CHAPTER 25

Cingulate Neuropathological Substrates of Depression

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Chapter contents

Since antiquity, mood disorders were considered to be among the most common diseases. Hippocrates (460–357 BC) was the first to describe melancholia ('black bile'); Aretaeus of Cappadocia (ca. 150 AD) described manic-depression (bipolar disorder—BD), and Robert Burton's *Anatomy of Melancholy* published in 1621 was the first modern text on mood disorders. Cartesian thinking separated mind from body, and in this way it provided medicine with autonomy over the body, free from interference by the Church, but this had profound consequences on the way mental disorders were conceptualized in the centuries that followed. This situation was changed by Adolf Meyer (1866–1950) who introduced the term 'psychobiology' to emphasize that both psychological and biological factors are relevant for the etiopathogenesis of mental disorders. Depression is still viewed as a major health problem with nearly 20% of women and more than 10% of men suffering from at least one mood disorder episode during their lives. Depression was ranked as the fourth most urgent health problem worldwide by the World Health Organization (Murray & Lopez, 1996). Although effective treatments exist for decades now, the disability induced by mood disorders is comparable with and often exceeds those of pain, hypertension, diabetes mellitus, and coronary artery disease (Wells *et al.*, 1989).

Current classification systems (DSM-IV and ICD-10; WHO, 1993; APA, 1994) classify mood disorders into unipolar (major depression and dysthymia) and BD (includes the alternation of depressive and manic, hypomanic, or mixed episodes, and cyclothymia). The clinical picture includes also vegetative and psychomotor disorders, and there is a great overlap with anxiety disorders (mainly with generalized anxiety disorder, panic disorder, obsessive–compulsive disorder, and social phobia; Ahrens *et al.*, 1995). The current concept of mood disorders includes many conditions previously diagnosed as schizophrenia, personality disorder, or neurosis. The term 'mood' which refers to the internal tone was chosen today as more suitable in comparison with the term 'affective,' which refers to the external expression or 'emotion' that is the transient present state. It should be stressed that whereas depression has traditionally been considered a benign episodic disorder, recent observations suggest that many patients are only partial responders to therapy or become chronic. Thus, the presence of structural abnormalities is possible.

The psychological approach (Gabbard, 1995; Agras & Wilson, 1995; Konner, 1995), which was the strongest approach to psychiatry during much of the 20th century, has three major schools: psychoanalytic (Freud, 1975; Abraham, 1948), cognitive (Beck, 1967), and behavioral (Seligman, 1975). The most important models for depression proposed by them were the Aggression-Turned-Inward Model in which depression is viewed as an epiphenomenon of the transduction of thanatotic energy; a reaction that took place in the closed hydraulic space of the mind. The Object Loss model refers to traumatic separation from significant objects of attachment, while the Loss of Self-Esteem model suggests depression originates from the narcissistic injury because of the ego's inability to give up unattainable goals. The Cognitive model incorporated negative thinking about self, the environment, and the future, while the Learned Helplessness model viewed depression as learned from past situations in which the person was unable to terminate undesirable situations. Finally, the Reinforcement model viewed depression as associated with the lack of appropriate rewards and non-contingent rewards. Although psychological models were generally proposed without specific brain mechanisms, some lead naturally to biological models including the Learned Helplessness and Reinforcement models.

Biological Models and Cingulate Cortex

Schildkraut (1965) was the first to publish a formal hypothesis connecting the central nervous system neurotransmitter norepinephrine and clinical depression. The role of serotonin was emphasized by subsequent research, but in spite of extensive research, no deficiency or excess of any biological substance in specific brain structures has been shown to be necessary or sufficient for the occurrence of mood disorders. However, there are sufficient data to support the approach of biological theories that the cause of depression lies in disturbances of the central nervous system (Changeux & Dehaene, 1993; Lewis & Oeth, 1995; Brown *et al.*, 1994; Kling *et al.*, 1995; Cooper & Martin, 1995; Copolov & Rubin, 1987).

