CHAPTER 35

Cingulate Neuropathology in Anterior and Posterior Cortical Atrophies in Alzheimer's Disease

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Chapter contents

Introduction

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder that presents a heterogeneous array of cognitive and behavioral symptoms and it almost always involves the cingulate cortex. A common or typical form of AD presents early memory and visuospatial symptoms and often but not always begins in transentorhinal cortex. Early involvement of cingulate cortex in typical AD is well established in neuropathological studies showing that amyloid peptides and neurofibrillary degeneration involve subgenual anterior cingulate cortex (sACC) and retrosplenial cortex (RSC; Braak and Braak, 1991, 1993) and neurodegeneration occurs in posterior cingulate cortex (PCC) relatively early in the disease (Brun and Englund, 1981; Mountjoy *et al*., 1983; Vogt *et al*., 1990). Moreover, very early glucose hypometabolism in PCC has been demonstrated in patients with early memory impairment in a prodromal form and typical AD (Minoshima *et al*., 1997). This suggests that amnestic symptoms need not be initially attributed to medial temporal lobe structures, although they may be (Greicius *et al*., 2004), but involve the cingulate gyrus itself. Thus, early cingulate damage is a prominent part of the primary etiology of the disease rather than the result of secondary, deafferentation lesions in the temporal lobe.

An argument has been made that there are a number of AD variants on the typical form that present different onset symptoms and pathology beginning in cortical regions other than medial temporal cortex (von Gunten *et al*., 2005). One of the atypical forms of AD is the 'frontal' variant which has a higher density of lesions in prefrontal than parahippocampal cortex (Johnson *et al*., 2004). The name of this variant, however, obscures the fact that posterior midcingulate (pMCC) and dorsal PCC have greater lesion density than does frontal cortex (Chapter 33). Nestor *et al*. (2003) evaluated cases of mild cognitive impairment and concluded that early damage to limbic networks involved PCC first and suggested that the first clinical symptoms associated with this damage heralds the beginning of AD with damage to the amygdala and lateral cortical areas appearing later. These findings and nomenclature confusion can be accounted for by the fact that we still lack sensitive neuropsychological tests that differentiate between frontal and posterior cingulate impairments. Thus, by the time frontal damage can be detected with tests of executive function, the primary damage in cingulate cortex has already progressed beyond that in the frontal lobes. Until more sensitive tests of posterior cingulate impairments are available, there will be a continuing bias in the research literature to understand the onset and progression of AD in terms of lateral cortical structures rather than one of the main sites of early damage in cingulate cortex. Thus, cingulate cortex is critically and early involved in both typical and atypical forms of AD.

Braak stages and cingulate cortex

Although progressive lesions and neurodegeneration are most easily expressed by a linear function for large neuron losses (Terry *et al*., 1981), this model depends to a large extent on how the data are analyzed and interpreted. The most widely discussed linear approach is that of Braak and Braak (1991, 1997) and the various stages of lesion deposition in the cerebral cortex. Although AD is comprised of differing genetic, cognitive, and neuropathological variants or subgroups, efforts often seek to characterize a single disease according to stages. For example, the Braak stages have had an important influence by standardizing the density and pattern of disease markers in the temporal lobe. These stages, however, do not necessarily link to disease progression (Gertz *et al*., 1998) or to the initial onset of cognitive changes (Gold *et al*., 2000). Indeed, the staging methodology was based on a large population that was not clinically or neuropsychologically evaluated (Braak and Braak, 1991). Thus, if all cases of neuropathologically diagnosed AD began with a single memory impairment that could be traced to transentorhinal damage and changes in the cingulate gyrus always occurred in mild-moderate stages, further study of cingulate cortex in AD would not be needed to identify the primary etiology(ies).

Gertz *et al*. (1998) evaluated the pattern and densities of neurofibrillary tangles (NFT) in the context of the Braak stages. They found that 6 of 42 AD cases conformed in all regions to the expected hierarchy and that 90 per cent of cases had two or fewer order violations; presumably these are the typical AD cases. They concluded that an approximate validation was achieved. Jellinger and Bancher (1997) evaluated cognitive function with the Mini-Mental Score in terms of Braak stages and observed a highly significant and inverse relationship between them suggesting that the neuritic pattern and load reflected progressive cognitive impairments. Finally, Gold *et al*. (2000) evaluated 116 subjects over the age of 90 with either no cognitive impairment or very mild to severe AD and found a strong positive correlation between Clinical Dementia Rating (CDR) scores and Braak stages. However, the staging did not distinguish cases with normal cognition (CDR 0) from those with mild cognitive changes (CDR 0.5), and stage III overlaps with all CDR scores and stages of IV or greater were consistently associated with at least mild dementia. Their conclusion well summarizes the linkage between Braak stage and CDR, 'Braak staging represents a broad concept of the evolution of NFT rather

than a precise hierarchical model associated with a stepwise deterioration in cognitive abilities near the upper limit of life.'

In spite of these general agreements between staging of neuritic pathology and functional impairments, there are a number of explicit mismatches in what the Braak staging model predicts and the observed facts. (1) The staging scheme does not identify all cases of dementia (Gertz *et al*., 1996). (2) AD cases with stages V and VI did not show a positive relationship with age, while the limbic stages III and IV have a wide range of cognitive performance from almost normal to overt dementia as measured with the Mini-Mental Score (Gertz *et al*., 1996; Jellinger, 1997). (3) As noted above, most cases do not follow the same pattern of neurofibrillary degeneration as specified in the hierarchical model. (4) As discussed below, there is not a single pattern in the evolution of cingulate damage and this approach provides only a descriptive tool for reporting the level of pathological markers in an area. (5) Early-onset cases of AD (i.e. ≤age 65) often have the most severe neurodegeneration and even when cases are matched for disease duration, early-onset disease is associated with more extensive neuron losses (Vogt *et al*., 1990). Thus, staging in a single continuum is not possible in relation to neurodegeneration including that in cingulate cortex. Gold *et al*. (2000) state, 'Although Braak staging of AD cannot be rejected by available univariate analyses of functional imaging, a single linear evolution of the disease is not sufficient to explain the important heterogeneity in the data.' For these reasons and the statistical considerations below, linear models and simple staging schemes cannot be applied to neuropathological studies of cingulate cortex in AD.

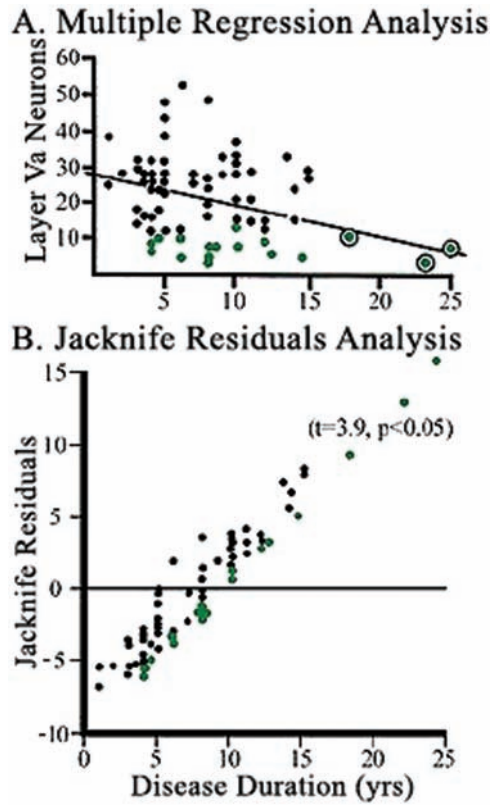

Fig. 35.1 Statistical assessment of layer Va neuron densities in 73 cases of AD. A. Least square fit of regression line to neuron densities and three outliers circled. Removal of these latter three outlier cases resulted in a non-significant ***r*** value. Cases coded green are all members of the most severe loss of neurons in layer Va (≤10 neurons). B. Jacknife residuals analysis shows a significant deviation of the severe group from remaining cases and confirms that the linear model is not appropriate for this data set.

Statistical Analysis of Cingulate Neurodegeneration

Neurodegeneration occurs in PCC relatively early in the disease (Brun and Englund, 1981; Mountjoy *et al*., 1983; Vogt *et al*., 1990). In the latter study, neuron densities were estimated in area 23b in a large sample of cases from drawings of neuronal somata in 160-µm wide strips of area d23b (also, Vogt *et al*., 1998). Counts from six strips for each case were averaged and Figure 35.1 plots them for layer Va of this area in each case. As predicted, there was an inverse relationship between densities of large neurons with disease duration as estimated from the first clinical symptoms. The linear least squares regression 'confirms' the linear model with a significant correlation coefficient (r = –0.27; F = 6.19; p = 0.015). Or does it? Two or three cases with disease durations of 18–25 years had an unusually large influence on the regression. If these three cases with most severe neurodegeneration are removed from the analysis (Fig. 35.1A, individuals circled), the linear regression is no longer significant (r = –0.16; F = 1.74; p = 0.19). Moreover, the 17 most severe cases form a separate group in this graph (green points in Fig. 35.1A) suggesting the total AD population is not homogeneous and there may be a progression of the disease from 4 to 25 years of duration for these cases that could be independent of the remaining cases.

The problem of outliers in linear regression is well recognized and has been discussed by Stevens (1984). Although all outliers do not necessarily influence the regression, one or more cases can be influential and alter the results significantly. Because outlier analysis provides important clues to the composition of the population, the issue needs further consideration for cingulate neuron densities. Analysis of jacknife residuals provides a means of evaluating the veracity of a regression model and, because they are linked directly

to a distribution, the significance of one or more outliers in a least-squares model can be determined. A residual e_i is the difference between the observed value of *Yi* from a regression model; i.e. the discrepancy that is still present after fitting with least squares. These error terms should be independent, have a mean of zero, a common variance, and follow a normal distribution (Kleinbaum *et al.*, 1988). A jacknife residual evaluates the residual variance computed with the *i*th observation deleted and the standardized residuals should not differ from predicted values, if the linear model holds for the sample. A graphic plot of residuals against predictor values is a way to evaluate the regression assumption and the influence of possible outliers.

A plot of jacknife residuals against disease duration for all cases has been reported (Vogt *et al.*, 2001a) and is provided in Figure. 35.1B. The dispersion of residuals plotted against the predictor variable is evidence that the basic regression assumption stating that the X variable must be known without error is not followed in this population. Furthermore, the coefficient b1 has a small but non-zero value that displaces individual points from the straight line. There is a systematic divergence of the 17 members in green with the most severe neurodegeneration; i.e. those cases with fewer than 10 neurons in layer Va. Thus, the regression model is inappropriate for this data and progressive neurodegeneration could occur in a non-linear and multivariate framework. Indeed, we have argued that laminar patterns of neurodegeneration in PCC does not occur according to a linear model but rather there are multiple laminar patterns of neuron degeneration and a linear progression is possible within each laminar pattern of neuron loss (Vogt *et al.*, 1998). The previous Chapter 34 also argues in favor of a multivariate approach characterized by lobar poles of the disease using functional imaging and correlation to particular categories of symptoms.

Since there is not a single population of vulnerable neurons in the cingulate gyrus or a single pattern of neurodegeneration, such as large neuron degeneration mainly in layer Va, we have used multivariate models to evaluate laminar patterns of neurodegeneration and the role of cingulate cortex in multifocal cortical atrophy (Vogt *et al.*, 1999). This multilobar and multilaminar approach to AD considers atypical or variant forms of AD, links particular symptoms to different cingulate cortical regions, and does not require an immediate involvement of medial temporal structures, although it can occur simultaneously or soon after onset particularly in the typical form of the disease. The multivariate approach seeks to link genetic risks such as expression of the three alleles of apolipoprotein E, neuron densities in particular layers, age at onset, and even transmitter receptor binding to subgroups of cases (Vogt *et al.*, 1998). Moreover, neuropsychological subgroups such as those described by Martin (1990) led us to consider the extent to which focal cortical atrophy might be linked to different patterns of neurodegeneration in the cingulate gyrus in a case of frontotemporal atrophy as well as PCC damage in AD with posterior cortical atrophy (Vogt *et al.*, 1999). The present discourse extends these analyses that were focused on PCC to other parts of the cingulate gyrus and links them with particular symptoms that extend beyond amnesia to include behavioral and mood impairments.

Four-region neurobiological model and AD symptoms

To make the transition from linking AD symptoms to damage in parts of cingulate cortex, we begin with a brief summary of the four-region neurobiological model and its implications for symptom patterns in AD. A key verification of the functional relevance of the model is the extent to which it predicts regional vulnerabilities to particular diseases and this is a theme in the present volume. Figure 35.2 presents the model and relationships to symptoms that might evolve along with damage to different parts of the cingulate gyrus (black arrows). Observations supporting this view are summarized throughout this chapter and the model provides specific hypotheses for clinicopathological correlations. Although there are many strong correlations between site of damage and symptom development, the structural boundaries of the AD pathology are not fixed to strict borders among cortical areas. Indeed, most studies of clinicopathological correlates evaluate only one or two parts of cingulate cortex; a study design that will hopefully change over the coming decade.

Although the Braak stages do not model disease progression in cingulate cortex, it is striking that the cases with least cingulate damage (stages III–IV) involve subgenual ACC and ventral PCC (Braak and Braak, 1991). As reported by Vogt *et al.* (2006) and summarized in Chapter 13, vPCC is not part of an emotion system in terms of storing episodic memories and regulating autonomic outflow, but rather is involved in extracting information from sensory experience for emotional content and context that is relevant for further processing in cingulate cortex. A study in the visual system clearly demonstrates this function of vPCC and adjacent RSC. An important aspect of emotional relevance is the context in which an object or event occurs and vPCC is active during context-dependent processing of visual stimuli (Bar and Aminoff, 2003). Context from the visual system and emotional valence from sACC combine in vPCC for transmission of self-relevant objects and events into cingulate cortex for emotionally relevant processing and movements. Since valences are assigned in ACC, this region stores those memories (George *et al.*, 1995; Mayberg *et al.*, 1999). Johnson *et al.* (2002)

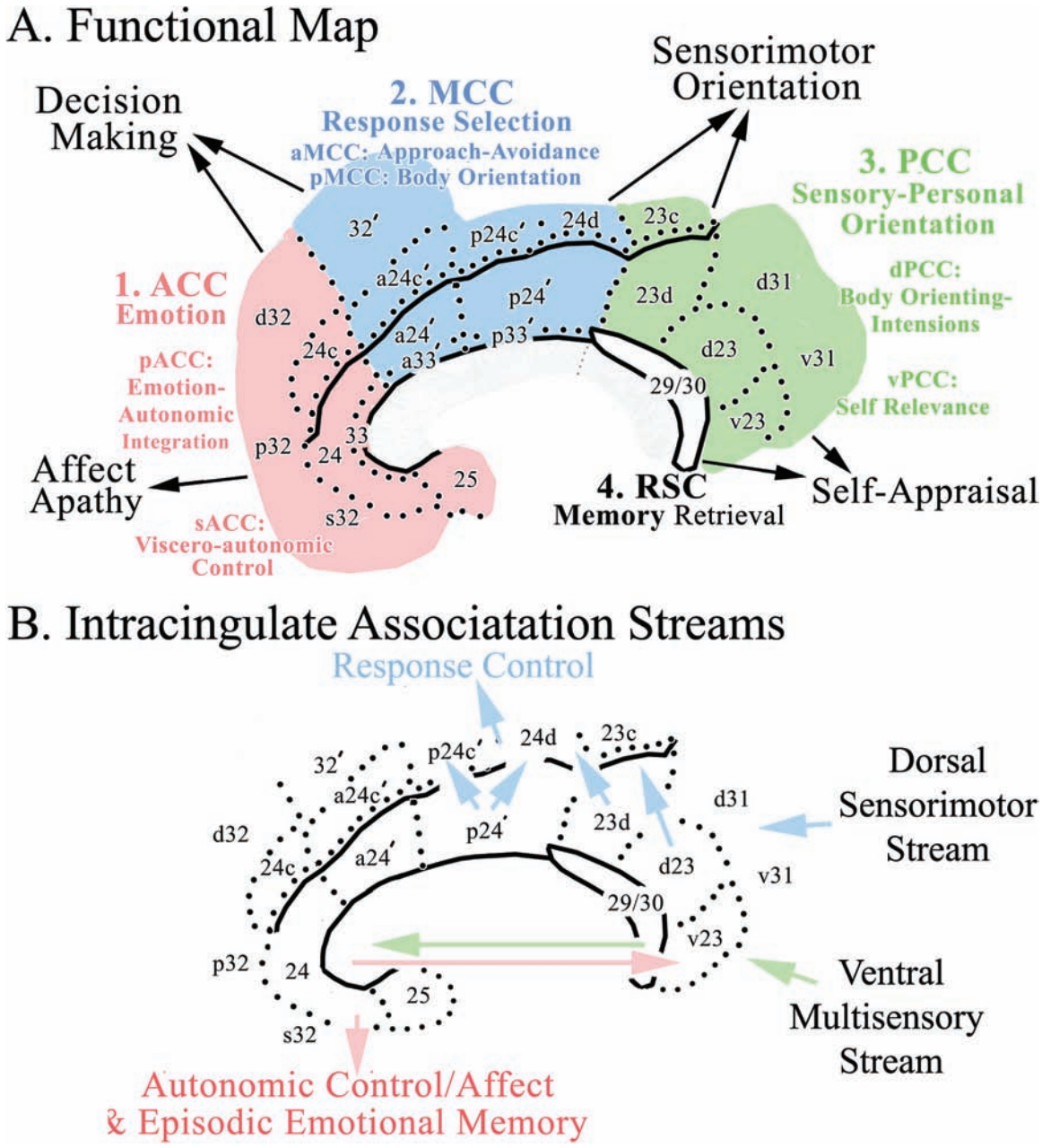

Fig. 35.2 A. Four-region neurobiological model and predictions of particular classes of vulnerable functions and symptoms that could emerge in AD depending on which region is involved. Each of five classes of symptoms include subregions in the rostrocaudal dimension of cingulate cortex: (1) Affect/Apathy, (2) Decision Making, (3) Sensorimotor Orientation, (4) Self-appraisal, (5) Memory Retrieval. B. Intracingulate information flow is impaired in AD by damage to pyramidal neurons and likely contributes to particular symptoms. The two primary streams are the dorsal sensorimotor stream that is focused on connections into the caudal cingulate premotor area in areas 24d and 23c and the ventral multisensory stream that interacts with ACC to engage context and valence dependent sensory information (personally relevant sensations), episodic emotional memories in ACC, and regulation of affect and autonomic outflow.

used such a task and vPCC was activated in a task involving self reflection. Other studies of one's own face (Kircher *et al.*, 2001; Sugiura *et al.*, 2005), familiar faces (Maddock *et al.*, 2001), and intentional self assessment (Kircher *et al.*, 2002) activate a similar region. The reciprocal connection between vPCC and ACC is very important as shown with one of the intracingulate processing streams in Figure 35.2B. The vPCC appears to select among emotion and non-emotion events, assesses their self relevance with sensory context and interacts with ACC before further processing ensues. Thus, vPCC is a gateway for multisensory information

processing and entry into the cingulate gyrus for processing as emotionally relevant information and damage thereto in AD likely contributes to impairments in assessment of emotionally relevant objects and contexts and associated behavioral output.

