biotin peroxidase method as described in Part One. A monoclonal antibody for glial fibrillary acidic protein (Lee et al, 1984) was used at a 1:1000 dilution and the Alz-50 antibody (Wolozin et al, 1986) was used at a 1:400 dilution. Control sections were subjected to identical procedures with the omission of the
Table III. Clinical and autopsy characteristics of study subjects. For legend see next page.

<table>
<thead>
<tr>
<th>Code</th>
<th>Age</th>
<th>Sex</th>
<th>Dx</th>
<th>Plaques/Tangles</th>
<th>BrWt</th>
<th>ChAT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mc148</td>
<td>41</td>
<td>F</td>
<td>NEO</td>
<td>-</td>
<td>-</td>
<td>1320</td>
</tr>
<tr>
<td>Mc196</td>
<td>52</td>
<td>M</td>
<td>PE</td>
<td>-</td>
<td>/</td>
<td>1390</td>
</tr>
<tr>
<td>Mc195</td>
<td>57</td>
<td>M</td>
<td>CHF</td>
<td>-</td>
<td>/</td>
<td>1240</td>
</tr>
<tr>
<td>Mc277</td>
<td>61</td>
<td>M</td>
<td>MI</td>
<td>-</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Mc213</td>
<td>64</td>
<td>M</td>
<td>MI</td>
<td>-</td>
<td>/</td>
<td>1410</td>
</tr>
<tr>
<td>Mc166</td>
<td>73</td>
<td>F</td>
<td>NEO</td>
<td>-</td>
<td>++</td>
<td>1150</td>
</tr>
<tr>
<td>Mc219</td>
<td>77</td>
<td>F</td>
<td>NEO</td>
<td>-</td>
<td>/</td>
<td>1050</td>
</tr>
<tr>
<td>Mc239</td>
<td>82</td>
<td>M</td>
<td>MI</td>
<td>-</td>
<td>/</td>
<td>1220</td>
</tr>
<tr>
<td>Mc168</td>
<td>83</td>
<td>F</td>
<td>CHF</td>
<td>-</td>
<td>++</td>
<td>1500</td>
</tr>
<tr>
<td>Mc146</td>
<td>86</td>
<td>F</td>
<td>RAA</td>
<td>-</td>
<td>-</td>
<td>1210</td>
</tr>
<tr>
<td>Mc152</td>
<td>93</td>
<td>F</td>
<td>MI</td>
<td>-</td>
<td>++</td>
<td>1150</td>
</tr>
</tbody>
</table>

Non-neurological cases

<table>
<thead>
<tr>
<th>Code</th>
<th>Age</th>
<th>Sex</th>
<th>Dx</th>
<th>Plaques/Tangles</th>
<th>BrWt</th>
<th>ChAT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mc224</td>
<td>18</td>
<td>M</td>
<td>CI</td>
<td>-</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Mc222</td>
<td>48</td>
<td>F</td>
<td>HE</td>
<td>-</td>
<td>/</td>
<td>1240</td>
</tr>
<tr>
<td>Mc186</td>
<td>58</td>
<td>F</td>
<td>CI</td>
<td>-</td>
<td>/</td>
<td>1425</td>
</tr>
<tr>
<td>Mc296</td>
<td>71</td>
<td>M</td>
<td>CI</td>
<td>-</td>
<td>/</td>
<td>1450</td>
</tr>
<tr>
<td>Mc143</td>
<td>60</td>
<td>M</td>
<td>HE</td>
<td>-</td>
<td>-</td>
<td>1445</td>
</tr>
<tr>
<td>Mc314</td>
<td>64</td>
<td>F</td>
<td>FAM</td>
<td>16 yrs</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc292</td>
<td>68</td>
<td>F</td>
<td>EA</td>
<td>1 yr</td>
<td>++</td>
<td>1040</td>
</tr>
<tr>
<td>Mc303</td>
<td>68</td>
<td>F</td>
<td>ID</td>
<td>5 yrs</td>
<td>-</td>
<td>1050</td>
</tr>
<tr>
<td>Mc150</td>
<td>69</td>
<td>M</td>
<td>ID</td>
<td>5 yrs</td>
<td>+</td>
<td>1300</td>
</tr>
</tbody>
</table>

Misc. neurological disorders

<table>
<thead>
<tr>
<th>Code</th>
<th>Age</th>
<th>Sex</th>
<th>Dx</th>
<th>Plaques/Tangles</th>
<th>BrWt</th>
<th>ChAT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mc310</td>
<td>48</td>
<td>M</td>
<td>FAD</td>
<td>2 yrs</td>
<td>+++</td>
<td>1309</td>
</tr>
<tr>
<td>Mc270</td>
<td>60</td>
<td>F</td>
<td>AD,SCZ</td>
<td>8 yrs</td>
<td>+++</td>
<td>950</td>
</tr>
<tr>
<td>Mc287</td>
<td>62</td>
<td>M</td>
<td>AD,SD</td>
<td>4 yrs</td>
<td>+++</td>
<td>1400</td>
</tr>
<tr>
<td>Mc132</td>
<td>63</td>
<td>M</td>
<td>AD</td>
<td>5 yrs</td>
<td>+++</td>
<td>1160</td>
</tr>
<tr>
<td>Mc316</td>
<td>66</td>
<td>M</td>
<td>AD</td>
<td>4 yrs</td>
<td>+++</td>
<td>/</td>
</tr>
<tr>
<td>Mc226</td>
<td>72</td>
<td>F</td>
<td>AD</td>
<td>3 yrs</td>
<td>+++</td>
<td>/</td>
</tr>
<tr>
<td>Mc167</td>
<td>73</td>
<td>F</td>
<td>AD</td>
<td>3 yrs</td>
<td>+++</td>
<td>1120</td>
</tr>
<tr>
<td>Mc217</td>
<td>74</td>
<td>F</td>
<td>AD,CI</td>
<td>5 yrs</td>
<td>+++</td>
<td>/</td>
</tr>
<tr>
<td>Mc153</td>
<td>75</td>
<td>F</td>
<td>AD</td>
<td>9 yrs</td>
<td>+++</td>
<td>880</td>
</tr>
<tr>
<td>Mc185</td>
<td>76</td>
<td>M</td>
<td>AD</td>
<td>2 yrs</td>
<td>+++</td>
<td>1350</td>
</tr>
<tr>
<td>Mc236</td>
<td>78</td>
<td>M</td>
<td>AD,WE</td>
<td>8 yrs</td>
<td>+++</td>
<td>1465</td>
</tr>
<tr>
<td>Mc159</td>
<td>79</td>
<td>M</td>
<td>AD</td>
<td>4 yrs</td>
<td>+++</td>
<td>1090</td>
</tr>
<tr>
<td>Mc174</td>
<td>80</td>
<td>M</td>
<td>AD</td>
<td>5 yrs</td>
<td>+++</td>
<td>1240</td>
</tr>
<tr>
<td>Mc212</td>
<td>85</td>
<td>F</td>
<td>AD</td>
<td>10 yrs</td>
<td>+++</td>
<td>1100</td>
</tr>
<tr>
<td>Mc183</td>
<td>87</td>
<td>F</td>
<td>AD,CI</td>
<td>2 yrs</td>
<td>+++</td>
<td>1220</td>
</tr>
<tr>
<td>Mc151</td>
<td>98</td>
<td>F</td>
<td>AD</td>
<td>8 yrs</td>
<td>+++</td>
<td>1240</td>
</tr>
</tbody>
</table>

Alzheimer’s disease
Legend for Table III.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Alzheimer's disease</td>
</tr>
<tr>
<td>CHF</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>CI</td>
<td>Cerebral infarct(s)</td>
</tr>
<tr>
<td>EA</td>
<td>Ethanol abuse</td>
</tr>
<tr>
<td>FAD</td>
<td>Familial Alzheimer's disease</td>
</tr>
<tr>
<td>FAM</td>
<td>Familial amyloidosis</td>
</tr>
<tr>
<td>HE</td>
<td>Hepatic encephalopathy</td>
</tr>
<tr>
<td>ID</td>
<td>Idiopathic dementia</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>NEO</td>
<td>Neoplastic disorder</td>
</tr>
<tr>
<td>PE</td>
<td>Pulmonary embolism</td>
</tr>
<tr>
<td>RAA</td>
<td>Ruptured aortic aneurysm</td>
</tr>
<tr>
<td>SD</td>
<td>Seizure disorder</td>
</tr>
<tr>
<td>SCZ</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>WE</td>
<td>Wernicke's encephalopathy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Plaques and tangles in hippocampus only</td>
</tr>
<tr>
<td>++</td>
<td>Condition as in +, with frequent neocortical plaques</td>
</tr>
<tr>
<td>+++</td>
<td>Condition as in ++, with frequent neocortical neurofibrillary tangles</td>
</tr>
</tbody>
</table>

/ Not evaluated

* Choline acetyltransferase activity is expressed as the mean value of seven neocortical sites. 100% is the mean value of normal control patients, adjusted for age (see methods section, Part One for further discussion).
Table IV. Presence of visual disorders and histopathology of area 17 in the primary visual cortex of Alzheimer and control patients. For legend see next page.

<table>
<thead>
<tr>
<th>Code</th>
<th>VisDis</th>
<th>Pla</th>
<th>Ta</th>
<th>AA</th>
<th>GFAP</th>
<th>Alz-50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-neurological cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc148</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>Mc196</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>Mc195</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Mc277</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>Mc213</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>Mc166</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc219</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Mc239 cat</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>Mc168</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc146</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc152</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Misc. neurological disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc224</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mc222</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mc217</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc226</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mc132</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc396</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc398</td>
<td></td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc150</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>/</td>
</tr>
<tr>
<td>Mc292</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Mc303</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc310</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Mc270 NLVF</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Mc287</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>--</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Mc312</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc316 NLVF</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Mc226</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mc163</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc153</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>/</td>
</tr>
<tr>
<td>Mc185 cat</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Mc236 cat</td>
<td>++</td>
<td>/</td>
<td>/</td>
<td>++</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Mc159 cat, BL</td>
<td>++</td>
<td>+</td>
<td>/</td>
<td>++</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Mc174</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc212</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc183</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>/</td>
</tr>
<tr>
<td>Mc151</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>/</td>
</tr>
</tbody>
</table>
**Legend for Table IV**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>amyloid angiopathy</td>
</tr>
<tr>
<td>BL</td>
<td>functionally blind</td>
</tr>
<tr>
<td>cat</td>
<td>cataracts</td>
</tr>
<tr>
<td>DR</td>
<td>diabetic retinopathy</td>
</tr>
<tr>
<td>NLVF</td>
<td>neglect of left visual field</td>
</tr>
<tr>
<td>Pi</td>
<td>senile plaques</td>
</tr>
<tr>
<td>Ta</td>
<td>neurofibrillary tangles</td>
</tr>
<tr>
<td>VisDis</td>
<td>visual disorder</td>
</tr>
</tbody>
</table>

+ sparse density  
++ moderate density  
+++ heavy density  
- condition not present or lack of staining  
/ not evaluated
primary antibody. Endogenous peroxidase activity was suppressed prior to immunoperoxidase staining with a 30 minute incubation in 1% H₂O₂.

The laminar distribution of stained features was determined by consulting nearby sections stained for cresyl violet, as well as by counterstaining selected sections with neutral red or cresyl violet. Immunohistochemical double-staining was performed to reveal the interrelationship of GFAP- and Alz-50-immunoreactive structures. This was accomplished by sequential application of the two methods with an intervening incubation in H₂O₂ to eliminate residual peroxidase activity from the first localization. The first reaction was done with 1% nickel ammonium sulphate added to the solution of di-aminobenzidine, giving a dark bluish-black colour, while the second was performed with di-aminobenzidine alone, giving a reddish-brown colour.

Plaque density analysis. Photomicrographs of sections of area 17 cortex from 12 of the 16 AD cases (those with staining of uniformly high quality), stained with the plaque-selective Bielschowsky method, were taken with the 2x objective of a light microscope and printed onto 8x10 sheets of photographic paper. The photomicrographs were centred on the longest non-curved segment of cortex present. Laminar boundaries were marked by drawing lines on a clear plastic overlay; fibre staining (myeloarchitectonics) visible in the section. Additional lines were drawn parallel to the laminar boundaries, subdividing each into two or more equal portions (see Figure 20a). The number of plaques intersecting each line was counted and expressed as plaques/mm. Analysis of variance was conducted to determine whether observed differences in the means for plaque density along the sampling lines could have been due to chance. Means for plaque density along individual lines were compared with each other and with the overall mean for plaque density along all lines by multiple, paired t-tests.
RESULTS

The presence or absence of visual disorders and histopathology in area 17 of individual cases is summarized in Table IV. There was no obvious pathological correlation with any of these conditions.

All cases of Alzheimer's disease had at least moderate numbers of plaques; even some non-AD cases displayed plaque formation. Most AD cases had neurofibrillary tangles, which were not seen in any non-AD case. Slightly less than half of the AD cases displayed some degree of amyloid angiopathy compared to only two non-AD cases, one of which was a case of familial amyloidosis. Gliosis was most common in the AD cases, but many non-AD cases also showed considerable gliosis. Staining with the Alz-50 antibody was strongly positive in seven out of nine AD cases examined. There was no positive reaction in any of the thirteen non-AD cases studied.