Ries *et al.* (2006) used an episodic recognition task and an autobiographical self-appraisal task in which control subjects and subjects with mild cognitive impairment rated themselves on a set of trait adjectives. The results of their conjunction analysis showed that vPCC was the sole region activated during both tasks and vPCC was active in the task-dependent response in the mild cognitive impairment group showed vPCC during self appraisal but not episodic retrieval. Amazingly, the pregenual ACC (pACC) was also active in the same task in the latter group. A study by Chételat *et al.* (2003) reported a role for memory retrieval in vPCC in mild cognitive impairment cases with a memory only deficit. These observations not only confirm an early task deficit in vPCC in mild cognitive impairment but also the importance of the reciprocal interaction of this subregion with pACC.

The dPCC subregion appears to be mainly involved with sensorimotor orientation in space. This has been shown with studies of visual feedback of moving hands (Inoue *et al.*, 1998), predictability of self-generated actions (Blakemore *et al.*, 1998), free exploration in a virtual reality maze (Maguire *et al.*, 1998), and complex London route recall (Maguire *et al.*, 1997). It may be impossible to separate visual orientation from an internal sense of self in dPCC. Vogeley *et al.* (2004) report that the first-person perspective is more effective in driving dPCC than is a third-person perspective and this emphasizes the role of dPCC in orienting the head and body in multisensory personal space and links to personal intentions. The pivotal role of PCC in intention implies an important role in guiding behavioral output that has often been attributed to supplementary motor areas. den Ouden *et al.* (2005) explored the role of intention in relation to actions (intentional causality) with a task involving one's intentions in relation to outcomes and outcomes in relation to physical events. They activated PCC more when subjects when thinking about intentional causality versus physical causality. This study supports the notion that PCC can directly modulate intentional outcomes and we hypothesize this occurs via the cingulate premotor areas as shown with a stream of blue arrows in the dorsal sensorimotor stream in Figure 35.2B. One of the demonstrations that dPCC pathology in AD is linked to visual agnosia in typical AD was provided by Giannakopoulos *et al.* (1999). They showed that neurofibrillary tangle densities in dPCC were positively correlated with scores on Ghent's overlapping figure and Gottschald's hidden figure tests in area 23 but not area 24. The involvement of PCC in sensorimotor processing is suggested below in the AD variant with Posterior Cortical Atrophy (PCA); however, due to the lack of cingulate-specific neuropsychological tests, the contribution of PCC to these deficits is still not known.

Apathy and Depression in MCI and AD: Early and Pivotal Role of Anterior Cingulate Cortex

Apathy and depression are common neuropsychiatric symptoms in mild AD including the amnestic subgroup of mild cognitive impairment (Modrego and Ferrandez, 2004). Geda *et al.* (2006) observed that depression in the elderly predicted an increased risk of mild cognitive impairment that was synergistic with the ε4 allele of apolipoprotein E. Involvement of medial prefrontal cortex and ACC was shown to be correlated with an Apathy Inventory by Benoit *et al.* (2004) and this symptom is linked to altered ACC function with multivariate analyses discussed in Chapter 34 by Salmon and Laureys. Finally, the presence of a diagnosis of depression in AD leads to a more severe cortical neuropathology as concluded from a database review by Rapp *et al.* (2008). Indeed, cases of Anterior Cortical Atrophy (ACA) discussed below had severe aggression and impulse control problems in the later stages of AD. This raises the possibility that alterations in mood alert to the presence of Anterior Cortical Atrophy and significantly enhances the progression of neuropathological changes during the later stages of the disease. Therefore, as reduced glucose metabolism in PCC is a pivotal sign predicting the amnestic form of mild cognitive impairment and AD, the same is true of ACC in predicting severe and negative outcomes associated with apathy and depression throughout the course of AD.

It has long been recognized that damage to ACC in AD is associated with neuropsychiatric outcomes, including the above observations. The four-region neurobiological model predicts that damage to ACC will be associated with depressive symptoms, apathy and possibly dysautonomia and this linkage will be discussed in detail below in relation to ACA. Thus, depression both signals an increased chance of developing AD and insures a more severe neuropathology in ACC when compared to AD patients with no signs of depression; i.e. typical AD.

Goals of This Chapter

In spite of the many detailed morphometric analyses of cingulate cortex in neurodegenerative disorders, little progress interpreting its relevance to clinical symptoms has been made due to a lack of a consistent and integrated perspective on its normal structure and

function. In contrast, studies of entorhinal cortex in AD quickly resolved the level of neuronal degeneration at different stages of AD and its role in brain dysfunction. The structural and functional heterogeneity of cingulate cortex are critical to understanding its diverse contributions to AD symptoms, early etiology and potential treatments. Before detailed stereology studies can be undertaken and interpreted, there is a need to define the parameters of degeneration and their links to particular toxic peptides; what classes of neurons should be studied, in what layers of what areas and with what clinical onset symptoms and disease durations. Without these types of information, an intelligent stereology design is not possible. Here we consider specific structure/function and impairment correlations in subgroups of AD along with an analysis of the extent to which the entire cingulate cortex may be involved in different lobar atrophies. The patterns of cingulate damage linked to particular symptoms are enhanced with detailed studies in carefully selected cases and new cases are introduced for this purpose. The present chapter seeks to accomplish the following specific goals:

1 Evaluate progressive laminar patterns of cingulate neurodegeneration and multivariate models in relationship to typical AD and age-matched controls.

2 Review the patterns of cingulate neuropathology in two cases of AD with Anterior Cortical Atrophy (ACA) and early mood/apathy and executive impairments. Since lobar atrophy appears at mid-to-late stages of the disease, an AD case with similar clinical symptoms without overt atrophy is explored to evaluate the status of subgenual ACC and dorsal PCC. This pathology is compared with reports of the behavioral variant of frontotemporal dementia because of clinical symptom overlap.

3 Provide the rationale for interpreting mood, autonomic and behavioral disorders based on anterior cingulate circuitry including projections to subcortical regions such as the periaqueductal gray in terms of a deafferentation model.

4 Summarize the visuospatial symptoms in Posterior Cortical Atrophy (PCA) in AD and the cingulate processing streams that are likely disrupted following posterior cingulate and retrosplenial damage.

5 Present a principal components analysis of posterior cingulate neurodegeneration in seven cases of PCA and immunohistochemical analysis of amyloid peptides that suggest profound Aβ43 deposition including involvement of ACC and MCC.

6 Consider PCA in the context of the subtypes hypothesis of AD including possible alterations in γ-secretase activity in PCA and propose a cingulate sampling strategy for studies.

Cingulate Neurodegeneration in Multiple and Progressive Subgroups

The null hypothesis for involvement of cingulate cortex in AD states there are no subgroups or subtypes and, therefore, marker deposition and neurodegeneration occurs along a single progression. In other words, AD initiates in essentially the same place in the gyrus in all cases and progressive loss of neurons occurs in the same layer(s) throughout its evolution. Early studies of neurodegeneration in the cingulate gyrus and other neocortical regions employed this hypothesis and it was thought that large neurons uniformly degenerate in layers III and V (Mountjoy *et al.*, 1983; Pearson *et al.*, 1985). It was surprising, in this context that layer III neurons in dorsal area 23 (d23) were often relatively intact in many AD cases and that small pyramidal neurons in layers II, IV, and VI were at risk for prominent death (Vogt *et al.*, 1992, 1998). It is possible that regions of interest and laminar histology were not fully appreciated for specific cingulate areas in early studies of neurodegeneration. Many issues arise simultaneously when contemplating a sampling strategy for cingulate neurodegeneration; Which region(s) are involved topographically and what is the vulnerability of neurons in PCC? Our early work emphasized a single suprsplenial level of area 23b (now area d23b) to insure that differences in neuron densities could be attributed to the disease rather than variability in dissection sites. The downside of this strategy is that many other areas were not sampled and could have been more prominently involved in a primary etiology than dPCC.

Figure 35.3 summarizes some of the key observations from this early work with 'strips' of area d23b in different cases. The case WJ is an example of focal temporoparietal degeneration that was reported in detail previously (Vogt *et al.*, 1999). Since he was a biochemist and married to a woman involved in special education, it is reasonably secure that the first signs of cognitive problems were impairments in word finding, substitution of the wrong words, and spelling and pronunciation problems at the age of 67. An MRI assessment subsequently showed profound atrophy in temporoparietal cortex in the left hemisphere and most atrophy was observed in this region in the postmortem brain. Since the language impairment began years before memory and visuospatial impairments, the first sites of disrupted function probably were in superior temporal and temporal pole cortices. A detailed summary of the clinicopathological correlations often observed in this variant of AD is provided by von Gunten *et al.* (2005) and this case shows the laminar patterns of cortical neurodegeneration at his death at age 77.

Case FG (Case 3 below) is an example of Anterior Cortical Atrophy in AD and is shown in Figure 35.3 for comparative purposes with Case WJ. It appears that

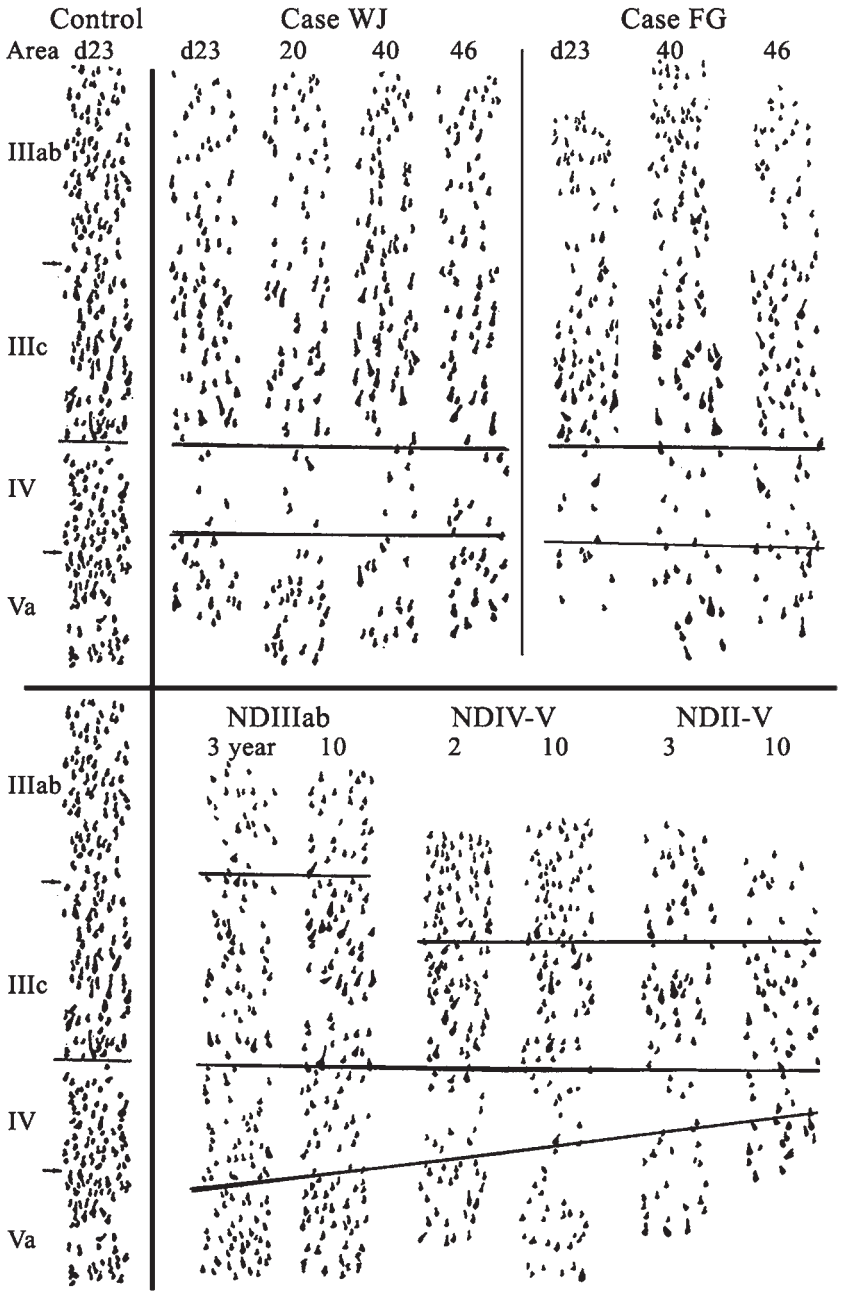

Fig. 35.3 Neuronal somata in area d23b of an age-matched control case to show clinically unique manifestations of neuron loss in focal parietotemporal (WJ) and anterior cortical (FG) atrophies in AD. The prominent damage to areas d23b and 46 are obvious in FG in layers III, IV, and V, while layers III and V of parietal area 40 are relatively spared. Damage throughout layer Va in case WJ is similar to that in Case FG, while WJ had clearing of neurons in layers IIIab in areas d23 and 20. The bottom 6 cases from three subgroups based on laminar patterns of ND from early and long duration cases provides evidence of three independent progressions that is pivotal to the argument for a multivariate model of AD.

layer IV is severely impacted in all areas sampled in both cases. Notice that the subgroup based on neurodegeneration mainly in layer IIIab in the typical cases did not experience this profound loss of neurons in layer IV. Thus, damage to layer IV might be an early vulnerability that is shared by the AD variants. Of the four sampled neocortical areas, layer III in area 20 of Case WJ had the greatest involvement and possibly links to the early language impairments in this patient. Finally, layer Va has some damage in WJ but is severely disrupted in Case FG and this is an important differentiation of Anterior Cortical Atrophy.

The bottom half of Figure 35.3 shows NeuroDegeneration (ND) for three subgroups with preferential damage to different layers in cases where no selective cortical atrophy was detected. Note the atrophy of area d23b in the two groups with broad laminar neurodegeneration in NDIV–V and NDII–V. A case is shown with duration of 2- or 3- versus 10-year durations for both to emphasize that these subgroups represent independent rather than linked progressions. This observation confirms the hypothesis that these are independent and progressive forms of AD and it is pivotal to arguing for multivariate models of the disease, since each subgroup must eventually be shown to have a unique disease progression.

Multivariate Models

Since the jacknife assessment of neurodegeneration in layer V and demonstration above of multiple and independent laminar patterns of neurodegeneration shows that AD is not uniform but comprised of multiple and independent subgroups, a multivariate approach to statistical analysis is necessitated. The use of such models and their assessment with eigenvector projections in complex biological diseases, such as gene-expression patterns in human breast cancers, has been demonstrated (Marx, 2000) and will likely be relevant to many neurological diseases in addition to AD.

Eigenvector projections of a model incorporating neuron densities in layers III–Va showed that, although most AD and control cases occupied different parts of the three-dimensional projection, the AD group was composed of at least five groups (Fig. 35.4A). The five cases with no neurodegeneration in PCC (ND0) occupy a part of the graph that overlapped with control cases. By removing the control cases from the assessment, a large proportion of the eigenvector projection is made available for more detailed analysis of AD and to explore the possibility of differentiable subgroups. Indeed, the five laminar patterns of neurodegeneration in the AD cases (coded with different colors) are observed in this projection (Fig. 35.4B).

The possible relevance of apolipoprotein E (ApoE) genotype on posterior cingulate AD neuropathology was suggested in a study by Reiman *et al.* (1996) that showed reduced glucose metabolism in this region as well as in temporal and parietal cortices. Although patients homozygous for the ε4 allele of ApoE have enhanced densities of senile plaques (Sparks *et al.*, 1990), the link to PCC neurodegeneration is not known. Ohm *et al.* (1999) showed that patients with disease onset below the age of 80 had an increase in amyloid peptide expression and neurofibrillary degeneration if they were homozygotic for the ε4 allele, while those over 80 years of age did not differ when characterized by their ε4 or ε2 alleles.

Inclusion of ApoE genotype in the multivariate model with neuron densities in dPCC did not alter the AD subgroups in any significant manner (Vogt *et al.*, 1998) as also shown in Figure 35.4C. However, it is noteworthy that there is the highest proportion of homozygous ε4 patients in the NDSev subgroup and this confirms that the ε4 allele contributes to the most severe pattern of neurodegeneration.

Thus, the allelic composition of ApoE expression influences the onset and progression of AD early and has a lesser influence as disease onset later in life. Interestingly, four of the subgroups based on neurodegeneration did not have a disproportionate influence of ApoE genotype.

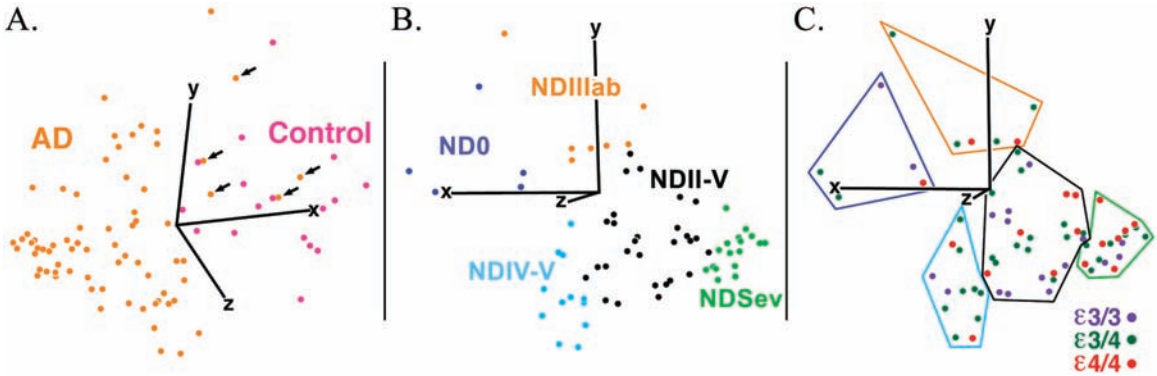

Fig. 35.4 Eigenvector projections of multivariate models based on neuron densities in layers IIIab, IIIc, IV, and Va that include controls cases (A.), AD cases only to enhance statistical comparisons within AD (B.) and ApoE genotype (C.). One AD subgroup/subtype overlaps with control cases because there is no NeuroDegeneration present in dPCC (ND0, arrows in A.). Segregation of five subgroups is demonstrated in B. Addition of ApoE genotype to the model does not further distinguish the groups (C.), although the highest density of ApoE ε4 homozygotes is in the group with most severe neurodegeneration (NDSev).

'Typical' AD with Two Poles of Cingulate Functional Impairment

As reviewed by von Gunten *et al.* (2005), the primary progressive amnestic syndrome characteristic of the initial stages of typical AD and amnestic mild cognitive impairment may be the consequence of changes in the medial temporal lobe. As a rule, cortical degeneration progresses throughout the cerebral cortex as diffuse atrophy and no lobar specificity can be detected therein. Nevertheless, patients with typical AD have particular deficits that are significantly associated with NFT deposition in different regions of cingulate cortex. Although typical AD cases experience deficits that are magnified in the lobar atrophies described below, these symptoms appear later in the disease after the memory impairments are well established (Jagust *et al.*, 1990).

Naming and identification of famous faces is correlated with NFTs in area 24 (Giannakopoulos *et al.*, 2000), while spatial disorientation was correlated with NFT in area 23 (Giannakopoulos *et al.*, 2000). There were no correlations between the development of psychotic features and senile plaques in typical AD (Farber *et al.*, 2000). Thus, even in typical AD, different regions express correlated levels of NFT deposition that are predicted by the four-region neurobiological model (Fig. 35.2). In cases with lobar specific atrophy, it appears that one of these trends is enhanced early in the disease to generate 'atypical' symptoms. These polar extremes may result from different mechanisms of neurodegeneration that certainly require different therapeutic and management approaches but also might suggest that there are subtypes of AD rather than simple subgroups.

Anterior Cortical Atrophy: ACC in Mood (Apathy), Autonomic, Executive Impairments

Damage to ACC in any neuron disease including AD can be heralded by three classes of neuropsychiatric symptoms; altered mood, behavioral or executive dyscontrol and autonomic changes. Although all are associated with damage to ACC, each requires a different mechanism and they are not automatically linked by a common pattern of neurodegeneration; as each likely results from degeneration of different circuits.

Early PET studies showed changes in frontal and cingulate cortex associated with altered executive functions. Grady *et al.* (1990) observed more inappropriate behaviors in patients with greatest prefrontal glucose hypometabolism and Mann *et al.* (1992) reported two groups of patients with equivalent parietal glucose hypometabolism but were differentiated by frontal lobe metabolism, measures of executive function and the rate of disease progression. Our Case FG (here Case 3) of AD with selective frontrotemporal atrophy (Anterior Cortical Atrophy, ACA) heralded his disease with paranoia and experienced profound pathology in ACC (Vogt *et al.*, 1999).

Impairments in mood have long been known in AD (Deutsch and Rovner, 1991) and it was noted above that apathy and depression are common neuropsychiatric symptoms in mild AD including the amnestic subgroup of mild cognitive impairment (Modrego and Ferrandez, 2004). Geda *et al.* (2006) observed that depression in the elderly predicted an increased risk of mild cognitive impairment that was synergistic with the ε4 allele of apolipoprotein E. Although Holthoff *et al.* (2005) found that orbitofrontal cortex had glucose metabolism correlated with apathy with no changes in ACC, involvement of medial prefrontal cortex and ACC was shown to be correlated with an Apathy Inventory by Benoit *et al.* (2004) and this symptom is linked to altered ACC function with multivariate analyses (Chapter 34). Moreover, all patients with major and bipolar depression experience neurodegeneration in sACC (Chapter 25). Thus, the integrity of this subregion is critical to stable mood and damage therein associated with apathy and depressive symptoms may alert to AD in elderly patients.

Autonomic impairments have been reported in AD and these include systolic hypertension (Prince *et al.*, 1994), vasomotor impairment (Algotsson *et al.*, 1995), and coronary artery disease (Breteler *et al.*, 1994). Sparks *et al.* (1995) observed a higher level of temporal lobe NFT in coronary artery disease; although the association of hippocampal formation pathology does not speak to specific links between cardiac function and CNS neuropathology. Aharon-Peretz *et al.* (1992) evaluated the power spectrum of heart rate variability in AD patients. They concluded that these patients manifested a relative hypersympathetic and hypoparasympathetic state. Finally, alterations in circadian temperature regulation (Printz *et al.*, 1984) and passive tilting-induced changes in blood pressure and heart rate have been reported (Aharon-Peretz *et al.*, 1992; Elmstahl *et al.*, 1992). These outcomes cannot be predicted from any model of CNS changes in AD, but they warrant consideration of autonomic regulatory systems in individuals with alterations in mood resulting from damage to sACC because this region directly regulates autonomic functions (Chapters 1 and 10).

In addition, the hypothalamus, amygdala, anterior insula and orbitofrontal cortex are connected with sACC and may contribute to autonomic impairments (Chapters 5, 6, 10 and 15). Sparks *et al.* (1988) reported a reduction in serotonin throughout the anterior, lateral and posterior hypothalamus, while no changes occurred in dopamine or norepinephrine. Changes in

cell number, volume and NFT densities in the suprachiasmatic nucleus and raphe nuclei have also been reported (Bondereff *et al*., 1982; Swaab *et al*., 1985). Kromer *et al*. (1990) observed extensive neurofibrillary damage in the accessory basal, cortical, and cortical transition area of the amygdala with much less in the central medial, lateral, and laterobasal nuclei. Taken together, this wide range of damage in autonomic regulatory systems may contribute to changes in body weight and food and fluid intake, sleep impairments and autonomic impairments in AD.

Two studies reported neurofibrillary changes in sACC in AD in reviews of a broad range of cases without reference to specific clinical symptoms. Braak and Braak (1991) reported this region had early though limited deposits of amyloid in stage A and the limbic stage III–IV was associated with neurofibrillary changes in this region. In both instances, therefore, relatively early damage to sACC was noted. In a second study by Chu *et al*. (1997), NFT were reported throughout the ventromedial frontal region including sACC, orbitofrontal cortex, and the anterior insula. Although case selection was *not* based on clinical symptoms and there was confusion of dysgranular area 32 with agranular areas 24 and 25 in the latter study, both studies confirm an early impairment in this region in AD.

We first recognized damage to cingulate cortex in the 'frontal' variant of AD with the report of Case FG in the broader context of multifocal cortical atrophy (Vogt *et al*., 1999; FG/Case 3 here). Another group of cases with preferential frontal lobe damage, i.e. greater in frontal than parahippocampal cortices, was reported as a frontal variant of AD (Johnson *et al*., 1999); although the latter study did not assess the cingulate cortex where damage exceeds that of frontal cortex (Chapter 33). These cases together with the present two cases form a growing body of literature suggesting that AD can either begin in cingulate cortex or simultaneously is disrupted with medial temporal areas to produce symptoms beyond amnesia to include autonomic and behavioral deficits. The important point here is that the greatest damage in these cases is in cingulate cortex rather than the frontal lobe *per se*.

Prefrontal syndromes include dysexecutive, mood, and behavioral disturbances. As noted by von Gunten *et al*. (2005), dysexecutive refers to the breakdown of a variety of higher cognitive functions that enable the logical and temporal coordination of actions, to inhibit inadequate and trigger appropriate actions in a particular context and there can be a transition to socially inappropriate behaviors. The presence of mood, apathy and behavioral symptoms characterize the first signs of Anterior Cortical Atrophy (ACA), although memory impairments can begin at the same time or later in cases presented here. Psychiatric symptoms including anxiety, obsessive ideation, decreased capacity of judgment, inappropriate joviality, poor hygiene, alimentary habit changes, coprolalia, emotional lability, pathological crying, impulsivity, disinhibition restlessness, wandering, hoarding, childishness, antisocial behaviors and violent behavior have been described in case reports (Wechsler, 1977; Snowden *et al*., 1992; Mizuno *et al*., 1996; Bak *et al*., 2001). Although many of these symptoms suggest a primary involvement of sACC, the deafferentation of massive projections to subcortical sites such as the hypothalamus and periaqueductal gray may also play a role in impulsivity, disinhibition and aggression. Although these latter sites have not been systematically studied, assessment of Case 2 supports secondary changes in these structures.

Cingulate Cortex in the Behavioral Variant of Frontotemporal Dementia

Frontotemporal dementia (FTD) can appear with or without lobar atrophy (Josephs *et al*., 2006) and is a preferred term for a spectrum of non-Alzheimer's dementias with a wide range of non-diagnostic histopathological changes including ubiquitin-positive inclusions, diffuse tau-immunoreactivity, and, in some cases, lack of distinctive pathology (Hodges *et al*., 2004; Josephs *et al*., 2006). One symptom group presents mainly with aphasia that is either progressive fluent or non-fluent (Hodges *et al*., 2004) and is unlikely to be confused with the presentation of AD cases reported here. A second group often referred to as the behavioral variant, however, presents with progressive personality changes including disinhibition, loss of empathy, changes in eating patterns, stereotypical behavior and apathy. Since some of these latter cases may have marked amnesia, there may be instances in which ACA in AD presents symptoms that overlap with the behavioral variant of FTD.

Although the present analysis does not attempt to differentiate the clinical symptoms in AD with ACA from the behavioral variant, it is important to note that cortical and subcortical intracellular inclusions are not present in AD with ACA or without atrophy as in Case 1. Although the semantic subgroup of FTD had a small involvement of sACC area 25 in a voxel-based morphometry study by Williams *et al*. (2005), the behavioral variant had a very large site of atrophy in midcingulate area 32′. The assessment of Case 1 below was extended to area 32′ to consider this issue and there was no significant damage there including detectable neurodegeneration or paired-helical filaments. Only the constant cortical pattern of amyloid-β42 (Aβ42) was present as elsewhere throughout the cerebral cortex. Even in sACC the damage is denser and extensive including area s24 and not limited to area 25. Thus, although the

clinical symptoms of the behavioral variant overlap to some extent with those of AD with ACA, even in the early case of definite AD with similar behavioral changes, it can be pathologically distinguished from the behavioral variant of FTD.

Methods and Case Summaries

The case review of AD patients with ACA is based on three detailed studies. Cases 2 and 3 had clear evidence of frontal and temporal pole atrophy. Since lobar atrophy occurs at mid-to-late stages of the disease, Case 1 was identified by a clinical records review for early neuropsychiatric symptoms and the lowest possible CDR compatible with a definite AD diagnosis. This patient had early major affective disorder with depression/dysphoria and emotional lability and we believe they provide a context for evaluating early changes associated with ACA.

The seven cases with Posterior Cortical Atrophy (PCA) are presented as a group for quantitative analysis rather than by individual case. Two groups of definite AD cases with no evidence of lobar atrophy were matched for age and total neuron densities in area d23b and are summarized in Table 35.1. These latter 'typical' AD cases are also presented as group data, since the individuals have been assessed previously as part of a larger study (Vogt *et al.*, 1998). Although Clinical Dementia Rating scores (Morris, 1993) were available for two ACA cases, the CDR scores were not available for the PCA cases and estimates of disease onset were based on the first clinical symptoms for each individual. The final case is one with early visual impairments that may reflect an early stage of PCA. The patient was too early in their disease to qualify as AD; however, this case provides important insight into what may be the earliest stage of PCA in AD.

The tissue blocks were cut into eight series of coronal, 50-µm thick sections and processed for thionin and the following antibodies: AT8 to identify early phosphorylation of tau at serine 202 and threonine 205 (Goedert *et al.*, 1995), AD2 to the hyperphosphorylated form of tau, PHF for paired helical filaments, Aβ40, Aβ42 and Aβ43 to demonstrate amyloid peptides in dense and diffuse plaques, SMI32 antibody to non-phosphorylated neurofilament proteins and neuron-specific nuclear binding protein (NeuN). The standard immunohistochemical procedures are provided by Vogt *et al.* (1998, 2001b) and Johnson *et al.* (2004).

Quantification of amyloid peptides for the PCA study was performed with microdensitometry using a Sony XC-77 video camera and NIH Image software for Macintosh computers (Vogt *et al.*, 2001c). Sections were placed on a light table and additional lens used for a final magnification of 200X for locating a rectangular sampling box in each layer. Relative optical density grey values were calculated in arbitrary units for thresholded dense plaques. All measurements were taken at the same time and the values for each layer in different groups are internally consistent though arbitrary. The background optical density was determined on each slide from a site without tissue and subtracted from the optical density determined for dense plaques. A similar measure was determined for diffuse plaques by subtraction of the dense plaque values in the same sampling box. Cases of AD were selected from the NDIV–V and NDII–V from the larger groups based on age at death and total density of neurons in area 23b. Finally, neuron densities were estimated with 160-µm wide strips of area d23b from which neuron somata were drawn with a camera lucida attached to the microscope according to previously published criteria (Vogt *et al.*, 1990, 1998). Some examples of these drawings are shown in Figure 35.3.

TABLE 35.1 Case material

	Gender	CDR	Age Death/onset	Brain Weight (gm)	Cause of Death
Anterior Cortical Atrophy					
Case 1	F	1	95/89	946	adenocarcinoma
Case 2	F	2.3	87/70	1145	cardiopulmonary arrest
Case 3 (FG)	M	NA	90/85	1270	cardiopulmonary arrest
Posterior Cortical Atrophy n = 7	2M/5F	NA	75 ± 3.5/66 ± 3.3	1134 ± 40	
Age-Matched AD; No lobar atrophy/total neuron densities similar to PCA					
NDIV–V n = 7	2M/5F	NA	75 ± 1.8/69 ± 1.7	1155 ± 55	
NDII–V n = 7	5M/2F	NA	80 ± 1.4/71 ± 1.2	1082 ± 39	

Cingulate Damage in Anterior Cortical Atrophy

Two cases of ACA (2 and 3) are presented and one with a CDR score of 1 and early neuropsychiatric symptoms but not a similar level of anterior cortical atrophy. The latter case is of definite AD and its pathology is presented as a possible precursor to that in the more severe cases. None of the three had clinical or neuropathological evidence for Lewy body, Pick or Parkinson diseases or multi-infarct dementia.

Case 1

This patient had early major affective disorder with depression/dysphoria and recurrent thoughts of death, insomnia, decreased appetite, psychomotor agitation and emotional lability. There was impairment in short-term memory and loss of long-term memories relating to personal history and common knowledge as well as impairments in judgment and abstract thinking. There was no evidence of alcohol abuse. Alzheimer's disease onset was approximately at the age of 88 and she died at 95 with adenocarcinoma and a brain weight of 946 g. Her disease lasted for 6–8 years and she had a Clinical Dementia Rating (CDR) score of 1 based on chart review (Morris, 1993).

Case 2

Her disease was announced by verbal abuse, irritability, aggression to family members and later to caregivers and impulsive behavior. Memory difficulties followed and she had a MMSE of 17/30 within about 3 years of onset. During the disease she had fears of falling, early panic attacks, violent temper tantrums, below normal weight, and alcohol addiction problems. She died at the age of 86 of cardiopulmonary arrest and failure to thrive and had a brain weight of 1145 g. The disease lasted clinically for about 10 years and she had a CDR of 2.3 during the last year. This case is of particular interest because it follows the general pattern of Case 1 in progression and because the history of alcohol abuse and low weight suggests involvement of autonomic systems.

Case 3 (FG)

He was an intelligent electrical engineer who experienced severe paranoia at the age of 85. Within 2 years he was evaluated neurologically and had increased problems with spatial disorientation, recent memory and forgetfulness and CT showed mild cortical atrophy and ventricular dilation. At 89 he suffered from mood instability and physical aggression toward his wife and he was over responsive to and agitated by television violence. There was mild psychomotor agitation, some loss of facial expression but speech was at a normal rate, volume, and tone. His thought was severely disorganized with most responses to questions being incoherent and non-sensical and he had visual and auditory hallucinations. His praxis was poor and he could not name colors, misnamed a pen but could name a watch and knew his name. Neuropsychological testing showed a global deterioration in all cognitive domains including attention, memory registration, and recall. There was a decline in total speech output to the point where complete sentences were not used and he frequently spoke with adjectives before his death.

Gross Morphology and Deposition of Marker Peptides in ACA

As reported for Case 3/FG (Vogt *et al*., 1999), Case 2 also had focal prefrontal atrophy that extended into the temporal pole as shown in Figure 35.5A–C. The atrophy is particularly prominent when viewed from the frontal and temporal poles (A.) as well as by the separation in the Sylvian fissure in the lateral view (B.). This pattern of damage is consistent with previous postmortem studies of AD cases that express early psychiatric symptoms including impulse control problems, panic and violent temper.

Since Case 2 had a CDR of 2.3 and Case 1 a CDR of 1, direct comparison of these two cases with the same methods provides insight into how pathology in this subgroup of cases may progress; particularly in conjunction with Case 3 which is much further along, although a CDR score is not available. Although Aβ42 in sACC is about the same in both cases in terms of laminar distribution and approximate density (Fig. 35.5I and E), phosphorylated tau (AT8 antibody) is much lower in Case 1 (Fig. 35.5H and D). Interestingly, a more profound differentiation occurs in PCC where Case 1 has a relatively weak expression of both AT8 and Aβ42 in comparison to that of Case 2. It might be concluded that the progressive impairment of cognitive functions in the cases may be more closely linked to PCC damage than that in ACC.