Staining patterns were often highly lamina-selective and even selective for subdivisions of laminae and laminar boundaries. These patterns are described below.

Staining for gliosis

Gliosis was studied by immunohistochemistry for GFAP. In most AD cases, gliosis preferentially affected laminae II-IVa, and there was a thin line of gliosis at the lower border of layer IVc. In a few cases, generally those with severe Alzheimer's changes, another thin, gliotic line was present at the upper border of IVc (see Figure 18). Non-AD cases often displayed considerable gliosis, but did not display the specific pattern noted in AD cases.

Staining and quantification of senile plaques

Senile plaques were studied with three methods: Thioflavin S staining, and the two modifications of Bielschowsky's method (see methods). Of the three techniques, the Tago modification of Bielschowsky's method was the most sensitive, and the
following descriptions are derived from sections stained with this method.

Qualitatively, the most obvious feature noted upon scanning slides from AD cases was the frequent disposition of plaques in a linear formation along the interface of layers IVc and V (Figure 19a).

In the remainder of area 17, plaques appeared to be distributed randomly amongst the cortical laminae, except for a relative sparing of layer VI (Figure 20).

Quantitative analysis of plaque density confirmed the qualitative impression. The number of plaques per millimetre differs significantly among sampling lines ($F_{17,196} = 2.04, p < .01$). The mean plaque density along line 14, drawn at the interface between layer IVc and V, was more than double that of the mean density along all sampling lines ($p < .05$; Figure 20b), and was significantly greater than the mean of any other individual line ($p < .05$). Means for line 12 (interface of layer IVb and IVc) and line 15 (through the centre of layer V) were significantly greater than the means for several other lines but were not significantly greater than the mean of all lines. Plaque density was significantly less than the mean for all lines along lines 17 (upper half of layer VI) and 19 (interface of layer VI with the white matter.

Staining for neurofibrillary tangles

Neurofibrillary tangles were studied with two techniques, Thioflavin S and the standard modified Bielschowsky method. Although the two techniques gave similar results, the Bielschowsky sections were studied more intensively and the following description is derived from them.

Tangles were not present in all AD cases, and when they were present, they were often obviously less numerous in area 17 than in adjoining area 18 (Figure 21a,b). In a few AD cases they were very numerous in localized areas of layers II-III (Figure 21c). Tangles were most common in layers II-III, and were also seen in layers IVa and V. Occasional tangles were seen in layer VI. Tangles were not seen in layers I, IVb or IVc (Figure 21d).
Staining with Alz-50

The microscopic appearance of the Alz-50 staining was consistent with previous descriptions (Wolozin and Davies, 1987), with positive staining of an interweaving network of short, thick fibres, presumably those of degenerating neurons, and also of neurofibrillary tangles and the neuritic halo of senile plaques. Seven out of nine AD cases displayed a densely stained, intricate pattern in area 17 (Figure 22a). Staining was extremely lamina-selective, with intense staining of layers I-IVa and V, moderate staining of layer VI and a nearly-complete absence of staining in layers IVb and IVc (Figure 22b). Alz-50-positive plaques were seen mainly in layers I-IVa, with relatively few plaques seen in layers IVb, IVc and VI. Alz-50-positive neurofibrillary tangles were seen only in layers II-III, V and VI. There was an abrupt change of staining pattern at the boundary of area 17 and 18 (Figure 22c). As in area 17, layers I-III and V also were the most heavily-stained in area 18, the major difference between the two areas being the presence of the wide, unstained band in layer IV in area 17.

There did not appear to be an accentuation of Alz-50-positive staining at the border of layer IVc and V, as seen with Bielschowsky and GFAP staining, but double-staining for GFAP and Alz-50 revealed a linear array of reactive astrocytes at the upper border of the intense band of Alz-50 staining in layer V (Figure 22d).

DISCUSSION

The results presented here demonstrate that the primary visual cortex displays considerable histopathology in Alzheimer's disease, and that this histopathology is highly lamina-selective. The selectivity suggests that the pathogenetic process affects specific neuronal types.

It is apparent that the AD visual cortex is "spared" only in relative terms, since recent quantitative (Lewis et al, 1987) and qualitative (Braak et al, 1989a) studies,
as well as the present study, show that Alzheimer's changes and other indicators of pathology are abundant. Staining with the Alz-50 antibody reveals that extensive neural networks within area 17 harbour the abnormal A-68 antigen, which is associated with AD pathology (Wolozin and Davies, 1987). Despite these prominent signs of disease, there are still objective indications in the literature of a relative sparing, compared to other cortical areas. Plaque density is less than that of temporal or cingulate cortex and neurofibrillary tangles are less numerous than in neighbouring occipital association cortex (Rogers and Morrison, 1985; Lewis et al, 1987). This study also found obviously decreased numbers of tangles in area 17 as compared to neighbouring area 18.

The line of gliosis at the interface of layers IVc and V in area 17 is matched by a line of plaques at the same site. (The existence of this line of plaques has been confirmed recently by Braak et al, 1989a). This discrete disposition, highly respectful of neuroanatomical boundaries, argues strongly for a neuronal causation of plaque formation, and thus at first seems compatible with the theory of neuroanatomical spread of AD. A search of the literature, however, failed to reveal an afferent system that is predominantly concentrated along this interface. In particular, the afferents from neighbouring area 18, the cortical area which projects most heavily into 17, are not concentrated along this line; this incongruity argues against the spread of AD along corticocortical pathways, at least in area 17.

The failure of plaque distribution to correspond with particular afferent systems in area 17 must be interpreted as being a possible failure of the theory of neuroanatomical spread. The obvious dependence of plaque distribution upon laminar organization, on the other hand, supports a neuronal, rather than vascular, basis for plaque formation. Capillary density in area 17 is greatest in layer IVc, but there is no apparent enhancement of vascularization at the border of IVc and V (Bell and Ball, 1985).
The molecular layer of the dentate gyrus also harbours a linear formation of plaques, and an hypothesis advanced to account for its existence (Geddes et al, 1986; Hyman et al, 1986; Crain and Burger, 1988) may be applicable to area 17. In both locations a line of plaques forms between a layer of strong Alz-50 immunoreactivity and a layer strongly staining for AChE. In the dentate, this line of plaques is situated at the boundary of the inner third and outer two thirds of the molecular layer, which, as in the different cortical laminae, are well-defined and distinct with respect to their afferent inputs. The outer two-thirds of the dentate molecular layer contains the termination of the perforant pathway, which is the innervation of the dentate gyrus by the entorhinal cortex (Van Hoesen, 1975, 1982; Steward, 1976). This pathway is known to undergo massive degeneration in AD, with marked neurofibrillary tangle formation in the neuronal cell bodies and strong Alz-50 immunoreactivity in the terminal field (Hyman et al, 1986, 1988). There is a dense AChE-rich fibre network adjacent to this, in the inner third of the molecular layer (Amaral and Campbell, 1986; Green and Mesulam, 1988). The line of plaques forms between these two layers. In area 17, the line of plaques forms at the boundary of layer IVc with V, where, as in the dentate gyrus, an AChE-rich layer (IVc; Mesulam et al, 1984) directly abuts a layer selectively marked by heavy Alz-50 staining and neurofibrillary tangle formation (layer V).

It has been suggested that the line of plaques in the dentate gyrus is the result of an attempt by AChE-positive fibres, normally located in the inner one third of the molecular layer, to reinnervate the adjacent degenerate perforant pathway in the outer two-thirds (Geddes et al, 1985, 1986; Hyman et al, 1987). This is based on the observation that, in some AD cases, there is an altered distribution of AChE staining, with strong staining in the outer two-thirds of the molecular layer, the denervated area, rather than in the inner third, as is normally the case. By analogy with the well-characterized animal model of the deafferented dentate gyrus (Lynch et al, 1972; Cotman et al, 1973; Storm-Mathieson, 1974), where such a redistribution of
cholinergic afferents has been well documented, this alteration of AChE staining in AD has been explained as a sprouting response of cholinergic fibres, which, attracted by trophic factors, grow into the deafferented area (Geddes et al, 1986). The plaques, which are themselves strongly positive for AChE and form a line where sprouting cholinergic fibres meet the zone of degeneration, are suggested to be a byproduct of this sprouting response, and perhaps an indication that entry into the denervated zone leads to the destruction of the sprouting fibres (see Figure 17, next page).

This scenario could equally well be invoked to account for the line of plaques in area 17. At least it might be surmised that the interaction of Alz-50-positive and cholinergic neural elements is conducive to plaque formation. Comparison of the anatomy of the dentate gyrus with that of area 17 suggests another possibility, however. In both areas the layer of strong AChE staining also harbours a capillary bed of distinctly high density (Bell and Ball, 1985; Duvernoy, 1988). Therefore, it may be that the component which "reacts" with the Alz-50 positive layer is vascular-related, rather than cholinergic. Conceivably, this could be a substance which diffuses out of vasculature, such as a toxin.

The distribution of neurofibrillary tangles and Alz-50 staining in area 17, in contrast to that of senile plaques, does support the theory of neuroanatomical spread. If AD does spread from area to area along connecting nerve fibres, it is most likely that the disease would spread to area 17 from area 18. Area 18 provides the major cortical input to area 17 (Tigges et al, 1977), and, in terms of neurofibrillary tangle formation, is more severely affected in AD than area 17 (Lewis et al, 1987). In non-human primates, afferents from area 18 go to all layers in 17 except IVb and IVc (Tigges et al, 1977). This does not match plaque distribution, but does closely match that of neurofibrillary tangles and Alz-50 staining. Therefore, it is possible that the axons of area 18 neurons may induce tangle formation and Alz-50 immunoreactivity in area 17. In further support of this is the fact that it is
Figure 17. Diagram of linear plaque formation by aberrant sprouting.

ZONE OF DEGENERATING NEURITES

ZONE OF CHOLINERGIC TERMINALS

sprouting cholinergic fibres

senile plaques

(PhF-positive neuropil)
precisely the layers of origin of area 18 cortico-cortical projections that contain the
greatest numbers of neurofibrillary tangles (Lewis et al, 1987) and stain the
strongest for Alz-50. On the other hand, the distribution of tangles in area 17 also
matches that of noradrenergic afferents (Lewis et al, 1986). Therefore, if tangles are
created sequentially in brain areas by a spreading of disease along nerve fibres, the
correlations noted here suggest that either cortico-cortical neurons (presumably
glutamatergic) or noradrenergic neurons are the conduits of spread.

The pattern of histopathology revealed here does not support a selective
involvement of either the magnocellular (M-cell) or parvocellular (P-cell) portion of
the geniculostriate pathway. The fibres of these parallel pathways are spatially
segregated from the optic nerve through the LGN, and terminate in different layers
of the striate cortex. Losses of retinal ganglion cells and optic nerve fibres have
been reported in some AD patients (Hinton et al, 1986; Bassi et al, 1987; Blanks et
al, 1989), and it is primarily the M-cell pathway which is affected (Bassi and
Lehmkuhle, 1990). The M-cell geniculostriate projection terminates mainly in layers
II, III, IVb and IVc alpha, while the P-cell pathway is distributed to layers II, III,
and IVc beta. There was no evidence of lesion concentration within either pathway’s
termination.

As an incidental finding, it is noted that the remarkably lamina-specific staining
pattern of Alz-50 immunoreactivity in area 17 is identical to that reported for
neuropil threads by Braak et al (1989a). Neuropil threads, first described by Braak
and co-workers in 1986, are revealed with the Gallyas silver stain and antibodies to
paired helical filaments (PHF) (Braak et al, 1989b), and presumably represent the
occurrence of PHF's in axons and dendrites. The staining of neuropil threads shown
by Braak and co-workers in area 17 is identical in character and distribution to that
shown in this study using the Alz-50 antibody. Therefore it appears likely that the
Alz-50 antibody recognizes an antigenic determinant of paired helical filaments. This
is also supported by the electron microscopic localization of Alz-50 solely with paired
helical filaments in AD (Love et al, 1989). In both the present study and that of Braak and co-workers, neurofibrillary tangles were noted to be restricted to the laminae which are positive for neuropil threads or Alz-50 staining.
FIGURES

Figure 18. Low magnification photomicrographs of sections of area 17 in a case of advanced Alzheimer's disease (Mc153). Calibration bar in (A) equals 1 mm and serves also for (B). The section in (A) was stained with an immunoperoxidase method for GFAP, showing the pattern of astrocytic gliosis. Note scattered foci of gliosis in laminae II-IVa and the two dense lines of gliosis at the upper and lower edge of layer IVc. There is also a dense band of gliosis at the interface of grey and white matter. The section in (B) is taken from the same block as in (A), and is stained with cresyl violet, showing the characteristic laminar organization of area 17.
Figure 19. Low magnification photomicrographs of section of area 17 in a case of Alzheimer's disease (Mc159). Calibration bar in (B) equals 0.25 mm and serves also for (A). The section in (A) is stained with a modified Bielschowsky method which selectively demonstrates senile plaques (Tago method, see Methods). Note the scattered plaques in laminae II-IVa and the line of plaques at the boundary between layers IVc and V. (B) This section was taken from the same block as (A) and has been stained with cresyl violet to illustrate the cytoarchitecture for comparison with (A).
Figure 20. (A) Senile plaque density was assessed by counting the numbers of plaques intersected by lines running across area 17 cortex as depicted. Lines drawn are numbered 2 through 19 (Line 1 represents the pial surface and Line 19 is the grey/white boundary); heavy lines also represent laminar boundaries. (B) Graph of plaques/mm for the sampling lines depicted in (A), and for the mean of all lines (M). Note that line 14, which corresponds to the interface of layers IVc and V, has a plaque density which is more than twice the mean value, and is significantly greater than the plaque density of the line with the second-greatest number (line 12). Lines 17 and 19, which correspond to the upper part and lower boundary of layer VI, respectively, have significantly lower densities than the overall mean. Error bars represent the standard error of the mean; * denotes significant difference from the mean density of all lines (M) (p < .05).
A. Schematic Representation of Sampling Lines for Plaque Density Analysis

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

B. Plaque Density Analysis by Intersection with Sampling Lines

Sampling lines

Plaques/mm
Figure 21. Photomicrographs of sections of area 17 stained with a standard Bielschowsky method (see methods section). (A) In this low-magnification view, and at a higher magnification in (B), note the abrupt change in density of tangles at the boundary between area 17 and area 18 (arrows). Area 18 is on the left, area 17 on the right. Calibration bars in (A) and (B) equal 0.5 mm and 100 μm, respectively. (C) Medium-magnification view of layer III of area 17, showing that very high densities of tangles were present in some AD cases. Calibration bar equals 50 μm. (D) Montage of photomicrographs depicting the typical distribution of neurofibrillary tangles in a column of area 17 cortex. Tangles are present in laminae II-IVa, V, and VI, and are absent from layers I, IVb, and IVc. Calibration bar equals 100 μm.
Figure 22. Photomicrographs of sections of area 17 from AD cases stained immunohistochemically with the Alz-50 antibody. (A) is a low-magnification negative print (intensely-stained areas appear white). Note the intricate laminar pattern. Calibration bar equals 2 mm. (B) Positive print at a higher magnification, showing the laminar distribution of the staining. Note that layers I-IVa and V are intensely stained, layer VI is moderately stained, and layer IVb and IVc are devoid of staining except for scattered plaques. Calibration bar equals 0.5 mm. (C) Low-magnification positive print showing the abrupt change of staining pattern at the border (arrow) of area 18 (on the left) and 17 (on the right). Calibration bar equals 1.0 mm. (D) Medium-power view of a section double-stained with the Alz-50 antibody and with a monoclonal antibody for GFAP (see methods). Note the line of gliosis at the upper boundary of layer V, which otherwise displays a uniform "neuritic" quality of staining typical of that seen with the Alz-50 antibody. Calibration bar equals 0.2 mm.
PART THREE

AN INVESTIGATION OF AFFERENT SYSTEMS TO LAMINAE IVc and V
IN AREA 17 OF ALZHEIMER'S DISEASE PATIENTS
In Part Two of this thesis, it was shown that neurofibrillary tangles, but not senile plaques, have a distribution within area 17 which matches that of afferents from area 18. Since the theory of neuroanatomical spread of Alzheimer's disease (Pearson et al, 1985) would predict spread into 17 along those afferents from 18 (the direction of spread is postulated to be from hippocampus and entorhinal cortex to association cortex and finally primary sensory and motor cortices), the observed distribution of tangles is supportive of this theory.

It is becoming increasingly likely, however, that neurofibrillary tangles represent a secondary phenomenon in Alzheimer's disease and that senile plaque formation is closer to the primary event. Recent improvements in methods for staining senile plaques, including enhanced silver techniques (Campbell et al, 1987; Hedreen et al, 1988; Yamaguchi et al, 1990a) and immunohistochemical staining for β-amyloid (Kitamoto et al, 1987; Yamaguchi et al, 1988a), have indicated that plaques are generally much more widespread in the brain than tangles. Autopsy studies of Down's syndrome patients, who are known to develop inevitably the changes of AD, have shown that plaques appear at a younger age, before tangles are present (Giaccone et al, 1989; Mann and Esiri, 1989). In autopsy series of normal old people, some of whom are undoubtedly preclinical AD cases, neocortical plaques are common while tangles are rare to absent (Tomlinson, 1982). Paired helical filaments are rare in plaque "haloes" in normal aging and early AD (Duyckaerts et al, 1988; Barcilowska et al, 1989). In up to 30% of AD cases, the neocortex is devoid of tangles, with senile plaques as the sole lesion (Terry et al, 1987). Tangles, unlike plaques, are not specific to AD, occurring in almost every category of neuropathologic disease. They are seen in degenerative (progressive supranuclear palsy), infectious (subacute sclerosing panencephalitis, post-encephalitic parkinsonism), toxic (lead poisoning), traumatic (dementia pugilistica), dysgenetic (tuberous sclerosis), metabolic (lipofuscinosis), malformative (meningeal angiomatosis), and neoplastic (ganglioglioma) conditions (Wisniewski et al, 1979; Terry et al, 1987). Even the
characteristic cortical laminar distribution of neurofibrillary tangles in AD appears to be non-unique. Mandybur (1990) and Robertson et al (1990) have reported that the same distribution of tangles occurs in subacute sclerosing panencephalitis and in cortex which has been subjected to hypoxia. This evidence suggests that, in AD, tangles form as a response to some other, as yet undefined, initiating event. While it is not certain that the primary event is senile plaque formation, it appears at this point in time that plaque formation at least is closer to it.

If neurofibrillary tangles are not a morphologic counterpart to the early events in disease initiation, then they can not be considered capable of "spreading" the disease. Hence the distinctive laminar distribution of tangles and neuropil threads indicates only that this secondary change is restricted to certain neuronal types, such as the neocortical pyramidal neurons of layers II, III and V.

Although the distribution of senile plaques in area 17, unlike that of neurofibrillary tangles, did not correlate with any known afferent systems, the striking concentration of plaques along an interface between cortical laminae indicates that neuronal architecture is highly influential in the process of plaque formation. The possibility of rearrangement of afferent systems in AD (Geddes et al, 1985; Hyman et al, 1987; see Figure 17, p. 73, Part Two) means that plaque distribution may correlate with an abnormal afferent distribution rather than a normal one. For this reason, a study of some of the afferent systems to area 17 in AD might reveal more directly a relationship with senile plaques.

Three separate area 17 afferent systems were chosen for examination, all bordering on the IVc/V interface, where plaques have been previously shown to congregate (see Part Two). These afferent systems are: the lateral geniculate nucleus (LGN) input to layer IVc, the cholinergic input to layer IVc, and a pyramidal neuron input (putatively glutamatergic) to layer V. Evaluation of the LGN afferents to area 17 was done using cytochrome oxidase enzyme histochemistry. The distribution of this enzyme within area 17 matches that of LGN afferents, and its presence, in a
characteristic laminar pattern, indirectly indicates that the LGN input is intact (Horton and Hubel, 1981; Horton and Hedley-Whyte, 1984). The state of the LGN was also examined directly by staining for plaques, tangles and gliosis in AD and control cases. Evaluation of the cholinergic input to area 17 from the nucleus basalis of Meynert (nBM) was assessed with acetylcholinesterase enzyme histochemistry, which has been shown to be a specific marker of nBM-derived cholinergic fibres in this area (Bear et al, 1985). The intrinsic pyramidal neuron projection from layers II and III to layer V was examined by anterograde staining after placement of crystals of a carbocyanine dye (Godement et al, 1987) in layers II and III (See Methods, below, and Burkhalter and Bernardo, 1989).

MATERIALS AND METHODS

Case selection. Cases were chosen from those in brain tissue banks at the Kinsmen Laboratory of Neurological Research, University of British Columbia and at the Department of Pathology, St. Louis University School of Medicine. Selection criteria included a clinical history of dementia and a neuropathological confirmation of the presence of Alzheimer's disease, or, for control cases, a clinical history excluding dementia. These cases are listed in Table V and VI.

Tissue processing. Brains taken from the Kinsmen Laboratory were treated as described previously in the methods section of Part Two of this thesis. Fixation of all Kinsmen Laboratory tissue (for immunohistochemical and enzyme histochemical staining) consisted of immersion of 0.4-1.0 cm slices in buffered 4% paraformaldehyde at 4° C for two days. Brains taken from St. Louis University were fixed either as 0.4 cm slices for 8 hours in 5% buffered formalin at room temperature (for cytochrome oxidase staining) or intact in 10% buffered formalin for periods ranging from two months to 18 months (for tract-tracing).
Tissue was sectioned either on a sledge-type freezing microtome at 40 μm (for immunohistochemistry and silver staining), a cryostat at 50 μm (for silver staining and enzyme histochemistry), or a vibratome at 50μm (for tract-tracing).

Staining procedures. Sections were mounted on slides and stained with cresyl violet, or processed free-floating by a standard Bielschowsky's method or Tago's modification of Bielschowsky's method (see Part Two of this thesis), by the Karnovsky and Roots (1964) or Tago (Tago et al, 1986) modifications of acetylcholinesterase histochemistry, for cytochrome oxidase histochemistry (Horton and Hedley-White, 1984), or for immunohistochemical staining for GFAP (see Part Two of this thesis).

Immunohistochemical control sections were prepared by omitting the GFAP antibody from the staining procedure. Histochemical control sections were prepared by omitting substrate from the protocol. Endogenous peroxidase activity was suppressed prior to immunoperoxidase and AChE histochemistry (Tago method only) with a 30 minute incubation in 1% H2O2. Pyramidal neuron axonal ramifications in layer V of area 17 were demonstrated by anterograde staining after placement of dye crystals (diI: 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) in layers II-III of area 17 (Burkhalter and Bernardo, 1989). After crystal placement, tissue blocks (approximately 1.5 cm side length) were held in 10% formalin for seven months at room temperature. After sectioning on a vibratome (50 μm), sections were viewed under rhodamine-selective fluorescence with epifluorescence, standard reflected fluorescence, and confocal scanning laser microscopy. Selected sections were counterstained for plaques with Thioflavine S (15 minutes free-floating in a 0.001% solution followed by three 5 minute rinses in 0.1M phosphate buffer, pH 7).
RESULTS

Comparison of histopathology in the lateral geniculate nuclei and primary visual cortex of AD cases revealed a consistently lesser degree of pathology in the LGN (see Table VI, next page). Senile plaques were observed only in the LGN's of patients with AD. In the AD group, 5 out of 7 cases stained with the Tago modification of Bielschowsky's method possessed plaques in the LGN, while all 11 cases had plaques in area 17 (Table VI). Using a standard modification of Bielschowsky's method, LGN plaques were detected in only 2 out of 5 AD cases. Plaques in the LGN were always of the primitive or diffuse type, without an accompanying neuritic reaction. In every case in which LGN plaques were detected, area 17 from the same brains showed considerably greater numbers of plaques (Table VI). Neurofibrillary tangles were not detected in the LGN of any case; 5 of 9 AD cases displayed tangles in area 17.

Gliosis was present to a variable degree in most LGN's examined, and in fact was generally more severe in non-AD cases than in AD cases. Amongst AD cases, gliosis of area 17 was more consistently present and usually more severe than that seen in the LGN. Area 17 gliosis was, on average, far more severe in AD cases than non-AD cases.

Cytochrome oxidase (CO) staining was performed in five cases (Table V), two of which had a neuropathological diagnosis of AD. The other three had died without a history of neurological disorder. The intensity and distribution of staining was similar in all cases (Figure 23), and was identical with previous findings (Horton and Hedley-Whyte, 1984). Staining of nearby sections with the Tago modification of Bielschowsky's method showed that, despite the presence of numerous plaques in the AD cases, the pattern and intensity of CO staining was unaffected (Figure 24). The wide band of intense staining in layers IVb and IVc, as well as the "blobs" in layers II-III were easily visualized and without defects.

Acetylcholinesterase (AChE) staining was performed in 12 cases, six of which had a neuropathological diagnosis of AD (see Table V). All AD cases had numerous
Table V. Patient characteristics: major autopsy diagnoses, presence or absence of dementia, tissue utilized and procedures used.