Deposition of phosphorylated tau and Aβ42 are compared for Cases 1 and 2 for two levels of the cingulate gyrus in Figure 35.5. The following points are about these cases and rostrocaudal differences: (1) Both markers are greatly reduced in dPCC compared to sACC. Indeed, AT8 is generally in localized aggregates with highest densities in ectosplenial area 26, retrosplenial area 29l and the superificial layers of area 30 with less dense aggregates in layers III and Va in parts of areas d23ab. (2) Although AB42 forms a continuous band in dPCC it is quite narrow and limited to layer III, while that in sACC has a more dense band and extends through

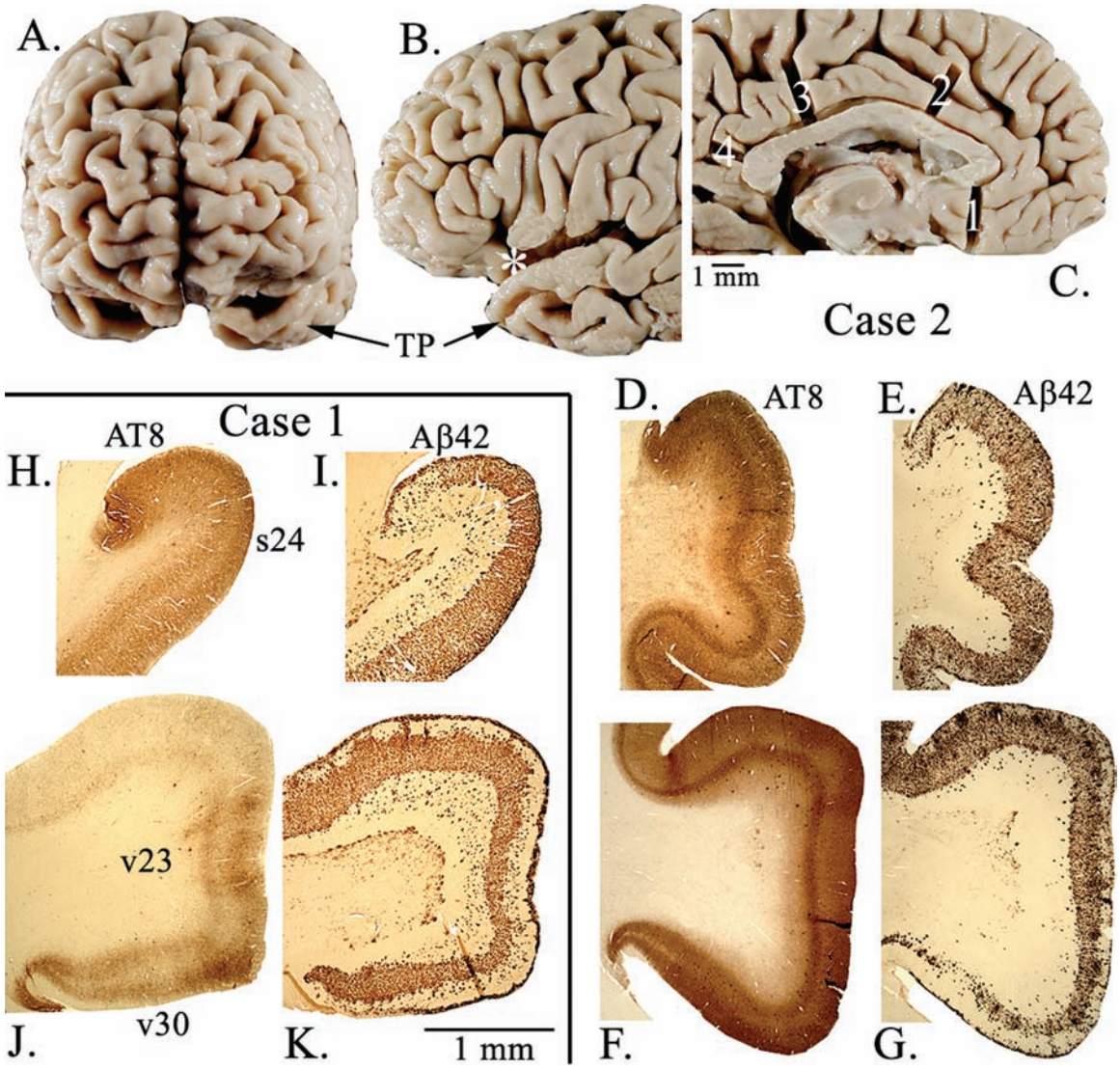

Fig. 35.5 Surface morphology of Case 2 shows the prefrontal and temporal pole atrophy in rostral (A.) and left hemisphere lateral (B.) and medial (C.) views. The numbers in C. identify the site of block removal for histological analysis. Although deposition of Aβ42 in both sACC and vPCC appear similar, AT8 shows a substantially higher level in Case 2 than Case 1 (H. vs D.). Differentiation of both cases with both markers is apparent in PCC where each are at substantially lower levels in Case 1 (J./K.vs F./G.). The sACC is comprised of area 33 near the genu of the corpus callosum and area s24.

layers I–III (there is no layer IV in ACC). At both levels, there is almost none in deeper layers including layer Va as marked with asterisks in Figure 35.6. (3) Case 2 has much greater densities of AT8 immunoreactivity throughout the entire cingulate gyrus, however, the relatively high layer Va density remains and the distinction between superficial and deep layer AB42 remains. As this was also true for later stage case FG (Vogt *et al.*, 1999), it appears that AB42 never invades layers V and VI. (4) Considering aMCC in Case 2 shown in Figure 35.6, there is a very dense deposition of Aβ42 that appears to extend into layer Va; however, there was no AT8 immunoreactivity in this area a24c′.

The wider cortical distribution of disease markers in Case 2 is shown in Figure 35.6. Phosphorylated tau (AT8 antibody) is very high in the CA1 sector of the hippocampus (asterisk), while almost no Aβ42 is expressed in the hippocampus including this sector. The temporal lobe also has very high levels of AT8 including layer V of area 22 (asterisk). There is a moderate level of diffuse Aβ42 in the same cortex mainly in layers I and IIIc–IV. Given the overall density of markers in the temporal lobe it can be expected that early memory changes were due in part to these changes in PCC as noted previously.

An important differentiation of markers occurs in sACC and prefrontal area 46. Both AT8 and Aβ42 are very

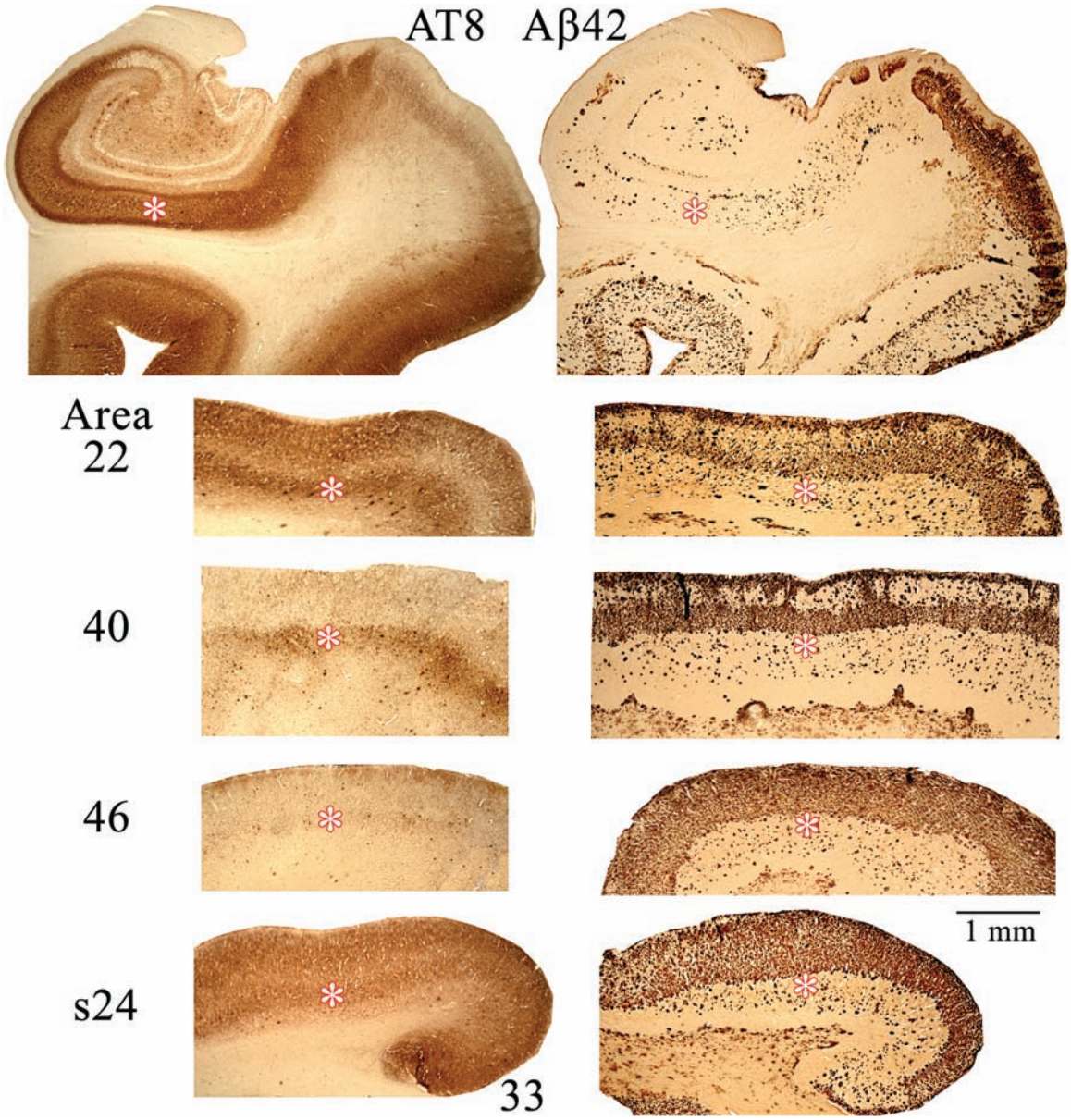

Fig. 35.6 Distributions of phosphorylated tau (AT8) and amyloid-β 42 (Aβ42) in Case 2: The hippocampal formation has extensive AT8 including the CA1 sector (asterisk), while almost no Aβ42 was deposited in this region. The temporal lobe also has very high levels of AT8 including layer V of area 22 (asterisk). An important differentiation of markers occurs in area s24 of sACC and prefrontal area 46. Both markers are very high in sACC, although layer V has high AT8 and low Aβ42 (asterisks). Area 46 has a low level of AT8 expression but very high Aβ42.

high in areas s24 and 33, although layer V has high AT8 and low Aβ42 as noted at the asterisks. Area 46 has a low level of AT8 expression but very high Aβ42 and this is of interest when considering neuron losses in both areas below. Finally, area 40 generally has low levels of AT8 and almost no neurodegeneration. Differentiation of expression of both peptides and neurodegeneration in area 46 but relatively spared neurons in area 40 may lead to understanding the mechanisms of *in vivo* toxicity of these peptides. Although prefrontal atrophy is a general feature of these cases in later stages of the disease, ACC is the most vulnerable to tau phosphorylation and Aβ42 expression and early neurodegeneration.

Laminar Patterns of Cingulate Neurodegeneration, Tau and Amyloid Peptides

An early assessment of large neuron loss and possible links with NFT showed that large neuron density in layers III and V was inversely correlated with NFT density

in cingulate cortex (Mountjoy *et al.*, 1983). This theme was repeated more recently by Chu *et al.* (1991) in sACC, although it has been known for some time that neuron loss is not limited to large neurons but small neurons in layer IV are vulnerable in PCC (Vogt *et al.*, 1990). Furthermore, the pivotal role of neocortical NFT in cognitive decline was demonstrated by Bierer *et al.* (1995) who showed that CDR score is critically linked to the density of NFT in neocortex but not in the hippocampus, amygdala, or entorhinal cortex. Thus, involvement of cingulate cortex is substantially more complex than the large neuron hypothesis suggests and includes different regions, areas, layers and neuron types depending on the earliest symptoms, age of onset, and genetic context. The task of interpreting the mechanisms of neuron death is amplified by the fact that we still do not know the mechanisms of cell death *in vivo*. To make progress on this front, we clarify the detailed laminar relationships of neuron death with disease markers.

Cingulate neurodegeneration occurs early in AD, since three cases within 1–2 years of onset have been reported (Vogt *et al.*, 1998); a few of these cases are shown in Figure 35.3. This latter study demonstrated an association with atrophy in cingulate drawings of neuron somata, NFT formation is associated with neuron loss, and there are groups of cases in which the very largest neurons in layer IIIc of PCC were preserved even when there was severe neurodegeneration in most other layers. Thus, large neurons can be resistant to death. There were also instances where the small neurons in layers II and IV were substantially damaged but no NFT were deposited in these neurons and the large neurons in layers III and V were relatively intact. An evaluation of ACA seeks to interpret some of the complex relationships among, neuron death, deposition of toxic peptides, cingulate regional topography, and disease progression.

Case 1

In addition to the documentation of clinical onset of AD with early major affective disorder, the patterns of cingulate neurodegeneration are compatible with the hypothesis of early sACC damage and less damage in PCC. Interestingly, comparison with Case 3 with overt atrophy suggests that the process of neuron loss in sACC and PCC slows (below). Two figures provide photographic documentations of neuron densities in Case 1 with an age-matched control and laminar co-registrations to PHF and amyloid peptide deposition, while a third documents changes in ventral midcingulate area 33′. Figure 35.7 shows thionin-stained sections from Case 1 compared to NeuN sections in a control because NeuN preparations set the standard for comparison. (Fig. 35.8 compares thionin-stained sections). As is true in earlier work (Vogt *et al.*, 1990, 1998), differences of less than 20 per cent in any particular layer are considered to be part of natural variation.

Neuron losses were substantial throughout area s24b as sites of neuron paucity are evident throughout its depth; asterisks highlight the most pronounced sites in the figure. In keeping with the relevance of this case to ACA, area 46 also has substantial neurodegeneration in layers II, III, V and VI. Amazingly, midcingulate area a24c′ had no apparent neurodegeneration, extensive Aβ42 deposition and almost no PHF immunoreactivity. Indeed, the thionin-stained section in the figure suggests that layer V may be intact in this case in spite of the extension of diffuse Aβ42 into layer Va and dense core plaques in layer Vb. It appears that Aβ42 plays at best a small role in cingulate neurodegeneration in this case. Since the skeletomotor control system was intact, there is a need to explain behavioral control problems in terms of other circuits such as those to the PAG originating in ACC where extensive deafferentation is possible subsequent to layer Va neurodegeneration. Figure 35.7 shows evidence of neuron loss in layers V and VI of areas s24 and d23b and this is shown in more detail at a higher magnification in the next figure.

Neuron loss throughout area s24b is shown again in Figure 35.8 and higher magnification shows some important links with PHF and Aβ42 immunoreactivity. First, layers II–IIIab and Va have numerous PHF-immunoreactive neurons. Second, the pattern of diffuse Aβ42 is homogeneous throughout layers I–III, while dense core-Aβ42-immunoreactiver plaques are most numerous in layers I–II and Va. Third, the MC1 antibody is to an early conformation change of tau and it was only observed in layer V. Fourth, the pattern of Aβ42 is quite different from that in area d23b where layer II is free of the peptide, while in layer V there are almost no dense-core plaques immunoreactive for Aβ42.

Neuron loss and the pattern of PHF in the posterior cingulate gyrus (PCG) raise a number of interesting associations that may reflect early vulnerabilities of this region in ACA. The PHF pattern increases progressively from the fundus of the callosal sulcus onto the gyral surface. Areas 29 and 30 have the overall highest density of PHF, while that in area d23a is lower and in layers II–III and Vb–VI. Also, the higher magnification of area d23b shows that significant neuron loss occurs in all layers with most in layers IV and Va. It is possible that the dense core plaques do not contribute to neurodegeneration in area 23. Finally, although neurons are degenerating in layers IIIc and Vb, the proportion is lower than in other layers.

Although neurodegeneration was generally normal in midcingulate cortex, the report by Williams *et al.* (2005) of atrophy in area 32′ of the behavioral variant of FTD suggested a further consideration is necessary to ascertain the extent to which it can be differentiated

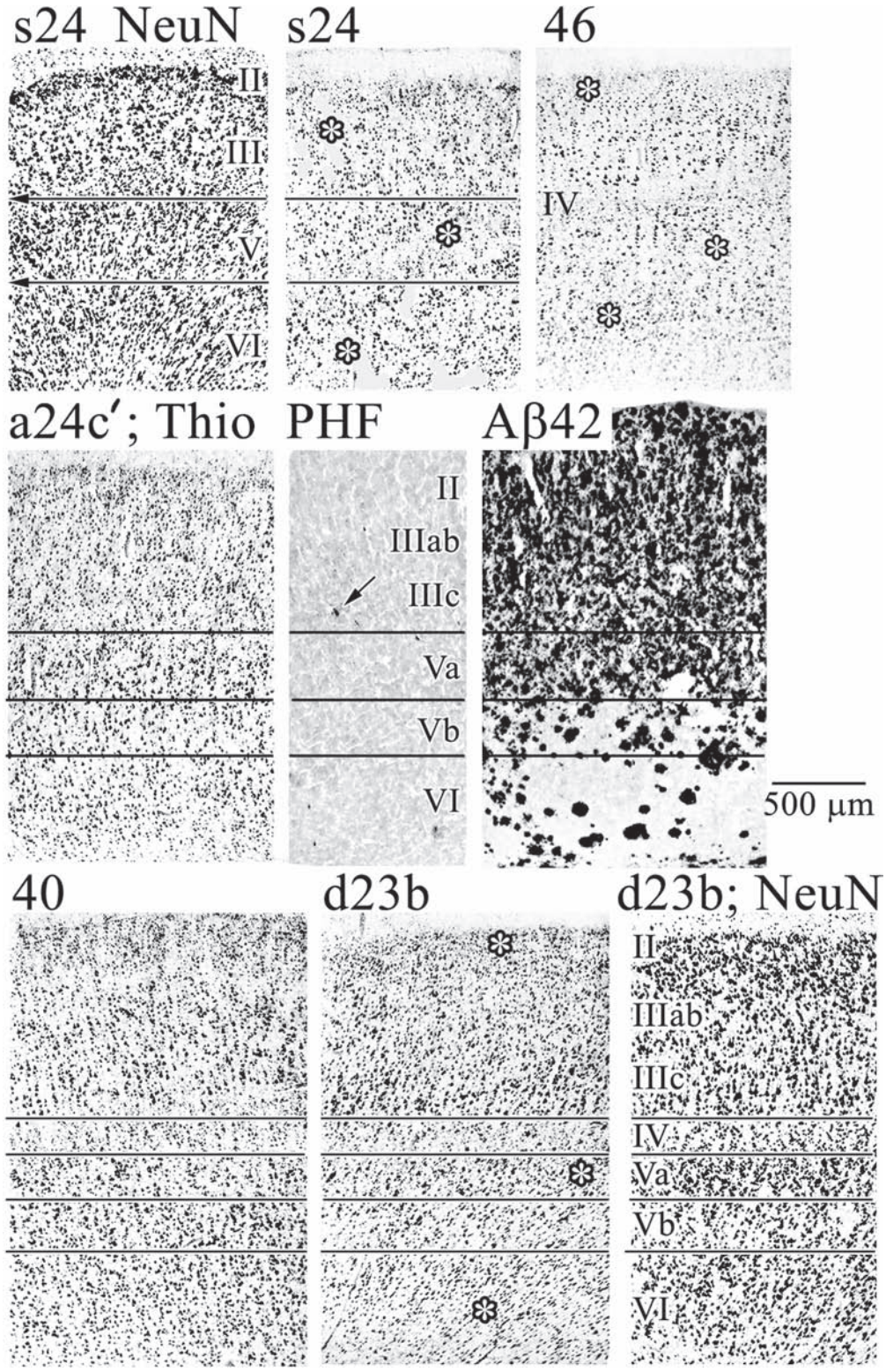

Fig. 35.7 Laminar patterns of neuron losses in Case 1 at three levels of cingulate cortex compared to an age-matched control case reacted for NeuN. Points with greatest neuron losses are marked with asterisks (areas 46, 40 and d23b normally have a layer IV; area s24 does not). Neuron losses were substantial throughout area s24, however, they were mainly in layers Va and VI in d23b with less so of layers II and IV (see Fig. 35.8 higher magnification of layer IV). Midcingulate area a24c′ had no apparent neuron losses, there was no PHF immunoreactivity except in one neuron (arrow), and heavy Aβ42 extended into layer Vb.