<table>
<thead>
<tr>
<th>Code</th>
<th>Age/Sex/Dx</th>
<th>Dementia</th>
<th>Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mc375</td>
<td>76/F/MI</td>
<td>-</td>
<td>AChE*</td>
</tr>
<tr>
<td>Mc372</td>
<td>54/M/MI</td>
<td>-</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Mc361</td>
<td>60/F/MI</td>
<td>-</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Mc386</td>
<td>78/M/COPD</td>
<td>-</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Mc348</td>
<td>63/M/MI</td>
<td>-</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Mc336</td>
<td>82/M/CI</td>
<td>-</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Mc403</td>
<td>88/M/AD</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Mc371</td>
<td>83/F/AD,CI</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Mc398</td>
<td>83/M/AD,CI</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Mc349</td>
<td>69/F/AD</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Mc393</td>
<td>67/M/AD</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Mc333</td>
<td>72/F/AD</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>SM89-84</td>
<td>64/M/PF</td>
<td>-</td>
<td>CO**</td>
</tr>
<tr>
<td>SM89-86</td>
<td>63/M/MI</td>
<td>-</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>SL89-99</td>
<td>56/M/NEO,PAN</td>
<td>-</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>SM89-88</td>
<td>77/F/AD</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>SL89-112</td>
<td>78/M/AD,CI</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>VA89-87</td>
<td>61/M/MI</td>
<td>-</td>
<td>diI***</td>
</tr>
<tr>
<td>VA89-88</td>
<td>63/M/PN</td>
<td>-</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>AD88-17</td>
<td>75/F/AD,CI</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>AD88-19</td>
<td>91/M/AD</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>AD88-20</td>
<td>82/F/AD/PD</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>AD88-24</td>
<td>78/F/AD,CI</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>AD88-28</td>
<td>100/F/AD,PD</td>
<td>+</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

AD = Alzheimer's disease
COPD = Chronic obstructive pulmonary disease
CI = Cerebral infarct(s)
MI = Myocardial infarct
NEO = Neoplastic disorder
PAN = Pancreatitis
PD = Idiopathic parkinsonism
PF = Idiopathic pulmonary fibrosis
PN = Pneumonia

* AChE histochemistry (Karnovsky & Roots; Tago)

** CO: Cytochrome oxidase histochemistry

*** diI crystal placement in layers II-III
Table VI. Neurofibrillary tangles, senile plaques and gliosis in the lateral geniculate nucleus and primary visual cortex. For autopsy and clinical characteristics of these cases, see Table III, Part Two of this thesis.

<table>
<thead>
<tr>
<th>Code</th>
<th>Lateral Geniculate</th>
<th>Area 17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tang</td>
<td>Plaq</td>
</tr>
<tr>
<td><strong>Non-neurological cases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc196</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mc195</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mc213</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>Mc219</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mc239</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mc152</td>
<td>/</td>
<td>-</td>
</tr>
<tr>
<td><strong>Misc. neurological disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc222</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mc186</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mc143</td>
<td>/</td>
<td>-</td>
</tr>
<tr>
<td>Mc292</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mc150</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td><strong>Alzheimer's disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc310</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Mc270</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mc287</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mc167</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Mc217</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mc185</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mc236</td>
<td>/</td>
<td>-</td>
</tr>
<tr>
<td>Mc212</td>
<td>/</td>
<td>++</td>
</tr>
<tr>
<td>Mc183</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>Mc151</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

+ light density
++ moderate density
+++ heavy density
- not present
/ not evaluated
AChE-positive senile plaques and marked reductions in AChE-positive fibre density, as compared to controls. In the best preparations, the numbers of plaques stained for AChE approached that achieved with the Tago modification of Bielschowsky's method. Fibres in the non-AD cases were most dense in layers I and IVc, as reported previously for monkeys; unlike in monkeys, a third band of staining was not seen in layer VI (Mesulam et al., 1984). Fibres in the AD cases were usually so scarce that it was not possible to assess their differential distribution amongst layers. Remaining fibres often exhibited bulbous deformities along their lengths. These bulbous enlargements were often found within senile plaques (Figure 25). Axonal deformities were rarely seen in non-AD cases.

Tract-tracing with dil was done in seven cases, 5 of which had AD while the remaining 2 were reportedly free of neurological disease during life (see Table V). All cases (3 AD cases, 2 control cases) with a successful dye crystal placement in layers II and III showed a similar pattern and intensity of fluorescent staining (Figure 26). The site of crystal placement was marked by a small ragged hole in the section, often with a crystal of dil remaining, and a surrounding region of intense fluorescence. The layer V staining was clearly seen as a "satellite" to the dye placement site, and was separated from it by a zone of relatively poor staining (Figure 26). Laminar boundaries were visualized by cresyl violet staining on the same section, after fluorescence photography.

Selected sections from the successfully-stained AD cases were counterstained with Thioflavine S to visualize senile plaques and their relationship to dil-outlined fibres in layers IV and V. All exhibited senile plaques in the regions of dil staining (Figure 27). Dense fibre populations, including many fine fibers with varicosities along their lengths, coursed amongst the plaques (Figures 27-29). Fibres never exhibited deformities, in the vicinity of plaques or elsewhere (Figures 27-29). It sometimes appeared that fibres were "pushed" off course by plaques, resuming their prior orientation after sweeping around them (Figure 28a). In most cases, however,
the fibres were seemingly unaffected.

DISCUSSION

It has been suggested that senile plaques are a product of degenerating axon terminals and that their distribution should therefore correspond to the distribution of defined neuronal systems. The prime candidates for the degenerating, plaque-producing systems have been the cholinergic system of the nucleus basalis of Meynert (Arendt et al, 1984, 1985; Tago et al, 1987) and the neuronal system or systems which display neurofibrillary tangle formation (thought to be primarily the glutamatergic, cortico-cortical system; Pearson et al, 1985; Rogers and Morrison, 1985; Hyman et al, 1986). In the case of area 17 a third possibility could be considered, that plaques are formed by degenerating terminals of the geniculocalcarine projection, since there have been reports of retinal ganglion cell and optic nerve fibre degeneration in AD (Hinton et al, 1986; Bassi et al, 1987; Blanks et al, 1989).

These three afferent systems were examined for evidence of pathology in Alzheimer's disease. Only the cholinergic system showed changes. This study was not quantitative, so small differences between AD and controls may have gone undetected, particularly in the diI and cytochrome oxidase staining, but there was an obvious and marked depletion of AChE-positive fibres which would clearly overshadow any changes in the other two systems.

The comparison of histopathology in the LGN and area 17 of AD cases, together with the impression gained from cytochrome oxidase staining, suggests that, if there is a spread of disease along visual pathways in AD, it would likely be from area 17 to LGN, rather than the reverse. The LGN consistently showed a lesser degree of pathological change, and the cytochrome oxidase staining pattern in area 17 was undisturbed, indicating the presence of a roughly intact LGN input. It must be
emphasized, however, that small losses of LGN projection fibres would not likely be easily detected using the cytochrome oxidase stain. Reports of losses of retinal ganglion cells and optic nerve fibres in AD cases (Hinton et al, 1986; Bassi et al, 1987; Blanks et al, 1989) suggest that there may be some loss of LGN neurons as well.

The diI technique was effective in demonstrating the projection of layer II-III pyramidal neurons to layer V. This projection is well known from degeneration and horseradish peroxidase studies in non-human primates (Spatz et al, 1970; Gilbert, 1983; Kisvarday et al, 1986). These same neurons are also known to project to area 18 (Gilbert and Weisel, 1981). Thus they are corticocortical neurons, and their axons are therefore the hypothesized conduits along which AD spreads (Pearson et al, 1985). Despite the presence of numerous labelled fibres around senile plaques, there was no evidence of special morphological interrelationships. The diI technique has limitations, however. The populations of very small fibres labelled with diI were not quite as dense as might have been expected, and, in particular, the number of enlargements indicative of axonal terminals was relatively small, leaving the possibility that the full extent of the plexus within layer V may not have been completely revealed.

The results of this study do not support an active role for pyramidal neurons in plaque formation, at least within layers IV and V of area 17. There was no morphological evidence for degeneration, sprouting, or deformation of pyramidal cell axons, either in the vicinity of plaques or elsewhere. This finding appears to contradict previous silver impregnation studies which have reported deformation and sprouting of pyramidal neuron dendrites in AD (Scheibel, 1983) and participation of tangle-bearing pyramidal neurons in the formation of dystrophic neurites present in classical senile plaques (Probst et al, 1983; Braak and Braak, 1988). It is now thought, however, that the enlarged, deformed, dystrophic neurites that form the halo of classical plaque are found only at later stages of plaque development, and
that the earliest form of the senile plaque consists solely of extracellular deposits of amyloid which are unaccompanied by morphologic changes (such as dystrophic neurites) in the surrounding neuropil (Probst et al, 1987; Dickson et al, 1988; Duyckaerts et al, 1988; Tagliavini et al, 1988; Giaccone et al, 1989; Mann and Esiri, 1989; Yamaguchi et al, 1989b). The dystrophic neurites of the classical plaque contain paired helical filaments, and, like neurofibrillary tangles, are likely a secondary, reactive component of the disease. As plaques of the diffuse type are probably the earliest form of the lesion, structures associated with them are better candidates for participation in plaque genesis. The layer IV and V plaques seen in this study were predominantly of the diffuse and compact types and therefore did not contain dystrophic neurites. Pyramidal neuron axons in the immediate vicinity of these plaques were not altered. Pyramidal neuron dendrites were not evaluated; layer IVc and V diI-filled fibres were assumed to be axons as layer II-III pyramidal neuron dendrites do not extend to layer IVc.

In support of this failure to find significant pathology of pyramidal neurons in AD striate cortex, it has previously been reported that total neuronal density is not decreased in this area in AD (Mountjoy et al, 1983). Even amongst a subset of pyramidal neurons found to be selectively depleted in other cortical areas, significant decreases in density were found in only two laminae (IVb and VI; 24.8 and 26%, respectively; Hof and Morrison, 1990).

In contrast to the apparent lack of changes in the other two afferent systems investigated, the cholinergic input to area 17 in AD was severely depleted, as evidenced by a severe reduction of the numbers of AChE-positive fibres. Marked depletions of AChE-positive fibres in other areas of AD cerebral cortex have been previously noted (Tago et al, 1987; Geula and Mesulam, 1989). As in other areas of the cortex (Tago et al, 1987; Geula and Mesulam, 1989) many of the remaining fibres in area 17 show large, bulbous swellings, which often appear to be particularly numerous in the immediate vicinity of senile plaques. These swellings may
represent a degenerative process, or, alternatively, a regenerative, or sprouting, response. Sprouting has been reported to be characterized by an increase in the number and size of presynaptic terminals (Steward et al, 1988). Axonal growth cones are also characterized by large, bulbous and varicose swellings (Hogan and Berman, 1990). Because the numbers of cholinergic terminals are much reduced in AD, rather than increased, if sprouting does occur, it must be considered ineffective.

An altered distribution of cholinergic fibres within area 17 of AD cases was not observed. In the dentate gyrus, cholinergic fibres have been reported to have an abnormal distribution in some AD cases. This has been interpreted as representing the invasion and reinnervation of the perforant pathway terminal field, which undergoes severe degeneration in AD (Geddes et al, 1986; Hyman et al, 1987). Layer V in area 17, like the perforant pathway terminal field, is the site of termination of axons from regions with large numbers of tangle-bearing neurons, and both show intense Alz-50 immunoreactivity. Both are bordered by a zone of rich cholinergic innervation (see Part Two), therefore one might have predicted that, in AD, there would be increased numbers of cholinergic fibres in layer V of area 17. The depletion of AChE-positive fibres was so profound, however, that it was difficult to appreciate any laminar preferences of the remaining fibres.

In conclusion, of three different neuronal inputs to specific laminae of area 17, only one, the cholinergic system, showed significant changes in AD. The severity of fibre depletion, the morphologic abnormalities of remaining fibres, and their intimate association with senile plaques suggest that, at the least, cholinergic axonal degeneration is an early secondary change in AD, and precedes degeneration of pyramidal neuron axons. The possibility remains open that cholinergic fibres contribute significantly to plaque formation.
FIGURES

Figure 23. Negative print of a section from area 17 of an AD patient (SM89-88) stained with an enzyme histochemical method for cytochrome oxidase (CO). The pattern and intensity of staining is identical to that seen in sections from patients without AD, and to published descriptions in the literature. Note the dense band of staining in layers IVb and IVc (asterisks) and the "blobs" in layers II and III (arrowheads). Calibration bar = 2 mm.
Figure 24. Low magnification photomicrographs of semi-adjacent sections of area 17 of an AD patient (SM89-88) stained for cytochrome oxidase (A) and for senile plaques (B). Despite the presence of numerous plaques (B) there are no observable defects or abnormalities of CO staining. Calibration bar in (A) = 0.2 mm and serves also for (B).
Figure 25. High-magnification photomicrographs of sections from area 17 of AD cases (A:Mc371; B:Mc333), stained with an enhanced enzyme histochemical method (Tago et al, 1988) for acetylcholinesterase (AChE). Note that the plaque matrix is positive for AChE (diffuse, amorphous, strong staining) and that AChE-positive fibres exhibit intimate associations with plaques (A) and unusual morphologies, such as large, distorted axonal swellings (B). Callibration bar in (A) equals 25 μm and serves also for (A).
Figure 26. Photomicrographs of the diI crystal placement site in area 17 of an AD patient (AD88-24). (A) represents the fluorescence pattern produced by diI diffusion along neuronal processes, while (B) is a nearby section stained with cresyl violet to illustrate laminar organization. Note the tear in the tissue at the placement site is located in layer III, and diffusion around the placement site affects laminae II-IVa. The asterisk in (A) marks the filling of axons in layer V, illustrating the pyramidal cell projection from layers II-III. The calibration bar in (A) equals 0.25 mm and serves also for (B).
Figure 27. (A) Fluorescence photomicrograph of diI-filled pyramidal cell axons in layer V of an AD case (AD88-24). Note the dense plexus of fibres. (B) Fluorescence photomicrograph of the same microscopic field, same section, stained for senile plaques with Thioflavine S. Note there are no apparent morphologic abnormalities in diI-filled fibres, including those that are in close proximity to senile plaques (asterisks). Calibration bar in (A) equals 100 μm and serves also for (B).
Figure 28. Fluorescence photomicrographs of diI-filled axons in layer V (A), and Thioflavine S-positive senile plaques (B) in the same microscopic field of an AD case (AD88-24). Note the lack of morphological abnormalities of fibres at plaque sites (asterisks). The only observed reaction of fibres to plaques is a tendency for fibres to "bend away" from some plaques (eg. plaque at left side of print. Calibration bar in (A) equals 50 μm and serves also for (B).
Figure 29. Fluorescence photomicrograph of diI-filled axons in layer V (A) and Thioflavine S-positive senile plaque (B) in the same microscopic field of an AD case. In (C) is shown a print from an image derived from confocal scanning laser microscopy. In both (A) and (C), note the lack of morphologic abnormalities of diI-filled fibres at plaque sites (asterisks). The confocal image also shows that even very small fibres, with diameters less than 1 \( \mu \)m, are filled with diI. Calibration bar in (B) equals 25 \( \mu \)m for (A) and (B), 10 \( \mu \)m for (C).
PART FOUR