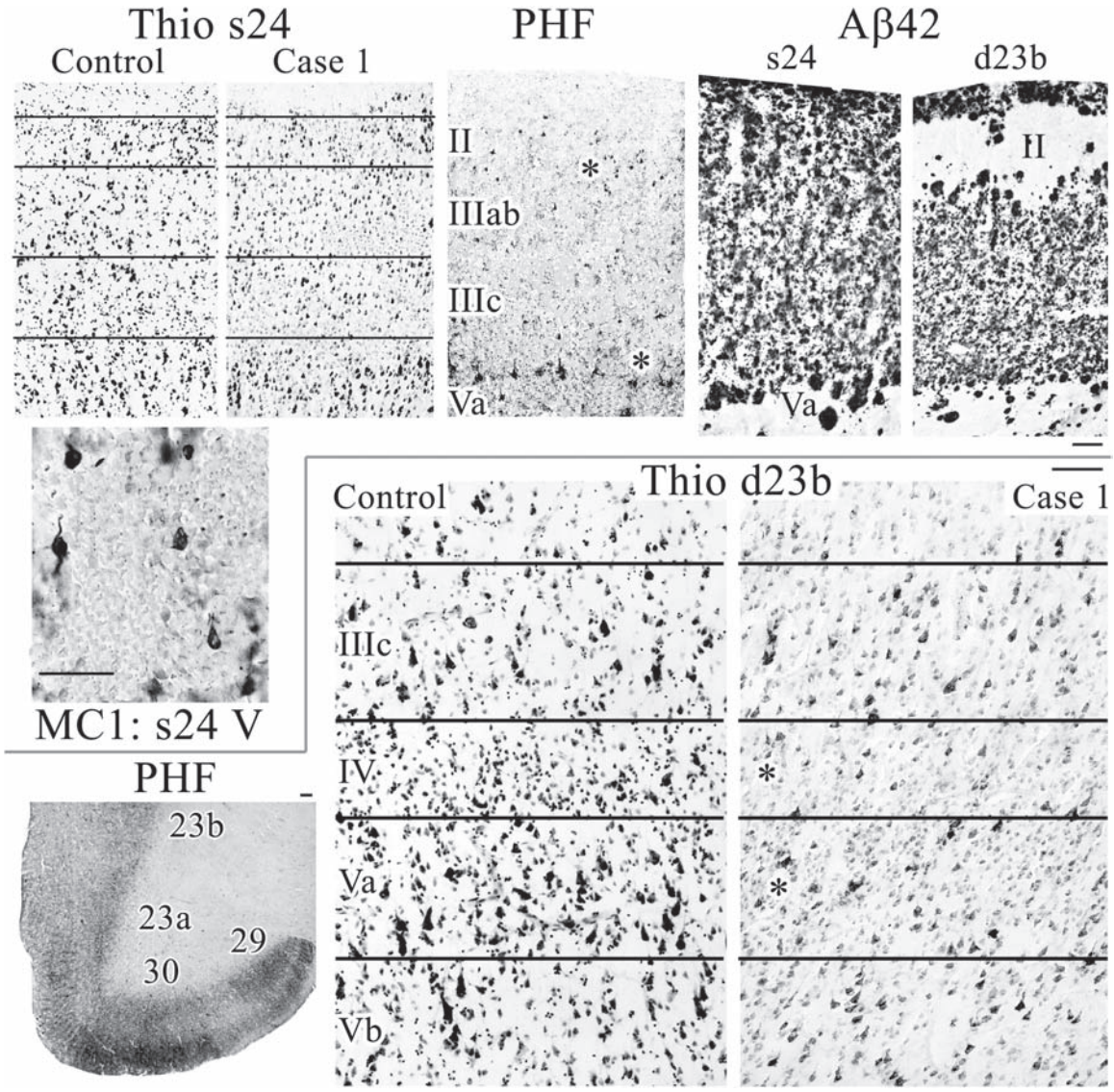

Fig. 35.8 Laminar patterns of neuron loss and disease markers in a control and Case 1. PHF are expressed mainly in layers III and V of area s24b (two asterisks) and Aβ42 is diffuse throughout layers I–V but also in dense-core plaques in layers I–II and V. MC1 was only expressed in layer V. PHF deposition shows a medial-to-lateral progression from highest in the callosal sulcus in areas 29 and 30 to lower levels on the gyral surface. Area d23b shows greatest proportionate neuron loss in layers IV–Va (two asterisks) and less in layers III and Vb. All scale bars = 200 μm.

from AD with similar behavioral presentations. Generally, neuron densities throughout midcingulate cortex appeared normal including area 32′. The density of diffuse, Aβ42-immunoreactive plaques was the same as in other cortical areas and there were almost no neurons with PHF. Review of area 33′ just dorsal to the corpus callosum, however, showed a high level of PHF-immunoreactive neurons and neuropil threads as shown in Figure 35.9. In this area the plaques were more dense and there was a generally lower level of diffuse Aβ42. Nevertheless, neuron densities appeared normal and at higher magnifications there was no evidence of intracellular inclusions such as Lewy bodies.

Case 3

The presence of ACA in Cases 2 and 3 in itself indicates a mid-to-late stage of AD. Case 3 provides a number of interesting findings to elucidate some events. First and to the extent these cases are part of a single temporal continuum, neurodegeneration appears to have stabilized in area s24. Indeed, degeneration in layers V and VI is not as severe as in Case 1 as shown in Figure 35.10.

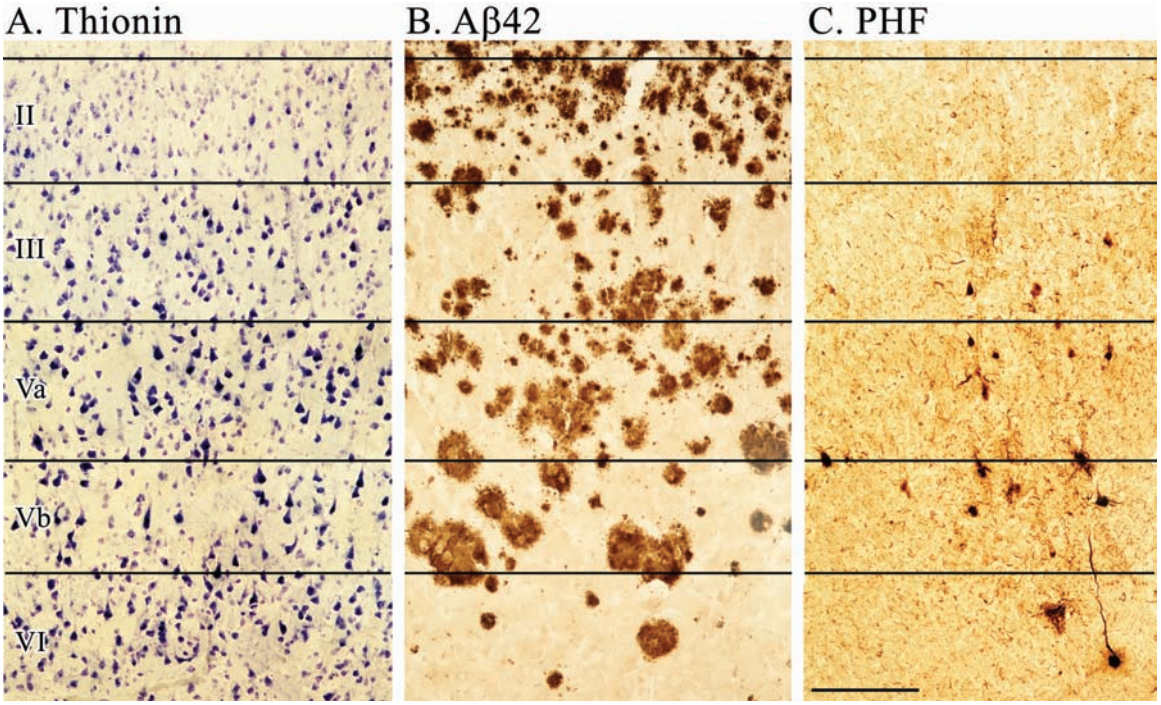

Fig. 35.9 Area 33′ of midcingulate cortex had the highest density of PHF-immunopositive neurons and neuropil threads (C.). Although neuron densities (A.) appeared normal for each layer, Aβ42-immunoreactive plaques (B.) were primarily dense core with some large diffuse plaques. Overall, the amyloid load is substantially lower in area 33′. Scale bar = 200 μm.

Non-phosphorylated-neurofilament protein expression is almost abolished in layers V and VI of area s24b as shown in the figure with the SMI32 antibody counterstained with thionin. Only few filamentous dendrites can be detected with the SMI32 antibody (arrows in Fig. 35.10). When compared to the control level of neurofilament proteins, expression of these proteins is almost completely abolished.

Case 3 also emphasizes that area d23 is at high risk for persistent neurodegeneration during the course of ACA and Figure 35.10 shows severe neuron loss in layers IV and Va with lesser amounts in other layers. Midcingulate neurodegeneration also is greater than in Case 1 (not shown). Thus, progressive neurodegeneration occurs in MCC and PCC in ACA and is not limited to ACC.

Case 2: Subcortical changes may contribute to impulsivity, verbal abuse and aggression

Although AD is likely a primary cortical neuron disease, degeneration of projections into subcortical structures must follow loss of deep layer V and VI projection neurons. In addition, there have been reports of typical AD (i.e. no selection according to lobar atrophy) of neurofibrillary degeneration in some midline thalamic nuclei, amygdala, nucleus basalis of Meynert, ventral tegmental area, dorsal raphe, and locus coeruleus (McDuff and Sumi, 1985; Sparks *et al.*, 1988; Kromer *et al.*, 1990; Braak and Braak, 1991, 1993; Tomlinson, 1992) and we have explored this issue in Case 2. To the extent that emotional motor systems are dennervated, it is likely that cingulate regions projecting to them likely contribute to impulse control problems, aggression and dysautonomia. Indeed, sham rage and other behavioral impairments have been evoked by electrical stimulation and small strokes in some parts of the emotional motor system such as the periaqueductal gray (Bandler, 1982; Bandler *et al.*, 1991; Depaulis and Bandler, 1991). Sham rage for example, involves excessive responsiveness to non-threatening stimuli that is an exaggerated responsiveness out of the context to the threat level. The rationale for this consideration is not yet to provide detailed mechanisms but rather to emphasize the crucial nature of deafferentation of these structures in ACA and the potential role of ACC therein.

Case 2 samples of the amygdala, hypothalamus, midbrain, and pons showed significant astrocytosis, neuron losses and NFT. Indeed, there were almost no neurons remaining in the locus coeruleus and those that were present were swollen and had condensed chromatin. Figure 35.11 shows some of the subcortical structures with neuron loss and NFT in the posterior ventromedial

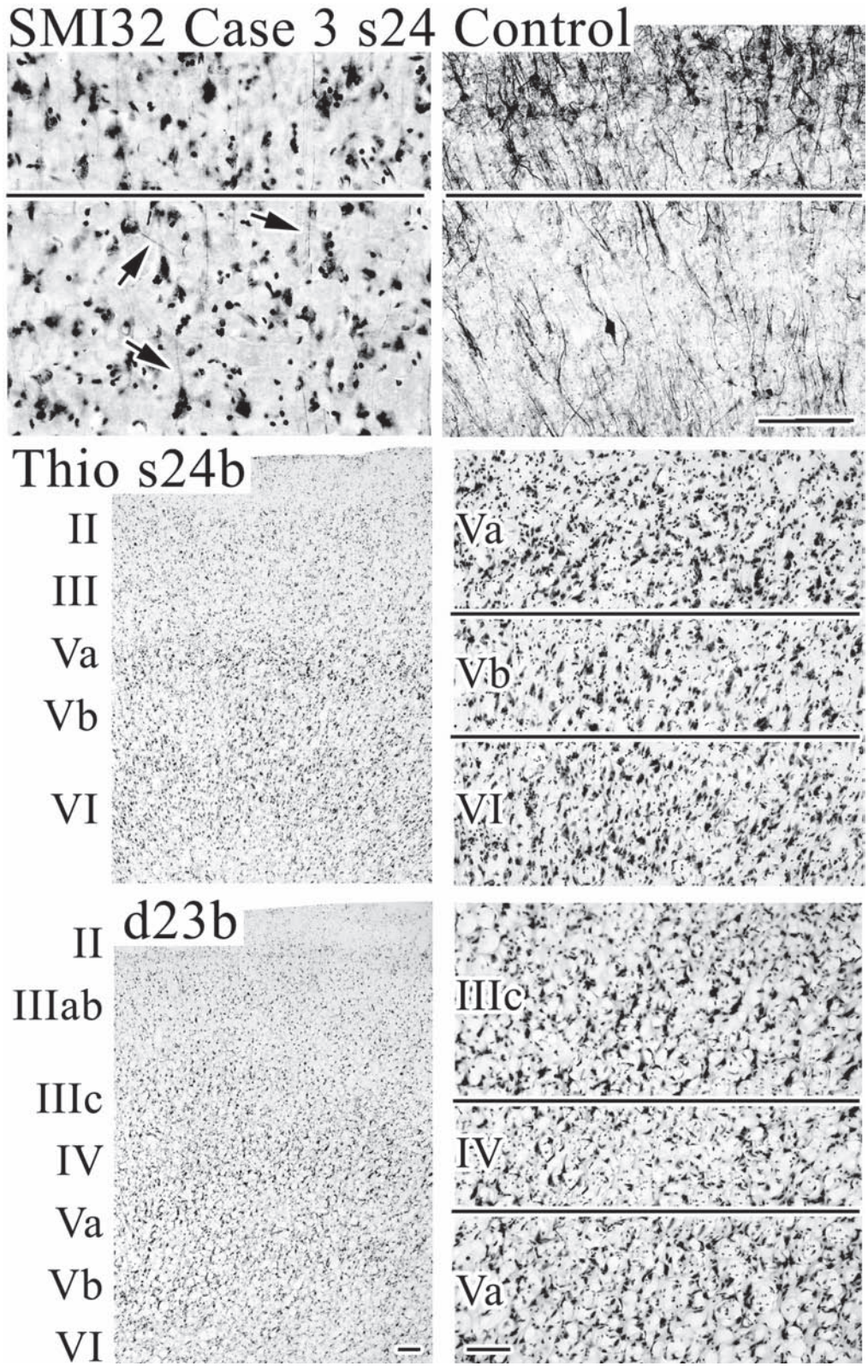

Fig. 35.10 Neurofilament protein expression and neurodegeneration in Case 3. SMI32 immunoreactivity is almost abolished in Case 3 when compared to a control case. The thionin counterstain of area s24 shows that even 'intact' neurons fail to express these proteins. Three arrows emphasize a few thin dendrites that still express the antigen. Although neuron loss is substantial in both sACC and dPCC, neurons in layers IIIc, IV and Va are significantly lost in area d23b. All scale bars = 200 μm.