THE LAMINAR DISTRIBUTION OF AMYLOID β-PROTEIN
WITHIN AREA 17 OF ALZHEIMER'S DISEASE
In Part Two of this thesis, the distribution of senile plaques (as seen with an enhanced Bielschowsky technique) within area 17 was examined in detail and found not to correspond to any known afferent system. This evidence was thus inconsistent with the theory that AD spreads along neuroanatomical pathways or that plaques represent degenerating axon terminals. Since that time, however, it has become increasingly apparent that a new type of plaque, for the most part not visualized by standard silver methods, is extremely common in the AD brain (Yamaguchi et al., 1988a,b; Wisniewski et al., 1989). This plaque type, which has been variously termed "diffuse plaque" (Yamaguchi et al., 1988a,b), "amorphous plaque" (Rozemuller et al. 1989), and "amyloid plaque" (Braak et al., 1989b), has been discovered very recently by investigators using enhanced silver techniques (Campbell et al., 1987; Hedreen et al., 1988; Yamaguchi et al., 1990a; Akiyama et al., 1990) and immunohistochemistry for the amyloid β-protein. Although the technique used in Part Two was an enhanced silver technique, and appears to compare favourably with others reported in the literature, the possibility remained that an immunohistochemical method might be more sensitive at detecting diffuse amyloid deposits, even though others (Braak et al., 1989a; Yamaguchi et al., 1988a) have reported equivalent results in comparisons of enhanced silver and immunoperoxidase methods.

The amyloid β-protein, or A4 protein, is now known to be the primary protein constituent of senile plaques and cerebrovascular amyloid in Alzheimer's disease and Down's syndrome (Glenner and Murphy, 1989; Masters and Beyreuther, 1989; Selkoe, 1989; Tanzi et al., 1989; Selkoe, 1990). Immunohistochemical studies of the distribution of this protein in the brains of AD and Down's patients (Rozemuller et al., 1989; Wisniewski et al., 1989; Yamaguchi et al., 1989a) revealed the existence of "diffuse" or "amyloid" plaques at about the same time that studies with the enhanced silver techniques did. Diffuse plaques were found to have a much wider distribution than the plaque types previously known to investigators. They were found in brain regions which were thought to be devoid of plaques (Ogromi et al., 1989), or they
were abundant where they were previously thought to be scarce (Akiyama et al., 1989; Braak et al., 1989b,c; Kalus et al., 1989; Wisniewski et al., 1989; Yamaguchi et al., 1988b, 1989b).

Diffuse plaques are thought to be the earliest morphologically-detectable lesion of Alzheimer's disease (Ogomori et al., 1989; Rozemuller et al., 1989; Yamaguchi et al., 1989b). This is based on three observations. The first observation is that they occur alone, in the absence of "classical" ("classical" plaques refers here to all plaque types known before the discovery of diffuse plaques) plaques, or tangles, as the first manifestation of Alzheimer's disease in cases of Down's syndrome (Mann and Esiri, 1989; Giaccone et al., 1989). The second observation is that diffuse plaques are commonly found, in the absence of other lesions, in the cerebral cortex of non-demented individuals (Duyckaerts et al., 1988; Delaere et al., 1989), some of whom are undoubtedly in the early, preclinical stages of AD. The third observation is that, in cases of full-fledged AD, they have a wider distribution than either "classical" plaques or tangles, appearing in many brain regions as the solitary change.

Because of the possibility that an immunohistochemical method for amyloid β-protein might be more sensitive in the detection of diffuse plaques than the enhanced silver technique used in Part Two of this thesis, the distribution of senile plaques within area 17 of AD brains was re-examined using such a method.

MATERIALS AND METHODS

Cases

Cases were selected from the brain tissue bank of the Kinsmen Laboratory of Neurological Research. Twenty-three cases were evaluated, of which eleven had AD, five had neurological conditions other than AD, and seven died without a history of neurological disease (see Table VIII, next page). Generally, tissue was obtained within 12 hours of death.
Tissue processing

Tissue was processed generally as described in Part One of this thesis. Fixation consisted of 48 hours immersion in buffered 4% paraformaldehyde at 4° C. Sectioning was done at 25 to 40 μm on a sledge-type freezing microtome.

Staining procedures

Sections were stained free-floating with an immunoperoxidase method (see Part One of this thesis for general method) for amyloid β-protein (AβP). The primary AβP antiserum was produced in a rabbit by injection with a synthetic peptide identical to the amyloid beta-protein sequence reported by Glenner and Wong (1984). This antibody was termed R17; details of its preparation and characterization have been reported previously (Ishii et al., 1989). The R17 antibody was used at dilutions of 1:5000 and 1:10,000.

To reveal laminar boundaries, comparison was made with nearby sections stained with cresyl violet. In addition, selected AβP-stained sections were destained after photography by immersion for 24 hours in 0.1 M HCl, followed by restaining with cresyl violet and repeat photography.

To correlate the laminar distribution of AβP with that for cholinergic fibres, sections were also stained with enzyme histochemical methods for acetylcholinesterase (Karnovsky and Roots, 1964; Tago et al., 1988).

RESULTS

Staining for amyloid β-protein revealed consistent patterns in the AD cases (Figure 30a, 2a). Senile plaques were the only structure stained. Dystrophic neurites and neurofibrillary tangles were not stained. Four out of twelve control patients showed light densities of senile plaques in area 17, while all AD cases except one showed moderate to heavy plaque densities (see Table VII, next page).
Table VII. Amyloid beta-protein-positive plaques and acetylcholinesterase fibre density in study subjects.

<table>
<thead>
<tr>
<th>Code/Age/Sex/Dx</th>
<th>Dementia</th>
<th>BrWt</th>
<th>ChAT</th>
<th>AB-P</th>
<th>AChE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-neurological cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc196 52/M/PE</td>
<td>-</td>
<td>1390</td>
<td>100%</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Mc372 54/M/MI</td>
<td>-</td>
<td>1580</td>
<td>21%</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Mc361 60/F/MI</td>
<td>-</td>
<td>1400</td>
<td>28%</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Mc348 63/M/MI</td>
<td>-</td>
<td>1482</td>
<td>75%</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Mc213 64/M/MI</td>
<td>-</td>
<td>1410</td>
<td>140%</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Mc375 76/F/MI</td>
<td>-</td>
<td>1110</td>
<td>36%</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Mc386 78/M/COPD</td>
<td>-</td>
<td>1498</td>
<td>/</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Misc. neurological disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc222 48/F/HE</td>
<td>-</td>
<td>1240</td>
<td>85%</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Mc314 64/F/FAM</td>
<td>16 yrs</td>
<td>/</td>
<td>54%</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Mc292 68/F/EA</td>
<td>1 yr</td>
<td>1040</td>
<td>90%</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Mc303 68/F/FAM</td>
<td>5 yrs</td>
<td>1050</td>
<td>54%</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Mc336 82/M/CI</td>
<td>-</td>
<td>1340</td>
<td>118%</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc310 48/M/FAD</td>
<td>2 yrs</td>
<td>1309</td>
<td>/</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Mc270 60/F/FAD,SCZ</td>
<td>8 yrs</td>
<td>950</td>
<td>10%</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Mc287 62/M/FAD,SD</td>
<td>4 yr</td>
<td>1400</td>
<td>/</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Mc316 66/M/FAD</td>
<td>4 yrs</td>
<td>/</td>
<td>15%</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Mc393 67/M/FAD</td>
<td>9 yrs</td>
<td>1098</td>
<td>11%</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Mc349 69/M/FAD</td>
<td>10 yrs</td>
<td>969</td>
<td>30%</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Mc333 72/F/FAD</td>
<td>6 yrs</td>
<td>1100</td>
<td>58%</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Mc371 83/F/FAD,CI</td>
<td>10 yrs</td>
<td>1110</td>
<td>21%</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Mc398 83/M/AD,CI</td>
<td>7 yrs</td>
<td>1400</td>
<td>14%</td>
<td>+</td>
<td>/</td>
</tr>
<tr>
<td>Mc212 85/F/FAD</td>
<td>10 yrs</td>
<td>1100</td>
<td>18%</td>
<td>/</td>
<td>++</td>
</tr>
<tr>
<td>Mc403 88/M/FAD</td>
<td>5 yrs</td>
<td>1010</td>
<td>14%</td>
<td>++</td>
<td>/</td>
</tr>
</tbody>
</table>

AD  Alzheimer’s disease
CI  Cerebral infarct(s)
EA  Ethanol abuse
FAD  Familial Alzheimer’s disease
FAM  Familial amyloidosis
HE  Hepatic encephalopathy
ID  Idiopathic dementia
MI  Myocardial infarction
PE  Pulmonary embolism
SD  Seizure disorder
SCZ  Schizophrenia
-  Absence of stained feature
+  Light density of stained feature
++  Moderate density of stained feature
+++  Heavy density of stained feature
/  Not evaluated
ChAT  mean choline acetyltransferase activity (see Methods, Part 1)
AB-P  amyloid beta-protein-positive senile plaques
AChE  acetylcholinesterase-positive fibre density
Plaque morphology varied with laminar location. Layer one exhibited numerous small, irregularly shaped plaques which stained intensely (Figure 31b). Layers II-IVb possessed fewer, medium to large size spherical plaques with a moderate to high staining intensity. Layer IVc plaques were very irregular in shape and size, lightly stained, and were often very large or were confluent with nearby plaques, occupying a large fraction of the laminar area (Figure 31b). Layer V and VI plaques resembled those of layers II-IVb.

In the cases with the greatest numbers of senile plaques, it was apparent that layers I and IVc consistently exhibited the greatest concentrations, to the extent that these two layers appeared as dense bands at low magnifications (Figure 30a). This pattern was readily apparent in six out of eleven AD cases. There was no apparent concentration within either of the sublayers of IVc (IVc alpha and IVc beta). In the remaining cases, layer I always exhibited heavy plaque densities, while the very lightly stained plaques in layer IVc either had a patchy distribution or were not seen at all. The line of plaques at the interface of layers IVc and V (see Part Two of this thesis) was apparent in some, but not all cases.

In support of these findings regarding the laminar distribution of AβP immunoreactivity within area 17 is a recent report by a separate group, who have independently described an apparently identical distribution (Takahashi et al, 1990; Takahashi et al, personal communication), using different antibodies.

Acetylcholinesterase (AChE) staining in non-AD cases revealed that cholinergic fibres were distributed preferentially to layers I and IVc, the same laminae favoured by AβP (Figure 30b, 31b). In AD cases, fibres were generally too sparsely distributed to allow estimation of their laminar preferences. Generally, fibre density was high in control subjects and low to nonexistent in AD cases (see Table VII). In AD cases, fibres often exhibited large bulbous swellings, particularly in the vicinity of AChE-positive senile plaques (see Part Three of this thesis). These deformities were rare in control cases.
DISCUSSION

These results demonstrate that amyloid β-protein-immunoreactive plaques and acetylcholinesterase-positive fibres are preferentially co-distributed to laminae I and IVc of area 17. The distribution of acetylcholinesterase-positive fibres in human area 17 thus resembles that reported for monkeys (Hedreen et al, 1984; Mesulam et al, 1984), although layers V and VI in the human do not appear as a third band of staining as they do in the monkey.

The distribution of AβP-positive senile plaques differs considerably from that of plaques stained with an enhanced Bielschowsky method (see Part Two of this thesis). Specifically, the Bielschowsky method did not reveal the great concentrations of plaques in layers I and IVc. This may be simply a reflection of decreased sensitivity of the silver method, as the layer IVc plaques were relatively lightly stained with the immunoperoxidase method, and thus they would disappear first if the general staining intensity was reduced. Indeed, this was observed while destaining the sections prior to cresyl violet staining. Alternatively, the antibody may recognize forms of the protein which are not argyrophilic.