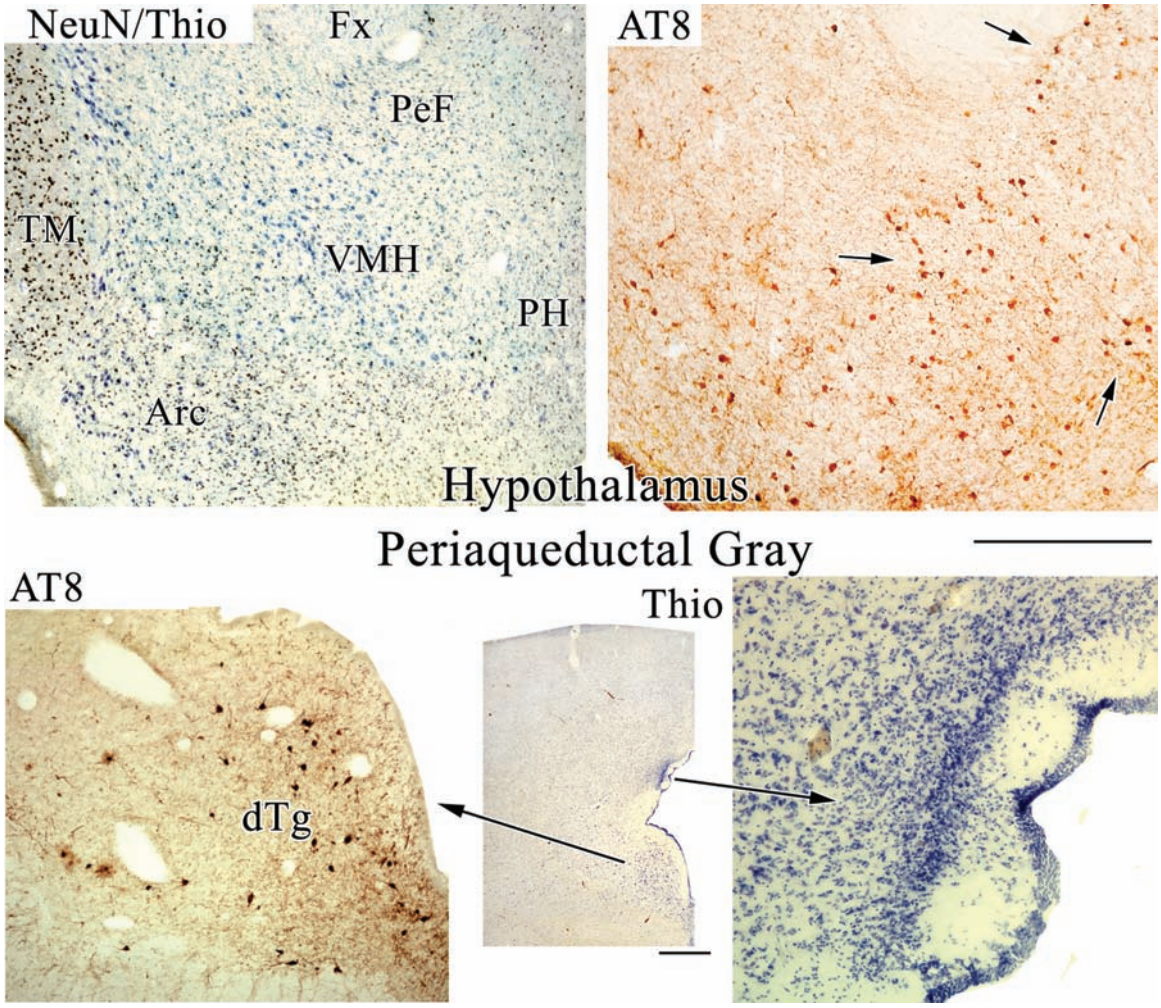

Fig. 35.11 Hypothalamic and midbrain structures in Case 2 showing substantial damage to the ventromedial hypothalamus (VMH), dorsal temental nucleus (dTg) and periaqueductal gray.

and perifornical hypothalamus and dorsal tegmental nucleus. An aberrant aggregation of neurons is apparent in the periaqueductal gray. Whether or not this is a pre-standing impairment or an active site of neurodegeneration cannot be determined and no markers were expressed by neurons in this region. There were some neurons expressing NFT throughout the remaining periaqueductal gray but at a low level. This pattern of subcortical damage may have contributed to the patient's symptoms including irritability, aggression, below normal weight, and difficulties regulating alcohol consumption.

Although a series of cases with a range of CDR scores will be necessary to evaluate the detailed progression in subcortical damage, there can be little doubt that as layer V projection neurons in sACC degenerate, and these nuclei are severely deafferented and remove cortical regulation that guides reflexive behaviors in specific contexts. These projections are reviewed in Chapters 10 and 15 but a few should be emphasized in the framework of ACA. First, the descending cingulate projections to the locus coeruleus have been shown in monkey and cat (Room *et al.*, 1985; Chiba *et al.*, 2001). Second, descending control of emotional motor systems for vocalization are critical for coordinated and goal-oriented behavioral output and the ACC is a pivotal player in selecting among behavioral outputs and emotional expression via its descending output systems. Layer V neurons of the ACC project to the periaqueductal gray (Müller-Preuss and Jürgens, 1976; Hardy and Leichnetz, 1981; Mantyh, 1982). Disease onset in Case 2 was announced by verbal abuse and aggression toward family members. The full organization and projections of the cingulate vocalization region in ACC are considered in Chapter 15 and descending control of vocalization is well established (Jürgens and Pratt, 1979; Vogt and Barbas, 1988). The periaqueductal gray also regulates

freezing and conditioned fear responses (De Oca *et al.*, 1998) and for these and many other reasons, it has been viewed as pivotal in coordinating emotional motor system outflow (Holstege *et al.*, 1996). Third, projections of ACC to the amygdala are involved in regulating responses to fear (Chapter 9) and visceromotor outputs (Neafsey *et al.*, 1993; Chapter 10). Finally projections to the hypothalamus provide another means of direct regulation of autonomic output by cingulate cortex; both hormonally and via projections of the hypothalamus to the periaqueductal gray (Chapter 22).

It is important to recognize that the amygdalar, hypothalamic and midbrain projections of ACC are not made by the PCC and this provides a pivotal dissociation between the clinical outcomes of ACA and PCA. Damage (lesion, stroke or neurodegeneration) in PCC will not have the same subcortical behavioral discontrol effect as that in ACC. The ACC has a wealth of descending projections to regulate many behavioral and autonomic outputs associated with emotional expression in relation to internal needs and anticipated outcomes. These projections regulate the pre-wired output of the brainstem emotional motor systems to produce context relevant behavioral output. The ACA variant of AD shows substantial impact of these systems at the cortical and brainstem levels of functional organization.

Finally, subcortical pathology is associated with changes in the cerebral cortex, although it is not clear to what extent the former generates the latter. Loss of cholinergic neurons in the basal forebrain in AD is associated with disease duration (Mufson *et al.*, 1989) and choline acetyltransferase activity is reduced throughout the cerebral cortex including the cingulate gyrus (Procter *et al.*, 1988). The binding of tritiated-oxotremorine-M in the presence of unlabeled pirenzepine showed that cingulate cortical binding to M2 sites was increased in some neurodegenerative classes of AD and the increase was greatest in layer V. Moreover, the increase was linearly and negatively correlated with neuron densities in layer Va in all AD cases but was enhanced according to which cortical layers had the greatest neurodegeneration (Vogt *et al.*, 1992). Although the ligand binding work was done in area d23, a similar pattern likely occurs in layer V of ACC in view of the diffuse loss of choline acetyltransferase activity throughout the cingulate gyrus. Furthermore, cases with elevated M2 binding experienced significantly higher levels of binding to β-adrenoceptors (Vogt *et al.*, 1991) implicating other subcortical structures. Neurodegeneration in AD has been reported for the locus coeruleus (Busch *et al.*, 1997) and raphe nuclei (Halliday *et al.*, 1992); indeed, the locus coeruleus could hardly be identified in Case 2. Although degeneration in the raphe was significantly greater in patients experiencing a rapid progression of symptoms, no information is available relating to specific classes of cortical neuron degeneration. In contrast, a positive relationship has been reported between aggressive behavior and the magnitude of rostral locus coeruleus cell loss and a reduction in norepinephrine concentration correlated with cognitive impairment (Matthews *et al.*, 2002). Thus, subcortical systems contribute to cingulate reorganization in typical AD including enhanced binding to postsynaptic receptors in cholinergic and adrenergic systems and this likely relates to classes of neurondegeneration; however, involvement of these systems in ACC still needs resolution as do links to ACA.

Laminar links between neuron death and disease markers and progression

There is little doubt that large, layer Va neurons in sACC are at the highest risk in ACA. They express a high level of PHF and are the only neurons with MC1 immunoreactivity. This layer has the highest density of Aβ42 in dense-core plaques, although high levels are also present in layers I–II. Although the severe reduction in neurofilament protein expression was shown in a late stage case, it is likely the inactivation of neurofilament protein synthesis in layer V occurs much earlier. Indeed, it is possible that this impairment alone disrupts the function of subcortical projections emanating from layer V neurons. A similar inactivation of neurofilament protein expression in late AD has been shown in areas 9 and 20 as well (Hof *et al.*, 1999; Hof, 2001). Neurofilament expressing neurons, however, are not the only ones to undergo degeneration. Indeed, neurons in layer III of area s24 express very little of these proteins yet they are vulnerable as are layer IV neurons in dPCC that also do not express them. Thus, other factors likely contribute to impaired neuron functions and overt neurodegeneration throughout the cingulate cortex in ACA.

Since neuron densities in layer V were relatively normal in MCC in Case 1 in the face of very high levels of Aβ42, it is possible that Aβ42 plays little or no role in neurodegeneration throughout the cingulate gyrus and, in the deep layers, may only play a minor role. This interpretation is supported by a unique case reported by Delaère *et al.* (1990) who observed that a psychometrically normal individual with a Mini-Mental Score of 27 had extensive amyloid deposits throughout neocortex and no evidence of neurofibrillary degeneration. Although they did not evaluate neuron densities, this case confirms the general lack of *in vivo* neurotoxicity of amyloid peptides. McKee *et al.* (1991) also observed non-demented patients with high amyloid peptide loads and no neurofibrillary degeneration. Finally, Bierer *et al.* (1995) showed that CDR score is not significantly linked to senile plaque densities in any region. Although none of these findings preclude a contribution

of amyloid peptides to neurodegeneration in some cortical areas in AD associated with other mechanisms of neuron loss, it does cast doubt on a necessary contribution of these peptides to neurodegeneration in all layers and areas.

Another view of functional inactivation is provided by dPCC. In area d23b there is a loss of deep layer neurons but it should be realized that a substantial loss of neurons in layer IV could seriously disrupt output from this area as many of the thalamic afferents to PCC terminate in this layer (see Chapter 4) and loss of neurons in this layer is essentially a thalamic and cortical deafferentation lesion that removes control of area 23 from the pulvinar and lateral posterior thalamic nuclei and multisensory processing centers in the parietal and temporal lobes. Since these neurons do not form NFT, many studies using this marker overlook this aspect of cingulate pathology in AD. The impact of ACA on PCC likely is associated with other deficits such as memory retrieval and visuospatial functions.

Although there are still only a few cases of ACA that thoroughly assessed cingulate cortex, it is possible to speculate on the progression of neurodegeneration in these cases and the secondary consequences of degeneration of subcortical projections. Figure 35.12 provides a summary of two general stages of cingulate neurodegeneration in ACA and the secondary deafferentation of pathways to subcortical structures. At the earliest

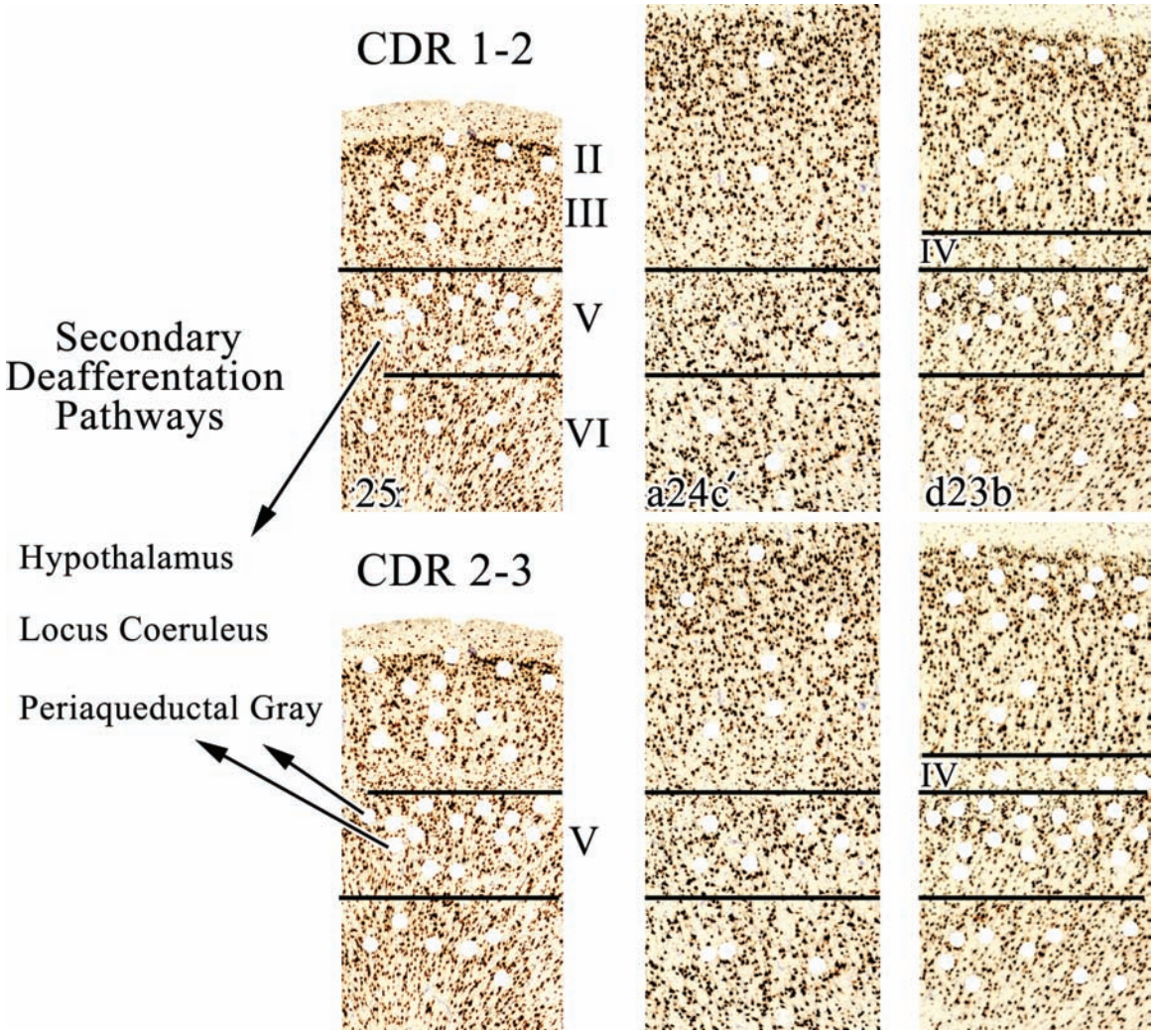

Fig. 35.12 Strips of NeuN reacted areas s24 (sACC), a24c′ (aMCC) and d23b (dPCC) used to remove neurons in a qualitative summary of the progression of ACA. Pivotal to the mood changes in ACA is destruction of layers II–V projection neurons and the relative sparing of MCC confirms the recruitment for subcortical control of emotional behaviors; dPCC has no such connections. Later stages show loss of layer V neurons and their projections as secondary deafferentation of subcortical structures that mediate autonomic and skeletomotor reflexes through the emotional motor systems. Also, PCC shows continued neuron loss and suggests that impairment of memory and visuospatial functions can be partially attributed to this damage in ACA.

stages substantial neuron inactivation and loss occurs in layer V of both ACC and PCC with less in superficial layers. At this point there appears to be almost complete loss of neurofilament protein expression in layer V and a serious functional impairment of ACC output to deep lying structures such as the hypothalamus, locus coeruleus and periaqueductal gray. Although there is a limited loss of MCC neurons, this increases later in the disease along with damage to other cingulate areas. The damage to cingulate cortex appears to stabilize in cases with CDR scores of 2–3. The cooperative imbalance of ACC and PCC information flow suggested in Figure 35.2 as well as impaired descending motor output leads to a loss of cortical control over emotional motor systems as well as loss of self-relevant sensory information flow via PCC to ACC. These events may combine in later stages of the disease to produce uncontrolled vocalization, aggression and impulse dyscontrol symptoms.

Posterior Cortical Atrophy and PCC in Visuospatial Impairments

Patients with typical AD and spatial disorientation for time and place are hypometabolic in PCC (Hirono *et al.*, 1998) and this impairment is associated with NFT in a number of visual areas and area d23d (Giannakopoulos *et al.*, 2000). The visual impairments expressed in typical AD include reading disturbances, becoming lost in previously familiar places, and interpreting complex visual scenes have been known for some time (Furey-Kurkjian *et al.*, 1995). However, when the visual disturbances occur before or during first signs of memory impairment and result in an ophthalmologcial examination first rather than a neurological examination, a lobar pattern of degeneration has been reported. Visual disturbances in this AD subgroup include visual agnosia and simultanagnosia (Sadun *et al.*, 1987; Kiyosawa *et al.*, 1989; Mendez *et al.*, 1990), spatial disorientation (Henderson *et al.*, 1989; Graff-Radford *et al.*, 1993) and impairments in oculomotor function (Hutton *et al.*, 1984). Patients with these impairments have posterior cortical atrophy (Benson *et al.*, 1988; Graff-Radford *et al.*, 1993).

There is extensive disruption of visual cortical circuits in AD cases with PCA (Hof *et al.*, 1989, 1993, 1997; Hof and Bouras, 1991). This includes high lesion counts (NFT and senile plaques) in primary and secondary visual areas and visual association areas that are not usually observed in typical AD. In contrast, areas 9 and 20 are less involved than is true for typical AD without prominent visual symptoms and PCA. It has been proposed that the symptoms in these cases reflect a visual disconnection syndrome in which association pathways originating in the corticocortical processing streams from neurons mainly in layer III but also layer VI Meynert cells that project to motion processing areas such as area MT, MST and parietal cortex (Hof *et al.*, 1989, 1993).

In the same framework of corticocortical denervation is the severe disruption of PCC and RSC also reported in postmortem studies (Hof *et al.*, 1993; Vogt *et al.*, 1997, 1999). Without specific neuropsychological tests for PCC/RSC functions, it is not possible to disentangle the contributions of cingulate regions to clinical impairments of visual functions. Nevertheless, predictions from the four-region neurobiological model suggest that disruption of sensorimotor integration, premotor planning and other executive functions of cingulate cortex contribute to aspects of this syndrome. Two visuomotor processing streams are summarized in Figure 35.3 including the dorsal sensorimotor stream that is focused on projections into the caudal cingulate premotor area in areas 24d and 23c and the ventral multisensory stream that interacts with ACC to engage context and valence dependent sensory information (personally relevant sensations), episodic emotional memories in ACC, and regulation of affect and autonomic outflow. Chapter 13 has a detailed consideration of PCC and its connections in sensorimotor and emotional functions. Thus, impairments in both of the intracingulate processing streams are consistent with the symptoms experienced by AD patients with PCA.

Histopathology of Posterior Cortical Atrophy

Complex biological diseases express so many phenotypical changes that no one or two variables can be used to identify groups of cases with common etiologies. Analysis of breast cancers, for example, with DNA arrays reveal many forms of the disease and symptom clusters represented with eigenvector projections serve as a basis for treatment (Marx, 2000). Salmon *et al.* (2007) and the previous chapter consider the multivariate framework for neuroimaging in AD and, as already argued above, neurodegeneration in AD is effectively described by multivariate models that include the number of neurons in 'descriptive' layers; that is to say, layers that have unique involvements in one subgroup and play an important role in differentiating some aspect of a subgroup's variance. For example, if the density of neurons in layer II are included in the model as was explored (Vogt *et al.*, 1998), there is no improvement in differentiating any subgroup. Neurons in area d23b layers IIIab, IIIc, IV, and Va are the ones that provide the most effective differentiation of AD cases with the fewest number of variables. Disease onset is another possible variable of interest; however, since there is a disease progression in each subgroup, this information provides no additional value to the model.