In studies by other investigators of plaque distribution in area 17, the results also varied with the method used. Three studies using Thioflavine S to mark plaques (Rogers and Morrison, 1985; Lewis et al, 1987; Bell and Ball, 1990) found layer IV plaques to be very numerous; this layer contained 46% of all plaques, according to Lewis and group. Bell and Ball (1990) reported that plaque density was greatest in layer IV of normal aging cases whereas in cases of AD plaque density did not differ significantly from layer to layer. When plaque abundance was expressed in terms of the fraction of neuropil occupied by plaques, layers II, III and V all surpassed IV (Rogers and Morrison, 1985). These analyses did not, however, extend to the subdivisions of layer IVc. Layer I plaques were found to be relatively scarce, accounting for only 3.9% of total plaque numbers in one study (Rogers and Morrison, 1985). A study using the Bodian-periodic acid-Schiff method (Rafalowska et al, 1988)
also found the greatest numbers of plaques in layer IV; subdivisions of IV were not
examined, and again, layer I plaques were reported to be relatively scarce. The only
published study of area 17 plaques which utilized an enhanced silver technique
(Braak et al, 1989a) also failed to reveal the heavy concentration of plaques in layers
I and IVc.

The results of the present study suggest that efficient immunohistochemical
methods are more sensitive than enhanced silver techniques in the detection of senile
plaques. This contradicts the reports of others (Yamaguchi et al, 1988b; Braak et al,
1989a) that the two methods are equivalent. Both the enhanced Bielschowsky
method used here, and the Campbell (Campbell, 1987) technique, used by Braak and
co-workers, failed to detect the large concentrations of amyloid in layers I and IVc of
area 17. Braak and co-workers (1989a) compared adjacent sections which had been
stained with either the Campbell method or an immunoperoxidase method for ABP
and saw no differences. The antibody used was raised against a synthetic ABP
(Masters et al, 1985). It is possible that the immunostaining obtained with their
protocol was less sensitive than that used in this study. Braak and co-workers did
not report their fixation conditions, and it seems likely that they used standard
neuropathological methods, which involves fixation in 10% formalin for 10-20 days.
Many antigens show decreased immunoreactivity with formaldehyde fixation times
longer than 48 hours (Beach et al, 1987). In addition, Braak and co-workers stained
paraffin-embedded sections mounted on slides, which, in the experience of this
laboratory, produces markedly inferior results compared to the use of free-floating
frozen sections. Furthermore, formic acid pretreatment, which has been shown to
greatly improve the sensitivity of ABP immunostaining on paraffin-embedded tissue
(Kitamoto et al, 1987), was apparently not used by Braak and co-workers. Another
possible reason for the differing results found by Braak and group, as compared to
the results of this study, is that different antibodies were used, which may detect
different portions of the ABP molecule. If all parts of the ABP molecule are not
present or accessible within all plaques, different antibodies may detect different populations of plaques.

The co-distribution of AβP and AChE staining to the same cortical laminae strengthens the already close association between the cholinergic system and Alzheimer's disease. A review of the literature reveals no other neurotransmitter marker or receptor which is distributed preferentially to layers I and IVc of the primate striate area (Berger et al, 1988; Hendrickson et al, 1981; Kosofsky et al 1984; Lewis et al, 1986; Rakic et al, 1988; Shaw and Cynader, 1986). In particular, the cortico-cortical afferents from area 18, which would, under a current popular hypothesis (Pearson et al, 1985) be the route of AD spread into area 17, are not implicated as they project to every lamina except layer IVc (Spatz et al, 1970). Capillary density is heavy in layer IVc, but very light in layer I (Bell and Ball, 1985, 1990) so the vascular theory of plaque formation is also not supported by this observation.

It is not known whether this correlation between AβP deposition and basal forebrain-derived cholinergic input may extend to other brain regions. Previous studies denying such a correlation (Agid et al, 1989; Rudelli et al, 1984; Rogers and Morrison, 1985; Lewis et al, 1987; Brashear et al, 1988; Masliah et al, 1989) were done without the use of sensitive techniques and are therefore inadequate. Three recent studies utilizing improved methods have found heavy diffuse amyloid deposits which are co-extensive with dense cholinergic terminal fields in the presubiculum (Kalus et al, 1989; Akiyama et al, 1989; Green and Mesulam, 1988) and amygdala (Brady and Mufson, 1990).
FIGURES

Figure 30. (A) Negative print of section from area 17 of an AD case (Mc371), stained with an immunoperoxidase method for amyloid beta-protein (AB-P). Note the intense bands of staining in layers I and IVc (arrows). (B) Negative print of section from area 17 of a control case (Mc361), stained with an enzyme histochemical method (Karnovsky and Roots, 1964) for acetylcholinesterase (AChE). Note that staining is enhanced in layers I and IVc. Calibration bar equals 2 mm and serves for both (A) and (B).
Figure 31. (A) Photomicrograph of section from area 17 of an AD case (Mc371), stained with an immunoperoxidase method for AB-P. Note the increased concentrations of plaques in layers I and IVc. The plaques in IVc differ from those in other laminae in that they have a more irregular shape and a lighter staining matrix. (B) Photomicrograph of section from a control case (Mc361), stained with an enzyme histochemical method for AChE. Note the increased fibre density in layers I and IVc. Calibration bar equals 0.5 mm and serves for both (A) and (B).
The objective of this thesis was to determine whether the histopathological lesions of Alzheimer's disease formed patterns which could be related to the neuroanatomical organization of the brain. Specifically, two theories of Alzheimer's pathogenesis were tested: one, that AD spreads throughout the brain along neuroanatomical pathways from an initially circumscribed location (Hyman et al., 1984; Pearson et al., 1985; Rogers and Morrison, 1985; Duyckaerts et al., 1986; Lewis et al., 1987; Mann and Esiri, 1988); two, that the lesions of AD represent the degeneration of one neurotransmitter-specific system (Arendt et al., 1984, 1985; Tago et al., 1987). These two theories are currently under serious consideration by many of those who study the disease. Both of these theories fall under the general class of neuronal theories of pathogenesis, theories with the common stipulation that the lesions of AD are the result primarily of neuronal degeneration. There are two other major classes of pathogenetic theories, which uphold either a primary glial (Wisniewski et al., 1989a), or vascular (Glenner, 1979) pathology. Although this thesis was not designed to test the glial or vascular hypotheses, the data will be reviewed to determine their compatibility with these theories as well.

These major hypotheses of lesion production do not directly speculate about the etiology of AD. It is assumed, however, that a knowledge of lesion production would serve to guide investigations which do focus on etiology. The advantage of studying the lesions lies in their accessibility and proven relevance to clinical parameters. It is possible that an understanding of plaque and tangle formation could lead to treatment methods which prevent these events, or ameliorate their damage, regardless of etiology.

In Part One of this thesis, the global cerebral distribution of lesions was examined using astrocytic gliosis as a marker. The findings reinforced the prevailing opinion that AD most severely affects the cerebral cortex and hippocampus. A
consistent gliotic response was not observed in any subcortical grey area except the substantia innominata. The cortical gliosis was distributed preferentially to laminae II-III and V (excluding area 17). This laminar distribution is identical to what had been previously reported by many investigators for neurofibrillary tangles (Rogers and Morrison, 1985; Pearson et al, 1985; Lewis et al, 1987; Braak et al, 1989).

The correlation of gliosis with senile plaques is less clear because there is at present no clear consensus on the laminar distribution of plaques. Lewis et al (1987) and Rogers and Morrison (1985) found plaques most numerous in layers III and V in areas 18 and 20 of the occipital lobe, and in layers II-III and V of the superior frontal gyrus and anterior cingulate gyrus. In the superior temporal gyrus, layers II, III and IV were most affected. Rafalowska and co-workers (1988) found that temporal lobe plaques were preferentially located in layers II-III and V in aged controls, but in II and III in AD cases. Duyckaerts et al (1986) found the greatest numbers in layers II and III, as did Wildi and Dago-Akribi (1968). Tomlinson, Blessed and Roth (1968, 1970) favoured layers III through V. Von Braunmuhl (1957) and Pearson et al (1985) found plaques to be randomly distributed in the cortical column. The difficulty in assessing these reports arises from the different techniques used and different areas of neocortex examined. All of these studies were done before the availability of antibodies to amyloid β-protein and therefore are potentially misleading (see Discussion, Part Four). Nevertheless, it is apparent that layers II and III are consistently implicated, with layer V involvement being somewhat less consistent. This does parallel the pattern of cortical gliosis observed in Part One, as layers II and III generally formed a heavier band of staining than layer V (Figure 9a,b). Furthermore, direct comparison of gliosis with plaque distribution in neighbouring sections (Part One, Figures 10 and 11), shows that, at least in some areas, the pattern of gliosis clearly parallels that of senile plaques.

The data from Part One, then, appear to provide support to the theory of AD spread along corticocortical connections, as the neurons involved in these projections
are situated in layers II-III and V. These neurons are believed to use glutamate and/or aspartate, so the data are also compatible with the theory that AD is a disorder of the cortical glutamatergic system.

The observation that gliosis of area 17 in AD displayed a different laminar disposition than other neocortical areas led to Part Two of this thesis, in which the distribution of plaques, tangles and gliosis within that area was examined in detail. The findings were as follows. Neurofibrillary tangles were not always present in AD cases, as opposed to the constant presence of plaques. Tangles, and staining with the Alz-50 antibody, which detects paired helical filaments, were distributed exclusively to layers II-IVa, V, and VI. Plaques, as detected with an enhanced Bielschowsky method, were quantified along intersect lines and found to be distributed relatively evenly to all layers, with the exception of a striking aggregation along the border of IVc and V, and decreased numbers in layer VI.

These data confirmed again the highly specific disposition of neurofibrillary tangles within the layers associated with corticocortical connections. Plaque distribution within area 17, however, did not correlate with any known afferent or neurotransmitter system. As there is a consensus emerging from the literature that plaques are the primary lesion in AD, with tangles representing a secondary reaction, this was potentially a refutation of the theories that this thesis set out to test. The alignment of plaques precisely along neuronally-determined boundaries, however, was evidence that formation of plaques is dependent on neuronal architecture. Neither the glial (Wisniewski et al, 1989b), nor the vascular (Glenner, 1979; Miyakawa et al, 1982) theories could account for this line of plaques, or for the clear respect that plaques show for neuroanatomic boundaries in other brain regions (eg. presubiculum: Akiyama et al, 1990, Kalus et al, 1989; dentate gyrus: Hyman et al, 1986, Crain and Burger, 1988). Glia and vasculature, unlike neurons, have not been shown to have the necessary region-dependent functional variation. Arterioles and capillaries, for instance, have not been shown to differ physiologically from one
brain area to another (Katzman, 1986; excluding area postrema and periventricular regions, which lack blood-brain barrier). There may be relationships between neuronal elements and vasculature which are important in AD, however.

Morphological abnormalities in AD cerebral capillaries have been described, and have been hypothesized to be secondary to denervation (Scheibel, 1987). The cholinergic and noradrenergic innervation of intraparenchymal cerebral blood vessels is thought to be derived, at least in part, from the nbM and locus coeruleus, both of which are known to undergo significant neuronal loss in AD.

A line of plaques in the molecular layer of the dentate gyrus (Hyman et al, 1986; Crain and Burger, 1988) has many parallels with that observed here in area 17. It has been proposed that the dentate plaques are created as sprouting cholinergic fibres invade a denervated zone (the perforant pathway terminal field), find it hostile, and die (Geddes et al, 1986). Support for this scheme comes from the finding that the normal laminar arrangement of cholinergic fibres in the dentate gyrus is altered in some AD cases, with an apparent redistribution of cholinergic fibres to the denervated zone (Geddes et al, 1985; Hyman et al, 1986). In both the dentate gyrus and area 17, a line of plaques occurs at the border between a cholinergic terminal field and a zone of degenerating neurites immunoreactive for Alz-50, a marker of paired helical filaments.

Part Three of this thesis was designed to determine if a redistribution or sprouting of cholinergic terminals or other afferent systems occurs in area 17. Of three afferent systems normally found in layers IVc and V, only one, the cholinergic system, showed any alteration. This consisted of a marked depletion of fibres with deformities of remaining fibres. Deformed fibres were frequently associated with senile plaques. A redistribution of cholinergic fibres, which has been taken as evidence of sprouting in the AD dentate gyrus, was not observed. Bulbous axonal enlargements might possibly represent sprouting, but the major change was axonal loss. Afferents from corticocortical pyramidal neurons were not visibly depleted in
numbers, did not show altered morphology, and were not associated with senile plaques.

The results of Part Three thus reinforced those of Part Two; senile plaques within area 17 do not appear to be spatially related to corticocortical afferent fibres. There was still no explanation for the line of plaques, however. Cholinergic fibres were markedly depleted, and often showed deformed morphologies in the vicinity of plaques, but the laminar distribution of plaques did not correlate with that of cholinergic input. Cholinergic fibre concentrations in monkey area 17 have been reported to be concentrated in layers I, IVc and VI (Hedreen et al, 1984; Mesulam et al, 1984). The line of plaques occurs at the lower border of IVc; other parts of IVc have only average plaque concentrations (see Figure 3, Part Two). Layers I and VI did not show increased numbers of plaques.

Part Four of this thesis re-examined the distribution of senile plaques in area 17, using an immunohistochemical method for amyloid β-protein. The results were striking in that an entirely new distribution of plaques was revealed. Layers I and IVc appeared to be almost filled with diffuse, irregularly-shaped amyloid formations. Staining of control brains for acetylcholinesterase revealed that cholinergic fibres are preferentially distributed to these same layers (layer VI, which is seen as a third band in monkeys, did not form a third band in humans). No other known afferent or neurotransmitter system has this distribution. There is no correlation with vasculature; capillary density in area 17 is greatest in layer IVc, but least in layer I (Bell and Ball, 1985, 1990).

The significance of the codistribution of cholinergic input and amyloid deposition in area 17 depends upon the generality of this relationship. If this codistribution is observed throughout the brain, a role for cholinergic fibres in amyloid formation may be inferred. Until extensive mapping studies of amyloid deposition are carried out with sensitive immunohistochemical methods, the full extent of codistribution can not be fully determined, but the available literature suggests that it is considerable.
In the cerebral cortex, amyloid is deposited preferentially in layers I and IVc of area 17; in the remainder of neocortex amyloid deposition has not been fully investigated with sensitive techniques, but the available information suggests that, in addition to layers II-III and V, already known to be the laminae of preference for textbook plaques (see earlier discussion, paragraph four), layer I is the site of a previously undetected but impressive deposition of diffuse amyloid in various neocortical areas (Majočha et al, 1988; Wisniewski et al, 1989b). Thus laminae I-III and V appear to contain the most extensive neocortical amyloid deposits.

The laminar distribution of markers for cholinergic terminals in the neocortex is, like the distribution of plaques, difficult to ascertain due to conflicts in the literature. Table VIII (next page) lists the findings of several groups. Despite the lack of agreement between studies, it is evident that most found a bilaminar arrangement of cholinergic terminals, with a superficial and a deep band of concentration, most often corresponding to layers I-III and V. Another general consensus regarding cholinergic lamination is that layer I contains a dense terminal field.

This comparison of cholinergic and amyloid laminar preferences in the non-striate cerebral cortex demonstrates that, although considerable uncertainty exists about both, there is a significant overlap in the reported ranges of the two distributions. Lack of agreement between observers clouds any possible conclusions however.

In subregions of the hippocampal formation and entorhinal cortex, the correlation between cholinergic fibre density and plaque distribution is poor (see Table IX, next page). The studies on plaque distribution generally agree that plaques are more abundant in CA1 than CA2-CA4. This is the complete reverse of cholinergic density. The ranking of the subiculum with respect to plaque density varies considerably, while both studies on cholinergic indices rank it last. Again, no detailed AβP immunolocalization studies are available, except for the subicular complex, so this correlation is based on possibly incomplete information. Within the
Table VIII. Results of studies describing the laminar distribution of cholinergic terminal indices in the neocortex.

<table>
<thead>
<tr>
<th>First Author</th>
<th>Species</th>
<th>Areas Examined</th>
<th>Laminae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesulam, 1984¹</td>
<td>monkey</td>
<td>various</td>
<td>I-III/V,VI</td>
</tr>
<tr>
<td>DeKoskey, 1985²</td>
<td>human</td>
<td>frontal</td>
<td>I-III</td>
</tr>
<tr>
<td>Lysakowski, 1986³</td>
<td>rat</td>
<td>retrosplenial</td>
<td>I, IV-VI</td>
</tr>
<tr>
<td>Luiten, 1987⁴</td>
<td>rat</td>
<td>various</td>
<td>I-II, V</td>
</tr>
<tr>
<td>Brady, 1988⁵</td>
<td>rat</td>
<td>motor, non-motor</td>
<td>I-II/ V-VI</td>
</tr>
<tr>
<td>Eckenstein, 1988⁶</td>
<td>rat</td>
<td>various</td>
<td>I-III, V</td>
</tr>
<tr>
<td>Friedman, 1988⁷</td>
<td>monkey</td>
<td>premotor</td>
<td>II, V</td>
</tr>
</tbody>
</table>

1  acetylcholinesterase enzyme histochemistry
2  choline acetyltransferase enzyme assay
3  choline acetyltransferase immunohistochemistry
4  PHA-L anterograde tracing from magnocellular basal nucleus
subiculum, as noted in Part Four, there is a well-defined dense cholinergic terminal field, which, in AD cases, contains a heavy deposition of diffuse amyloid (Kalus et al, 1989; Akiyama et al, 1990). In the amygdala two studies of classical plaque distribution (Brashear et al, 1988; Unger et al, 1988) reported a complete lack of correlation between acetylcholinesterase staining intensity and plaque density. A third study, however, which specifically looked for diffuse plaques (Brady and Mufson, 1990), found that the subregions with intense AChE staining contained dense populations of diffuse plaques. Boundaries between these AChE-rich regions and neighbouring AChE-poor regions were marked by the sudden disappearance of these plaques.

In the remainder of the brain, diffuse plaques have been found to be widely distributed, being reported in the basal ganglia, diencephalon, brainstem and cerebellum (McDuff et al, 1985; Grossi et al, 1989; Iseki et al, 1989; Ogomori et al, 1989; Ohm and Braak, 1989; Braak and Braak, 1990). In concert with these findings, the projections of the basal forebrain, already known to include most regions of the telencephalon and diencephalon, are now known to extend to the brainstem as well (Tomimoto et al, 1987; Parent et al, 1988; Dinopoulos et al, 1989). Detailed correlations in these regions must await the appropriate studies. There is no evidence as yet to associate the amyloid deposits of the cerebellar cortex (Braak et al, 1989b; Joachim et al, 1989; Yamaguchi et al, 1989a) with degenerating basal forebrain-derived cholinergic axons; the origin of these cerebellar cortical cholinergic fibres is unknown at present (Ojima et al, 1989).

It may be seen, then, that a perfect match of plaques and cholinergic basal forebrain innervation does not exist in every subregion, although this may change when detailed, sensitive studies are done. Roughly considered, however, the abundance of senile plaques parallels that of basal forebrain cholinergic innervation, with the greatest densities of both being observed in the cerebral cortex,
Table IX. Subregional density of senile plaques and histological estimation of cholinergic terminal density in the hippocampal formation.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Rank Order of Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senile plaques</td>
<td></td>
</tr>
<tr>
<td>Gellerstedt, 1933</td>
<td>CA1 &gt; subiculum, CA4 &gt; CA2, CA3</td>
</tr>
<tr>
<td>Meencke et al, 1983</td>
<td>CA1 &gt; CA2, CA3, subiculum</td>
</tr>
<tr>
<td>Casanova et al, 1985</td>
<td>CA1 &gt; CA2 &gt; CA3, CA4, subiculum</td>
</tr>
<tr>
<td>Hyman et al, 1986</td>
<td>subiculum, CA1 &gt; CA2 &gt; CA3</td>
</tr>
<tr>
<td>Davies et al, 1988</td>
<td>subiculum &gt; CA1 &gt; CA3 &gt; CA2, CA4</td>
</tr>
<tr>
<td>Ransmayr et al, 1989</td>
<td>subiculum &gt; CA1 &gt; CA4 &gt; CA2, CA3</td>
</tr>
<tr>
<td>Cholinergic terminals</td>
<td></td>
</tr>
<tr>
<td>Green et al, 1988¹</td>
<td>CA2, CA3, CA4 &gt; CA1 &gt; subiculum</td>
</tr>
<tr>
<td>Ransmayr et al, 1989²</td>
<td>CA2 &gt; CA3, CA4 &gt; CA1 &gt; subiculum</td>
</tr>
</tbody>
</table>

1  acetylcholinesterase histochemistry, human  
2  choline acetyltransferase immunohistochemistry, human
hippocampus, entorhinal cortex and amygdala. Plaque densities and basal forebrain innervation elsewhere in the brain are both generally light. A less-than-complete match could be explained if the degree of plaque formation by cholinergic terminals was at least partially dependent on variable neuropil properties (undefined at present) of the innervated region. Alternatively, more than one neurotransmitter-specific system might be involved.

If AD could possibly be caused by the degeneration of a single group of neurons, however, the cholinergic system must be considered the top candidate. The non-random distribution of the histopathological lesions argues that AD does not affect all neurons equally. If only a subset of neurons is afflicted, it is logical and economical of effort to first exclude the possibility that the disease begins with involvement of a single neuronal system. When the cholinergic deficit in AD was first discovered, in 1976, the major working pathogenetic hypothesis was, indeed, that cholinergic degeneration was the major significant event. As more data were acquired the cholinergic hypothesis became unpopular; at the present time, most investigators do not favour it. This re-examination of the evidence for and against the cholinergic hypothesis, however, suggests that it has been abandoned prematurely.

The initial disenchantment with the cholinergic hypothesis came with reports of decrements of other, non-cholinergic, neurotransmitter systems in AD. These included those systems using noradrenaline, serotonin, somatostatin, and glutamate (Mann and Yates, 1986; Pearson and Powell, 1987). Alzheimer's disease became a multi-transmitter disorder. A new unified theory soon arose to replace the cholinergic hypothesis. This new theory posited cortical degeneration to be the primary event, accounting for the cortical neurotransmitter anomalies, with secondary subcortical degeneration accounting for the subcortical deficits (Pearson and Powell, 1986). This was attractive since the histopathology of AD is concentrated in the cortex, and the only subcortical systems which showed deficits
were the ones with cortical projections. The glutamate/aspartate pyramidal neuron system became the new candidate for the role of primary degenerate population because it is within these neurons that most neurofibrillary tangles form (Pearson et al, 1985).

It is now apparent, however, that the involvement of all neurotransmitter systems other than the cholinergic is controversial, or is manifest only at later stages of the disease (Bowen and Davison, 1986; Lowe et al, 1988; Perry and Perry, 1985; Palmer et al, 1987; Procter et al, 1988). Comprehensive neurochemical studies of biopsy tissue from AD cortex, which, in general, provide a cross-section through an earlier stage of the disease than post-mortem studies, have not found significant decreases in non-cholinergic neurotransmitter indices (Bowen and Davison, 1986; Lowe et al, 1988). In many cases, these biopsies were done within a year of symptom onset. There is even evidence that the cholinergic system is already involved when the disease is still preclinical, since cholinergic indices have been reported to decline with increasing plaque count in the non-demented elderly (Perry et al, 1978). No other neurotransmitter system has been implicated in the preclinical stage. The neurochemical evidence, therefore, is more consistent with a primary degeneration of the cholinergic system than with that of any non-cholinergic system. Involvement of other systems, therefore, would likely occur later in the disease, or inconsistently, as is seen with non-dopaminergic systems in Parkinson's disease, for example.

The evidence for primary cortical degeneration in AD is also now unconvincing. Some authors (Mesulam, 1986a; Pearson and Powell, 1987) have argued that cortical neuronal loss precedes that of subcortical structures; these authors suggest that subcortical pathology is a retrograde degeneration of cortically-projecting neuronal groups. Pyramidal neurons, the neurons that are most susceptible to tangle formation, were hypothesized to be also responsible for plaques, which would form from degenerating pyramidal cell axons (Pearson et al, 1985). This scenario is now,
in the light of recent findings, supported by very little evidence. The case against a primary pathogenetic status of neurofibrillary tangles has already been reviewed (see Part Four); most investigators agree that tangles are a secondary phenomenon. Senile plaques do not appear to arise from degenerating tangle-bearing pyramidal neuron axons; the results of this thesis, too, are strongly against this hypothesis, at least in area 17. Also, the earliest forms of senile plaques, the diffuse plaques, which are found throughout tangle-free parts of the cortex in many non-demented individuals, do not have the paired helical filament-containing neuritic component, which does not occur until later in the disease, when dementia has ensued (Barcilowska et al, 1989; Duyckaerts et al, 1988; Delaere et al, 1989). Although plaque distribution in the association cortex roughly corresponds to the distribution of cortico-cortical (pyramidal neuron) terminal fields, this distribution may actually be reflective of the distribution of cholinergic cortical afferents. As shown in Parts Three and Four of this thesis, the distribution of plaques and amyloid beta-protein in area 17 does not correspond to the distribution of corticocortical afferents, and there were no apparent morphological relationships between the two.

Other evidence for early involvement of intrinsic cortical neurons in AD is also unimpressive. Four studies (Terry et al, 1981; Mountjoy et al, 1983; Hubbard and Anderson, 1985; Mann et al, 1985) have reported cortical neuron loss in AD, but two studies found no such loss (Tomlinson and Henderson, 1976; Terry, 1977). The studies which did find losses found them mainly in the subset of larger neurons, and the fact that, in three of these studies (the first three cited above), the smaller classes of cells actually increased in density leaves the possibility, admitted by one group (Hubbard and Anderson, 1985), that the apparent loss of larger neurons may actually be due to their displacement, by atrophy, into a smaller size class. Neuronal atrophy has indeed been reported to occur in AD (Mann et al, 1981). The studies reporting loss of large neurons failed to correct their counts for the effect of this neuronal shrinkage; small neurons are less likely to be cut by the plane of the
section than large neurons and so their numbers will be relatively underestimated. The type of neuron thought to be lost in AD is also controversial, with the groups already mentioned claiming that it is the large pyramidal neurons, while Braak and co-workers reported that it is the (small) non-pyramidal neurons (Braak and Braak, 1986). Cortical atrophy undoubtedly does occur in AD, however, with estimates ranging up to 24% (Duyckaerts et al, 1985; Hubbard and Anderson, 1985; Bugiani et al, 1988; Hansen et al, 1988; De la Monte, 1989). Even without changes in neuronal density, therefore, an absolute neuronal loss probably does occur. These studies were all done post-mortem on end-stage AD cases and therefore do not inform us on the state of the cortex in early AD. There is only one study in the literature which addresses this question and its results suggest that cortical atrophy is not an early change in AD. De la Monte (1989) performed post-mortem cortical morphometry on 16 AD and 18 non-dementia cases. Four of the control cases were found to have tangles and plaques of sufficient densities to qualify them for a diagnosis of AD, despite reliable clinical information indicating that all were free of signs of dementia; in fact, all four were living independently until shortly before death. There was no significant cortical atrophy in this group, as compared to the age-matched control group without significant Alzheimer's pathology.