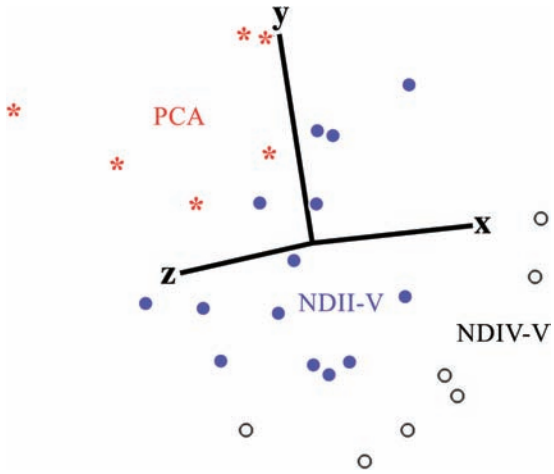

Fig. 35.13 Principal components analysis of six cases of PCA in AD and two other groups that had similar neuron losses and were age matched. The PCA group resides in a unique part of the eigenvector projection supporting the proposal that a unique laminar pattern of neurodegeneration occurs in this subgroup.

To evaluate the extent to which cases of PCA represent a truly unique subgroup of AD, neuron densities in layers III–Va were entered into a multivariate model and compared to AD cases with the same age at death and the same density of neurons in area 23b. Certainly, cases with less severe or more severe neurodegeneration will identify PCA as a separate group and matching for age and neuron density provides a higher standard against which to test the subgroups hypothesis; i.e. a unique laminar pattern of loss rather than simply a quantitative difference in neuron densities. Six cases of PCA with cases of AD that have a similar level of neurodegeneration in PCC and age-matched were selected to enter into the multivariate analysis; one case was an outlier with mean neuron densities of 3 standard deviations from the mean for all cases. As done previously (Vogt *et al.*, 1999), neuron densities in layers IIIab, IIIc, IV, and Va were entered into a principal components analysis. The eigenvector projection is shown in Figure 35.13 and confirms that this is a unique subgroup in terms of the laminar pattern of neuron loss. As noted below in Table 35.2, the difference between the PCA and NDII–V was based on

TABLE 35.2 Area d23b

Neuron densities	**PCA**	**%Con**	**NDII–V**	**%Con**	**Control densities**
IIIab	29 ± 5.9	43%	32 ± 2.2	47%	68 ± 3
IIIc	34 ± 3.5	56%	34 ± 1.2	56%	61 ± 3
IV	31 ± 2.4	**57%**	21 ± 2.1	**39%**	54 ± 3
Va	29 ± 2.8	**60%**	20 ± 1.8	**42%**	48 ± 4
Amyloid peptides	**Dense**	**Diffuse**	**Dense**	**Diffuse**	
Aβ40 IIIab	99 ± 13	13 ± 2	60 ± 13	18 ± 4	
IIIc	**103 ± 12**	14 ± 2	**46 ± 6**	19 ± 4	
IV	**66 ± 9**	14 ± 2	**32 ± 6**	18 ± 4	
Va	34 ± 4	7 ± 2	29 ± 7	7 ± 2	
Aβ42 IIIab	108 ± 15	28 ± 4	92 ± 17	35 ± 4	
IIIc	117 ± 25	30 ± 4	98 ± 13	37 ± 3	
IV	96 ± 21	31 ± 3	97 ± 17	37 ± 4	
Va	35 ± 9	10 ± 2	25 ± 4	12 ± 3	
Aβ43 IIIab	***112* ± 25**	14 ± 2	***63* ± *21***	9 ± 3	
IIIc	***70* ± *17***	3 ± 2	**33 ± 9**	8 ± 3	
IV	***53* ± *10***	12 ± 2	***17* ± 5**	7 ± 2	
Va	25 ± 6	8 ± 1	18 ± 5	5 ± 2	
Aβ43 in Layer IIIc					
Mean years from onset; *n* = 2/group	**5.5 years**	**9 years**	**12 years**		
Aβ40	116	116	113		
Aβ42	160	160	71		
Aβ43	104	75	61		

significant losses on neurons in layers IV and Va in the latter subgroup (bold in table), while changes from control values were relatively uniform in layers IIIab–Va.

Amyloid Peptides in PCA and Excess Aβ43 Deposition

Having identified the PCA group as unique in terms of its laminar pattern of neuron losses, one of two neuropathological hypotheses can be considered:

1 PCA is a subgroup of cases in which AD is initiated in different areas and layers, while the fundamental etiology of neurodegeneration is the same as that in other subgroups. This is essentially the null hypothesis; i.e. different place but same mechanism of neuron death.

2 PCA is a subtype of the disease because the variation in laminar pattern of neurodegeneration implies a different mechanism of neurodegeneration. Confirming a unique mechanism of neuron death in itself requires rejecting the first hypothesis particularly in light of the fact that the comparison AD cases were matched for age and total neuron degeneration. Since we have evidence that the latter is true, we begin with an assessment of amyloid peptide expression and then consider the laminar patterns in neuron losses with which they are associated.

Figure 35.14 shows coronal sections of amyloid peptide immunohistochemistry in the dPCG from one example of PCA and one age-matched AD case from the NDII–V subgroup. The densities and laminar

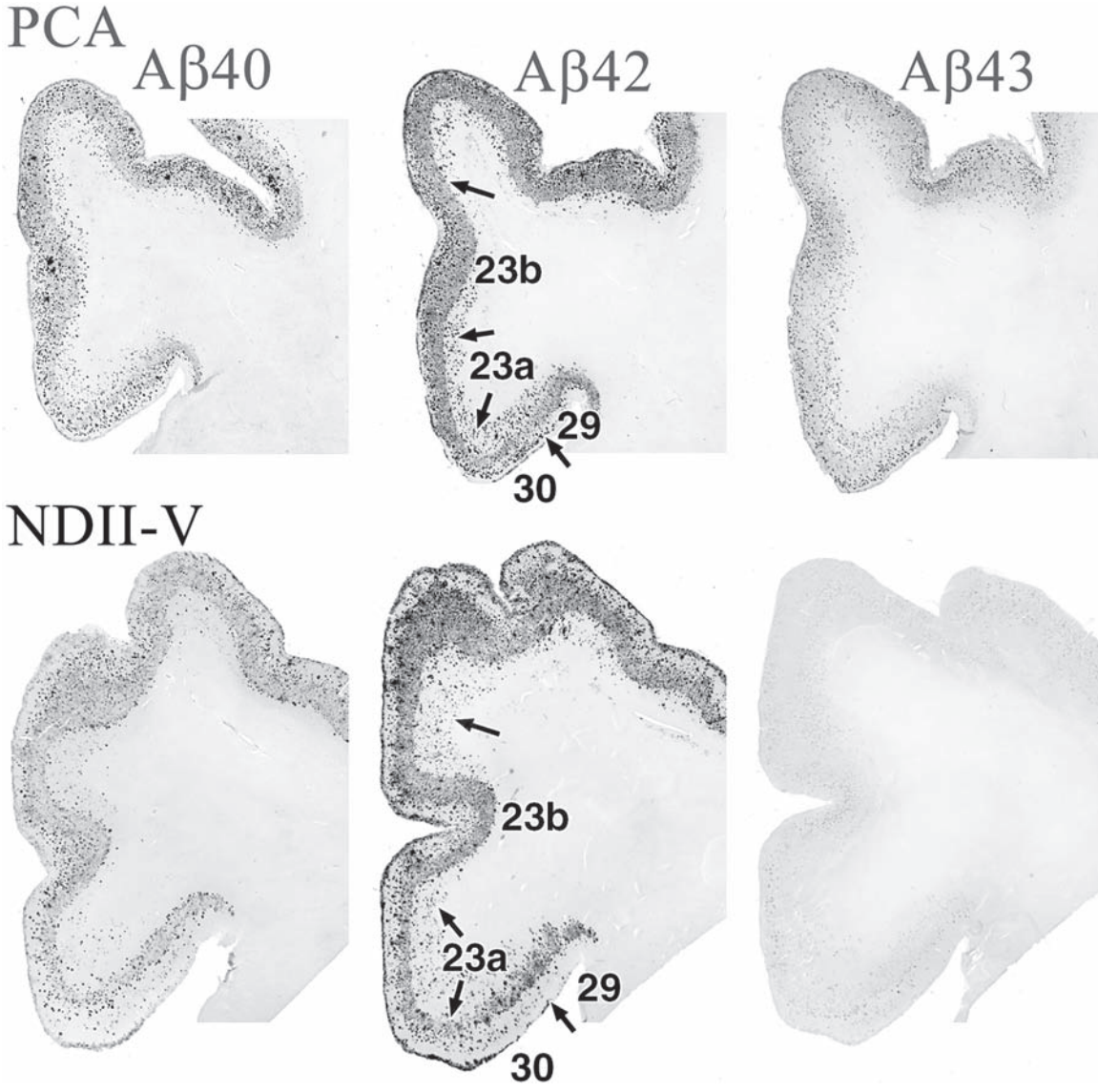

Fig. 35.14 Comparison of dorsal posterior cingulate gyrus in a PCA and age/neuron density-matched AD case with no evidence of specific lobar atrophy from the NDII–V subgroup.

distributions of Aβ40 and Aβ42 appear approximately similar in both cases, while that for Aβ43 is two to three times greater in PCA. Quantitative assessment of the optical density of each of these three peptides in each layer of area d23b is provided in Table 35.2. The average density of Aβ43 in PCA was twice that for NDII–V confirming the qualitative impression from each case individually. There was also a factor of two difference in Aβ40 in PCA but only in layers IIIc and IV. Finally, averaging of two cases per group based on the estimated duration of the disease showed that the highest level of Aβ43 was encountered early in the disease at 5.5 years duration (Table 35.2). While Aβ40 and Aβ42 were generally stable, that for Aβ43 decreased in density with durations of up to 12 years. This suggests that, if Aβ43 has neurotoxic properties or signals a common mechanism of neuron death, it may have a more prominent role early in the disease and this highlights the potential value of understanding the unique role of Aβ43 in the etiology of PCA in AD.

Parvathy *et al.* (2001) evaluated the three length Aβ peptides in relation to consensus CDR scores and observed that all, including Aβ43, were significantly linked to these scores. Of course, the CDR score at death may not be related to the estimated duration of the disease as used here and the tissue was sampled from prefrontal area 9 rather than cingulate cortex. These two studies together suggest that Aβ43 is clinically linked to disease symptoms, although peptide density decreases with disease duration.

Laminar Patterns of Neurodegeneration and Marker Peptides in PCC

A major difference between the PCA cases and the NDII–V shown in Table 35.2 is the increased loss of neurons in layers IV and Va. This loss extends throughout all deep layers as shown for an example of each group in Figure 35.15, where the brackets emphasize this difference; neurodegeneration occurs to a similar level in

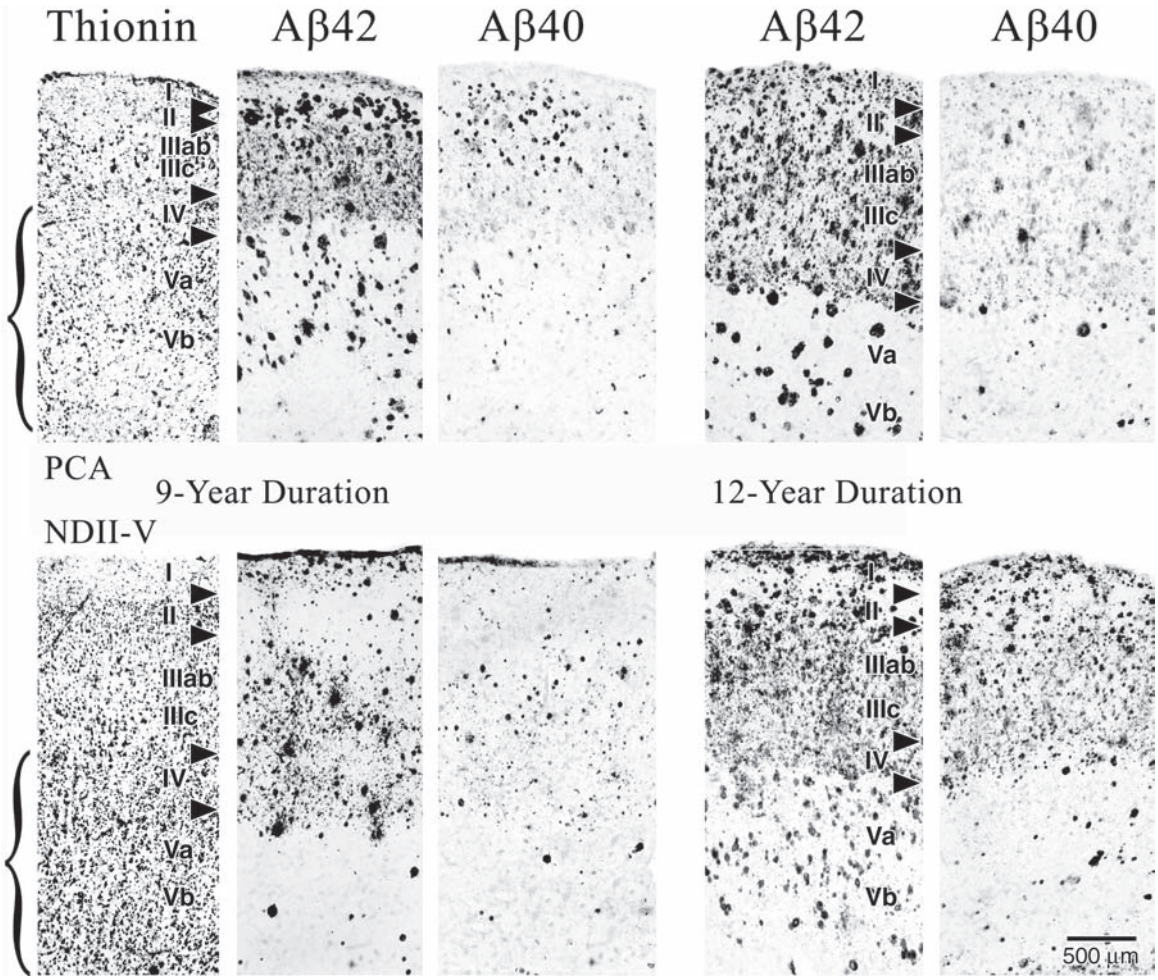

Fig. 35.15 Examples of PCA and disease duration-matched NDII–V cases. Amplified loss of deep layer neurons in PCA (brackets) is seen. Higher levels of Aβ40 and progressive increase in load of both peptides occurs.

superficial layers. Differences in disease duration suggest that in both subgroups there is a widening of the extent of superficial layer expression of amyloid peptides and in the NDII–V subgroup there is an increase in the overall load of expressed peptides.

The lateral surface atrophy in a case of PCA is shown in Figure 35.16 because that on the medial surface is less prominent. The AD2 antibody to hyperphosphorylated tau shows a progressive buildup in the PCG with greatest deposits in retrosplenial areas 29 and 30, less throughout area 23 and less in medial parietal cortex area 7 m. The three magnified histological photographs are of mid-layers of area 23a and show prominent deposition of tau in layer Va (AD2) and substantial loss of neurons in layers IV and Va compared to a control case (asterisks). Thus, severe neurodegeneration in layers IV and Va in dPCC is characteristic of PCA and the heavy burden of hyperphosphorylated tau may be directly linked to this deep layer degeneration.

Amyloid Peptides in ACC and MCC

Although PCC and RSC have high levels of neurodegeneration and amyloid peptides, this damage is not limited to these regions. Also, the last case of early visual impairments shows a profound influence on PCC where PCA may begin with a 'seed' in the posterior region but it also extends further rostral into other cingulate regions as shown in PCA. Figure 35.17 shows immunohistochemistry of the three amyloid peptides in ACC and MCC. Area 24 and area 24′ have high levels of amyloid deposition including the elevation of Aβ43 shown above for PCC. Neuronal damage is demonstrated with Aβ42 which extends into layer V of area 24d where there are examples of large neurons with intracellular deposits (two circles in Fig. 35.17F). As discussed in Chapter 5, layer V contains cingulospinal projection neurons and these neurons may be at risk in PCA. Indeed, it is possible that damage to these and other motor system projection neurons contribute to impairments of oculomotor behaviors in terms of premotor planning and coordination. Interestingly, a PET study of AD patients with and without visual symptoms showed no difference in these groups in PCC but significantly reduced glucose metabolism in ACC (Pietrini *et al.*, 1996). A similar comparison showed involvement of visual association areas and inferior temporal cortex.

These findings together suggest an involvement of ACC and MCC in an underlying decision-making process suggested by the four-region neurobiological model shown in Figure 35.3. Pietrini *et al.* (1996) also suggest that AD patients with early and prominent visual symptoms represent a subtype of AD. This hypothesis may be supported by the present observations showing an elevation of Aβ43 and indicating an impairment of amyloid precursor protein processing by a γ-secretase.

Amyloid-β peptides are critical for neuron viability (Plant *et al.*, 2003) and Aβ42 is made and retained in an insoluble form in the endoplasmic reticulum and it is packaged for secretion in the trans-Golgi network (Greenfield *et al.*, 1999). Mild cognitive impairment is associated with accumulation of intraneuronal Aβ42 (Gouras *et al.*, 2000) and a review by Braak and Del Tredici (2003) considers evidence that soluble, intracellular Aβ42 may be a precursor to NFT formation. Since we have observed intraneuronal Aβ42 in young cases of depression (ages 32–74; Chapter 25), cingulate neurons are capable of generating a soluble form of Aβ42 at ages that are compatible with diseases of aging. As suggested there, the intraneuronal accumulation may contribute to neuron death as also shown in Alzheimer's disease (Sheng *et al.*, 1998; Parvathy *et al.*, 2001) and this could put at risk neurons in layer V of MCC in PCA.

Early Visual Impairment and Cingulate Neuropathology

The seven cases of PCA reported above had a disease duration of 5–12 years which means there may have been early events that could not be appreciated with this sample and the conclusion that posterior cingulate damage is part of the leading edge in cortical damage in PCA. Therefore, we consider a case that was early in their visual impairments and visual pathology was compatible with the hypothesis that this is a very early case of PCA. This patient was a 67-year-old male when he first complained of visual difficulties; he 'bumped into things' and had 'split vision.' A neurologist observed elements of visual apraxia and simultanagnosia with no clear evidence of stroke or trauma. He was generally well oriented to time and space and he was to be followed for possible PCA or a mild watershed infarct. The neurological follow-up was never performed as he developed a pancreatic cancer and died 7 months later. Neuropathological examination showed NFT in entorhinal cortex and at the temporo-occipital junction in area 19. AD2-immunoreactive NFT were very rare throughout the remaining cerebral cortex including PCC. Amyloid-β42 was present in all cortical samples including areas 19, MT, medial 7m, retrosplenial areas 29 and 30 and posterior cingulate areas 23 and 31.