Quantitative studies of cortical neurotransmitter receptors and neuropeptides in AD also are inconsistent with major losses of intrinsic cortical neurons. The only receptors which are reliably known to be affected are the nicotinic and M2 muscarinic cholinergic receptors, both located mainly on cholinergic axon terminals, and the 5-HT-1 receptor (Procter et al, 1988), whose location is unknown [Procter et al (1988) hypothesize they are on non-pyramidal neurons, while Bowen and Davison (1986) favour a pyramidal cell location]. Of the many neuropeptides found in cortical neurons, only somatostatin is consistently reduced (Rossor, 1987), and early cases of AD studied by biopsy do not show even this deficit (Bowen and Anderson, 1986).
Morphometric studies suggest that atrophy of the cortical neuropil is a more consistent finding in AD than loss of neuronal perikarya (Hubbard and Anderson, 1985). In the earliest stages of AD, the only cortical changes appear to be the presence of diffuse plaques, which may be widespread even before the onset of dementia (Delaere et al, 1990). Within diffuse plaques, the cortical neuropil is almost entirely unaffected, at both light and electron microscopic levels (Yamaguchi et al, 1989b). Ultrastructurally, the only changes reported within diffuse plaques are wisps of amyloid fibres adjacent to neuronal processes, some of which contain accumulations of dense bodies (Yamaguchi et al, 1989b). Thus the earliest changes in AD cortex appear to consist of the degeneration of a small number of neuronal processes. If this information is considered together with the reports that both choline acetyltransferase levels (Perry et al, 1978) and cognitive function (Fuld et al, 1987) correlate inversely with increasing plaque count in the non-demented elderly (some of which are are undoubtedly in the early, preclinical stages of AD), it is evident that the small population of degenerating processes are quite possibly cholinergic, and their loss is significant functionally.

Mesulam (1986a) has argued against the cholinergic hypothesis because regional cortical plaque concentration does not correlate positively with the normal regional density of cholinergic innervation. His argument is as follows: if senile plaques are produced at cholinergic axon terminals, they should be most numerous in those cortical regions which receive the heaviest cholinergic innervation. The primary somatosensory and motor cortices, which have relatively high ChAT levels, however, have comparatively few plaques. This relative sparing of the somatosensory cortex is indeed an anomaly that must be explained by proponents of the cholinergic hypothesis. Again, as mentioned earlier in this section, these anomalies may occur due to regional variability of presently unknown neuropil factors which are either favourable or non-favourable to plaque formation. In any case, the bulk of the evidence, with some exceptions such as those pointed out by Mesulam, shows that
plaque density is greatest where cholinergic innervation is greatest (in the paralimbic temporal cortex; Mann and Yates, 1986), and that the overall pattern of morphological degeneration in AD mirrors closely that of cholinergic losses (Procter et al, 1988). In addition, three groups (Arendt et al, 1985; Mann and Yates, 1986; Etienne et al, 1986) have reported that regional cortical plaque density correlates significantly with neuronal losses within the respective innervating nbM subdivisions.

Finally, it has been argued that, because other diseases which have substantial losses of nbM neurons and cortical cholinergic indices do not show large numbers of cortical plaques, cholinergic fibres cannot be responsible for plaque formation. This argument does not allow for the reasonable expectation that disorders of nbM neurons may be variable in their histopathological appearance. In addition, it is now apparent that, in cases of Parkinson's disease (PD), the best-studied and most-cited example of a disease other than AD in which there is nbM neuron loss, cortical plaques and tangles are both commonly found and are significantly more numerous there than in the cortex of control patients (Hakim and Mathieson, 1978, 1979; Boller, 1983). A very recent study by Braak and Braak (1990), the first to use enhanced silver methods for plaque detection in this disease, describes large numbers of diffuse plaques, hitherto unrecognized, as a consistent finding in the cerebral cortex of Parkinson's disease cases.

This re-appraisal of the cholinergic hypothesis, therefore, reveals that it has been abandoned prematurely. The cholinergic neurons of the nbM are, as far as current evidence shows, the first and most consistently affected neuronal system in AD; a primary cortical degeneration is not supported by the literature. The results of this thesis also support the hypothesis that degeneration of the cholinergic nbM occurs early in the course of the disease, and that senile plaques are formed from degenerating cholinergic axon terminals.

Aside from providing further evidence to be incorporated into theories of AD pathogenesis, the results of this thesis may have some clinical usefulness. The
histopathology of area 17, in particular, may be useful in explaining some of observed visual deficits in AD, and in predicting the existence of others not yet reported.

It has recently been recognized that, amongst those in the early stages of AD, the first complaints registered with the physician may be that the quality of their vision is unsatisfactory. Frequently, they complain of difficulty in reading (Cogan, 1985; Katz and Rimmer, 1989). Specifically, they may say that they lose their place on the page, that the print is blurred, or "dancing". Preliminary ophthalmological examination invariably reveals normal acuity, full visual fields, and normal fundi. Alexia in AD has therefore been assumed to result from disease in the parietal lobe.

More detailed testing of visual function, however, has uncovered other deficits which implicate the central visual pathways themselves. The retinogeniculocalcarine projection is the major conduit of visual information, which is thought to be split into two parallel streams beginning at the retinal ganglion cell. These streams, known as the parvocellular and magnocellular (after the distinctive cell layers in the lateral geniculate nucleus through which the two streams pass), are anatomically and functionally segregated. The parvocellular pathway is thought to be concerned primarily with detail, form and colour, while the magnocellular primarily carries information about movement, visual attention, and with the gross features of the stimulus (Kandel, 1985). More simply, the parvocellular, or P system, deals with "what" an object is, while the magnocellular, or M system, deals with "where" an object is (Zeki, 1990). The P system has also been called the LTHS pathway, since it is optimally sensitive to stimuli with low temporal frequencies and/or high spatial frequencies, while the M system has been called HTLS since it is more sensitive to stimuli with high temporal and/or low spatial frequencies (Bassi and Lehmkuhle, 1990).

Reported deficits in AD implicate both systems. Difficulties with colour vision, and reduced contrast sensitivities at low to medium temporal frequencies (Katz and
Rimmer, 1989) suggest damage to the P system, while amplitude reductions in the pattern electroretinograms (PERG) at high temporal frequencies point to the M system (Bassi and Lehmkuhle, 1990). These functional deficits in parallel pathways may arise at multiple levels in AD; reduced PERG amplitudes are indicative of damage to retinal ganglion cells, while delayed late components of conventional visual evoked potentials (VEP) suggest damage to the extrastriate, visual association cortex (Katz et al, 1989). An absence of changes in the early components of VEP's has been taken as evidence that the geniculostriate projection, and the striate cortex, are relatively intact.

The histopathology of the visual system in AD supports the clinical evidence of involvement of both parallel pathways, at multiple levels. Degeneration of retinal ganglion cells (Blanks et al, 1989), especially the larger classes, loss of optic nerve fibres (Hinton et al, 1986; Sadun and Bassi, 1990), particularly those with larger diameters, and preferential damage to the magnocellular layers of the LGN (Bassi and Lehmkuhle, 1990; the data from this thesis would agree with this, but caution that this observation relating to the LGN is somewhat inconsistent), are highly suggestive of a relatively selective targeting of the M system within the retinogeniculate projection.

Damage to the striate cortex, as described in this thesis, is not so restricted, however. Amyloid deposition is concentrated in layers I and IVc, and at the border of IVc and V. The P and M systems pass through the lower (IVcβ) and upper (IVcα) portions of IVc, respectively. Amyloid deposition within layer IVc appeared to be uniformly distributed, not favouring either subdivision. The line of plaques at the IVc/V boundary may possibly be related to a subdivision of the P system; evidence for the existence of such a subdivision comes from the cytochrome oxidase staining patterns of normal squirrel monkey striate cortex and the striate cortex of enucleated macaques, both of which show a thin line of intense staining at the IVc/V boundary (Hendrickson and Tigges, 1985). The M system also passes through layers IVb and
the cytochrome oxidase-rich blobs of layers I-III, while the P system is distributed to both blob and interblob regions. Amyloid deposition in these areas was uniform, and, in particular, there was no concentration within blob or interblob regions (as assessed by sections taken both perpendicular and tangential to the pial surface).

Neurofibrillary tangles, and Alz-50 staining for paired helical filaments (PHF) are selectively found in layers II-IVa, V and VI. Again there was no evidence of concentration within blob or interblob regions and thus no evidence for selective involvement of either the P or M systems. Gliosis occurred in roughly the same laminae as tangles, and also in a line at the IVc/V border, corresponding to the line of plaques. There was no suggestion of selective involvement of the P or M systems.

The P and M systems continue to be segregated in their projections to higher cortical areas, for instance in the stripe and interstripe regions of area 18. There was no corresponding pattern of histopathology observed in sections including area 18. The P system "terminates" in the posterior temporal cortex while the M system ends in posterior parietal cortex. There was no apparent difference in the intensity of gliosis found in these two areas (Part One of thesis).

This review shows that there is ample anatomical evidence to account for the observed functional deficits in AD. In fact, the consistent presence of amyloid deposits and PHF accumulation would lead one to predict that deficits would be more severe and wide-ranging than reports indicate. It is possible that a constellation of specific visual defects may prove diagnostic for AD. Indeed, a characteristic electrophysiologic profile has already been suggested (the combination of a low-amplitude PERG, a normal flash electroretinogram, a normal pattern VEP, and a delayed P2 component of the flash VEP; Katz et al, 1990).

Of even greater interest would be a clinical diagnosis of early AD. This may be possible with tests which focus on visual functions mediated by the cholinergic system, since the results of Part III of this thesis suggest that loss of cholinergic fibres precedes general cortical involvement, at least within the striate cortex. The
cholinergic innervation of the visual cortex has been suggested to mediate the effects of arousal (Sillito and Kemp, 1983), participate in "plasticity" (Bear and Singer, 1986), improve the "signal-to-noise" ratio (Sato et al, 1987a), and contribute to spatial contrast sensitivity (DeBruyn et al, 1986). Lesions of the nbM, which deprive the striate cortex of cholinergic input, cause a generalized reduction in the visual responses of 50% of individually tested cells (Sato et al, 1987b). Of especial interest is the report that administration of muscarinic cholinergic antagonists to normal subjects duplicates the VEP results seen in AD (normal pattern VEP and delayed P2 component of the flash VEP; Bajalan et al, 1986). Further study of cholinergic effects on visual function, in combination with judicious selection of available clinical tests (or novel tests designed specifically for the assessment of cholinergic-related functions) may prove helpful in the early clinical detection of AD.
SUMMARY

This thesis has contributed a body of new observations on the histopathology of Alzheimer's disease. Among these, the following are considered to be most important, and have implications for our understanding of AD as noted:

1) Astrocytic gliosis is consistently seen in the cerebral cortex, hippocampus and substantia innominata, confirming the impression held by many workers that these are the primary affected brain areas in AD.

2) Astrocytic gliosis in the neocortex has a distinctive laminar distribution, being concentrated in layers II-III and V. These same layers have previously been identified by many workers to be most severely affected in terms of numbers of neurofibrillary tangles.

3) A line of gliosis and plaques is found in area 17, along the interface of two laminae. This indicates that plaque distribution is influenced by neuronal organization.

4) The laminar distribution of neurofibrillary tangles and Alz-50 immunoreactivity in area 17 correlates well with that of area 18 (cortico-cortical, presumably glutamatergic) and noradrenergic afferents. If AD spreads along nerve fibres, as current popular hypotheses suggest, these neuronal systems must be suspected as the conduits. Against this scenario, however, is the growing consensus that neurofibrillary tangles are a secondary phenomenon in AD.

5) The laminar distribution of amyloid β-protein immunoreactivity in area 17 correlates well with that of cholinergic afferent fibres. If plaques are formed from degenerating axon terminals, as proposed by several authors, this correlation suggests that the degenerating terminals may well be cholinergic. Since cortical cholinergic innervation is entirely derived from the nucleus basalis of Meynert, this observation strengthens the association between the nbM and AD.
BIBLIOGRAPHY


white matter changes and proton MR relaxation times in dementia. *Alzheimer's Disease and Associated Disorders*, 1:156-170.