Figure 35.18 shows some of the histological findings from this case. The highest density of Aβ42 was in area 23 but it was quite high in area MT. The latter area have dense plaques in layers I–II and diffuse deposits that extended into layer Vb. Area d23b, in contrast, had a higher density of dense plaques in layers I–IV suggesting an earlier impairment in PCC. Some dense-core Aβ40 plaques were also present in layer Vb. Counterstained Aβ42 showed a heavy reactive gliosis throughout dPCC. In spite of the lack of NFT, there was severe neurodegeneration in layers IV and Va (asterisks in Fig. 35.18) as

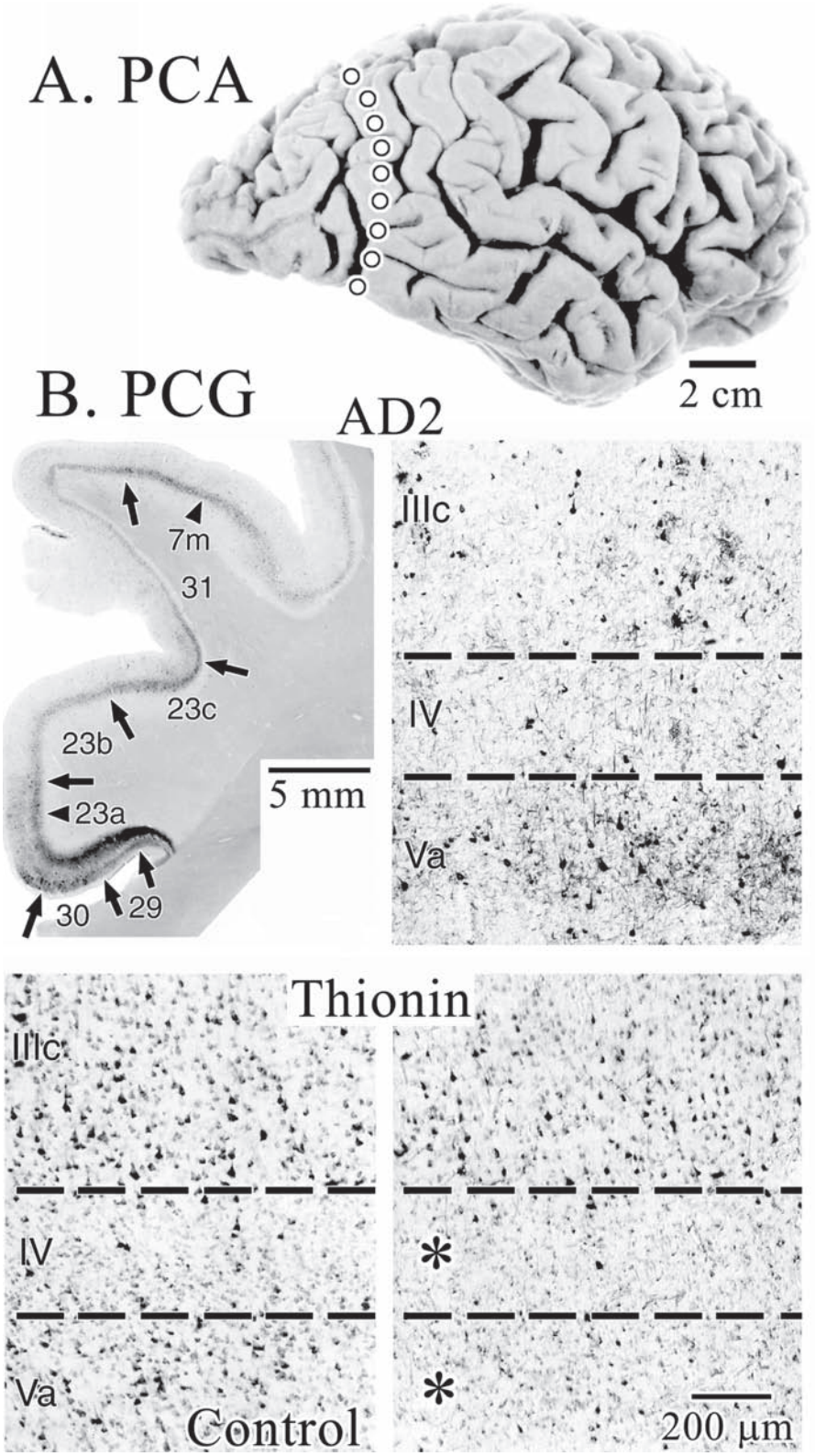

Fig. 35.16 PCA case showing the prominent atrophy on the lateral surface (A., posterior to the dotted line) and a section through the posterior cingulate gyrus (PCG). The AD2 antibody to hyperphosphorylated tau shows a progressive buildup in the PCG beginning with retrosplenial areas 29 and 30. The higher magnification histological photographs are of mid-layers of area 23a and show prominent deposition of tau in layer Va and substantial loss of neurons in layers IV and Va compared to a control case (Thionin, asterisks).

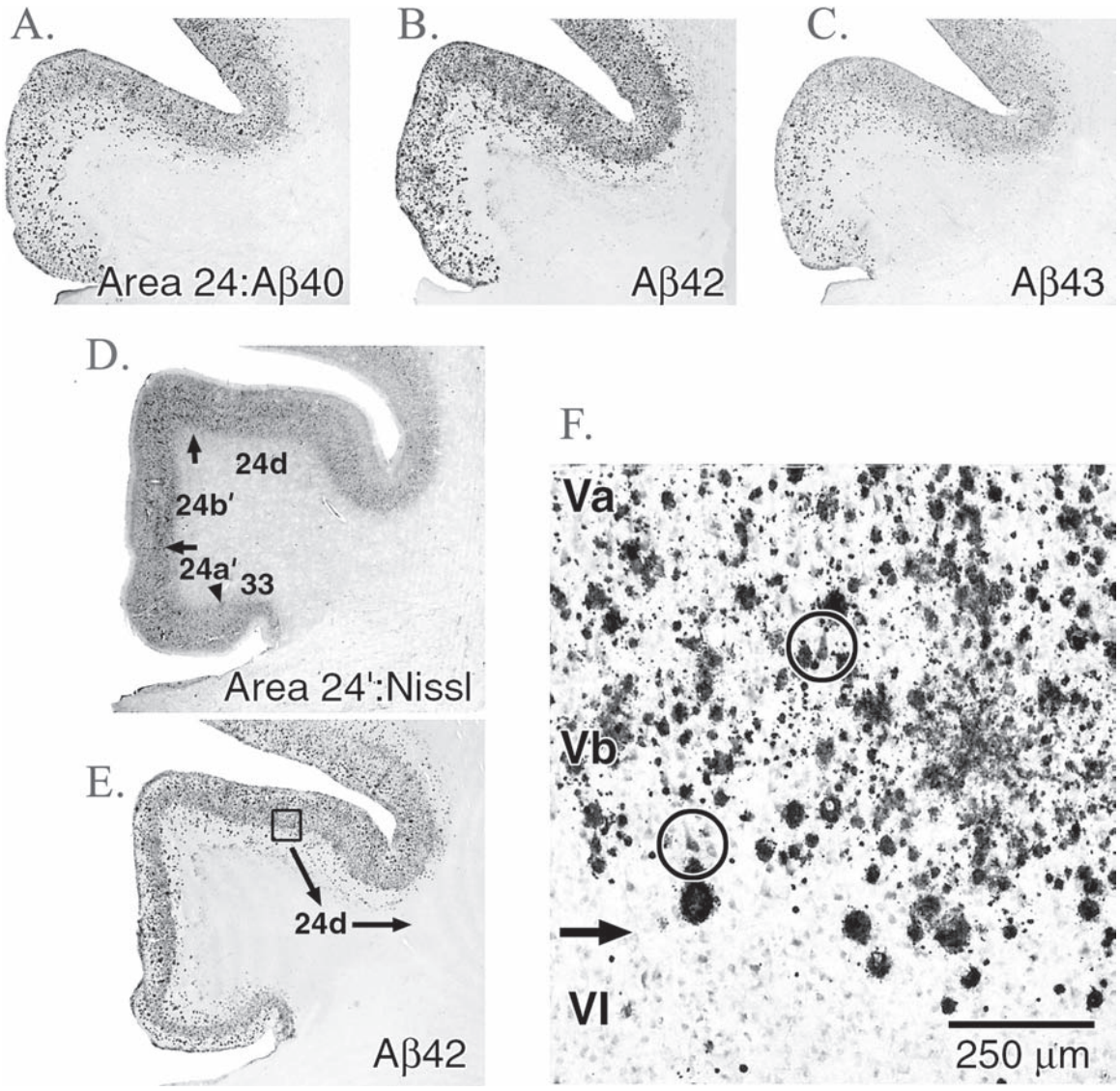

Fig. 35.17 Comparison of ACC area 24 and MCC area 24′ in PCA. Deposition of amyloid peptides is not limited to PCC and RSC but extends along the entire cingulate gyrus; elevated Aβ43 is widely distributed as well. The deposits of Aβ42 extend into layer V where there are examples of large neurons with intracellular deposits (circled in F.).

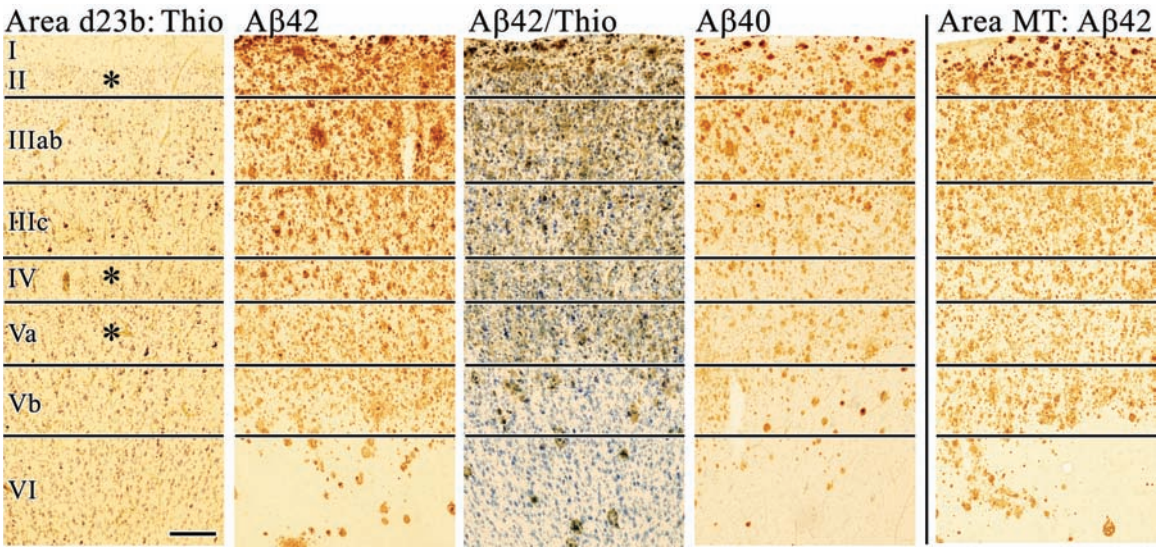

Fig. 35.18 Case with early visual impairment. Aβ42 was at a higher density in area d23 than in area MT in terms of both diffuse and dense plaques. Substantial neuron loss is in layers II, IV, and Va as noted with asterisks. An Aβ42 section was counterstained with thionin to emphasize its co-localization to particular layers. Scale bar = 200 μm.

predicted from other cases of PCA. Layer II also showed substantial neuron loss.

The pattern of amyloid peptide deposition and neurodegeneration in PCC in this case is compatible with the hypothesis it is a very early form of PCA. The substantial damage to PCC emphasizes the early role of this region in aspects of visuospatial symptoms in PCA and confirms hypotheses derived from the four-region neurobiological model.

Does PCA in AD Meet the Standard for an AD Subtype?

According to Jorm (1985), a variant of a disease constitutes a subtype when a unique mechanism can be demonstrated in association with a unique pattern of pathology. This approach may not go far enough when considering neurological disorders as it is not possible to determine if AD is comprised of subtypes with *in vivo* functional imaging and dominant/early symptoms because they do not provide information about mechanisms of neuron death. However, it may be possible with a combination of clinical, imaging, genetic and neuropathological findings to propose one or more subtypes. Thus, the first question is whether or not a subtype can be demonstrated in AD. This first issue is not a description of the most frequently observed clinical symptoms and associated pathology such as that of 'typical' AD. Once *any* subtype can be convincingly demonstrated, the question can be broadened to other possible subtypes and their frequency in the general population.

One AD subtype that fulfills the broader definition of a subtype is that of early spastic paraparesis associated with focal damage in cortical motor areas (Crook *et al.*, 1998). Certainly, motor cortex damage is not associated with typical AD or with ACA and PCA discussed above. An autosomal dominant mutation of the presenilin 1 gene with deletion of exon 9 is associated with the early spastic paraparesis and these cases have large senile plaques without cores and degeneration of the corticospinal tract which are not characteristic of typical AD. These findings suggest both a unique mechanism of neurodegeneration mediated by a presenilin 1 mutation or by altered amyloid precursor protein enzymatic processing that produces a unique pattern of cortical neuron loss and support the existence of a subtype of AD; even though such cases are rare.

Although the mutation(s) responsible for PCA have yet to be determined, PCA in AD is associated with early signs of visual and oculomotor symptoms, a unique laminar pattern of neurodegeneration as shown with principal components analysis including an excess of neuron loss in layers IV and Va, and two times greater expression of Aβ43 than in age-matched and neuron-density matched AD cases. Pietrini *et al.* (1996) suggested that AD patients with early and prominent visual symptoms represent a subtype of AD. Although we do not know the mechanistic links between the amyloid peptide/NFT and laminar neurodegeneration patterns, unique mechanisms of neurodegeneration may be active in PCA that qualify this subgroup as a subtype of AD. One explanation, for example, could be actions of γ-secretase.

γ-Secretase is a membrane-associated protease that cleaves the amyloid precursor protein to generate C-terminal amyloid-β peptides. The γ-secretase activity is catalysed by a presenilin 1 macromolecular complex (Li *et al.*, 2000), a deficiency of presenilin 1 inhibits the normal cleavage of the precursor protein (De Strooper *et al.*, 1998), and mutated presenilin 1 protein increases Aβ42 and Aβ43 (Duff *et al.*, 1996). Finally, nicastrin docking appears to be critical for amyloid precursor protein association with presenilin 1 and γ-secretase activity (Berezovska *et al.*, 2003). Although the *in vivo* links between amyloid peptide toxicity are still not fully understood, amyloid plaques may have neurotoxic properties (Sheng *et al.*, 1998) and there are reports of elevated amyloid peptides early in AD that are associated with cognitive decline (Näslund *et al.*, 2000). The significant increase in Aβ43 in PCA along with enhanced neurodegeneration in layers IV–Va suggest that one mechanism of neurodegeneration in the subgroup results from either an elevation in the actions of the γ-secretase or influences on the docking or other aspects of the enzyme's activity. Since a unique mechanism of neurodegeneration is necessary to designate a subgroup as a subtype, it is possible that PCA is a subtype of AD based on altered amyloid precursor protein processing.

Cingulate Circuitry and Damage Provide Clues for Solving the Alzheimer Puzzle

When AD was viewed as a single disease with clinical subgroups based on variability in cortical area involments and usually originating in parahippocampal cortex with memory and visuospatial impairments, the primary target of interest was the hippocampus and parahippocampal cortices. Extensive and detailed studies flowed from analysing the earliest damage in these regions. The problem has been slowly changing over the past decade as AD variants were identified, since minimal damage to parahippocampal cortex have been identified with different initial symptoms and cortical patterns of altered function. Although damage according to the Braak stages in medial temporal cortex will always be critical to documenting the status of an AD case, this approach provides little information about subgroups and subtypes of the disease. Even with

extensive genetic testing, clinical documentation and neuropsychological testing, a change in study design is required and this will focus attention on cingulate rather than temporal cortex. Damage to cingulate cortex can be early and pronounced as demonstrated here and in mild cognitive impairment (Chapter 33).

The relevance of cingulate circuits in AD has been appreciated by Braak and Braak (1999) and demonstrated in the above discourse. The four-region neurobiological model and functional imaging studies provide a framework for designing postmortem studies based on circuit impairments associated with different cingulate subregions. Since no single cingulate region can be used to evaluate the variant populations in AD, a minimal sampling strategy of three subregions is required beyond standard samples for the AD diagnosis. To the extent that dorsal motor areas may be involved as in spastic paraparesis in AD, coronal cingulate samples should include the complete medial cortex at each level. The three necessary levels include sACC, aMCC, dPCC and associated orbitofrontal, supplementary motor and medial parietal cortices, respectively. This approach provides documentation for studies of mild cognitive impairment and differential clinicopathological correlations in AD variants.

Mechanisms of neuron death and multivariate models need to be employed even though they enhance the burden of postmortem studies by requiring a wide and detailed analysis of neuron densities; not just marker pathology. This is particularly true of layer IV in PCC where neurofibrillary degeneration does not occur in the face of extensive neuron loss. Finally, we have used the ApoE genotype to show how genetic information can be entered into such models and it is not limited to this information alone as many clinical variables can be assessed in terms of their contribution to a multivariate model.

The pivotal problem associated with winkling out the contributions of cingulate subregions to AD is the lack of specific neuropsychological tests of cingulate functions including those of PCC and RSC. Without such tests, it is not be possible to identify their contributions to AD symptoms in any subgroup or subtype of AD and a persistent bias will continue toward structures such as prefrontal cortex for which tests are currently available. The message from thorough case studies of ACA, PCA, and mild cognitive impairment is that cingulate damage often appears earlier than expected and may be pivotal to determining the primary etiology of neurodegeneration in the variant AD populations. It may even be concluded that the lack of a consistent cingulate sampling strategy has left us with an incomplete understanding of 'typical' AD. The final pieces to the AD puzzle likely lie in the cingulate gyrus.

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