There is still a large gulf among specific symptoms, disease course, and biological models that seek to describe underlying brain mechanisms. The past decade of neuroimaging, however, leaves no doubt that alteration in the functions of a network of telencephalic structures including cingulate cortex is pivotal to depression. It has long been known that psychiatric symptoms, including depression, can be alleviated with cingulotomy lesions (Whitty *et al.*, 1952; Ballantine *et al.*, 1967). Recently, it is has been shown that higher preoperative rates of glucose metabolism in subgenual anterior cingulate cortex (sACC) was associated with better post-operative responses to anterior cingulotomy as measured by the Beck Depression Inventory (Dougherty *et al.*, 2003). Another direct link to impaired sACC is supported by the effectiveness of electrical stimulation in this region (Mayberg *et al.*, 2005). Finally, imaging studies showing deficits in glucose metabolism

and functional activation in this region have been reported and are discussed in Chapter 24.

An important proviso when considering functional impairments in depression is that normal brain function and that in depressed patients is whether or not induced sadness in normal volunteers share core mechanisms with clinical depression. They may not activate structures towards opposite directions and most studies assess emotion which is quite different from mood. Also, major depression (MD) and BD are not homogenous diseases and many subtypes have been proposed (Fountoulakis *et al.*, 1999; Roth, 1959; Overall *et al.*, 1966). Also, many phases of these illnesses have been recognized and most studies, especially those using neuroimaging or neuropathology, concern melancholic depression, while most studies on BD concern the depressive phase.

Depression-vulnerable network

Early efforts to synthesize theories and empirical data suggested that psychological, biological, and etiological factors converge in deficits in the diencephalic substrates of pleasure and reward (Akiskal & McKinney, 1973). As discussed in the previous chapter, neuroimaging observations also emphasize a pivotal role of sACC. One level of involvement is in the contribution of cingulate cortex to distributed network functions; as opposed to unique intracingulate information processing. The integrative model shown in Figure 25.1 links the central chemistry and physiology of structures that generate mood, episodic memory storage and retrieval, motor response programs, and reward mechanisms with behavioral impairments of depression. The amygdala and the cingulate cortex together play a key role in this model. In this simplified neurobiological model, 'mood' derives from the processes in the broader limbic network including the amygdala, insula, anterior cingulate cortex (ACC), and orbitofrontal cortex. Each of these sites is also involved in generating emotion and external affective responses, while the effortful regulation of movement likely implicates area 24' in midcingulate cortex (MCC) and the dorsolateral prefrontal cortex; between these regions it appears that 'affect' is generated at least partially (Phillips *et al.*, 2003a,b). As discussed in Chapter 10, sACC has direct projections to autonomic brainstem nuclei and is likely responsible for much or all of the final common autonomic output generated in the frontocingulate circuit.

Figure 25.1 provides an overview of part of the network that is involved in depression and the 'levels' of organization of the system from gene to cingulate cortex outputs. The short allele of the 5HT transporter (5HTT) is associated with altered serotonin (5HT) uptake and volumetric reductions in the ACC and amygdala (Pezawas *et al.*, 2005). The short allele generates transporter mRNA and transporter protein that is ineffective in transporting 5HT into the cell is reviewed by Caneli and Lesch (2007) in the context of emotion regulation and anxiety. The actions of this ineffective transporter are mediated by the axon terminals of the dorsal raphe nuclei that project throughout much of the rostral limbic system including ACC. A further consideration of 5HT function and the 5HT1A receptor in cingulate cortex is reviewed at the end of this chapter in the framework of neurodegeneration. Mood may be generated by the aggregate activity in this 5HT-regulated network and forms an endophenotype for depression. Emotion as an acute and internal response to various environmental cues and contexts is generated directly in cingulate cortex and may be modified by mood. One aspect of cingulate-mediated depression etiology could be the disruption of valence-coded sensory information that flows through posterior cingulate cortex (PCC) as discussed in detail in Chapter 13 and the final common pathway of emotional expression is mediated by autonomic activity via sACC and skeletomotor outputs from the cingulate premotor areas.

The amygdala has been implicated in fear and anxiety and contributes in a profound way to this network function. For this reason, the projections of the amygdala are shown in Figure 25.1B and two important points are emphasized. First, the major amygdala projections terminate in ACC, although there is a small extension in anterior MCC (aMCC). Second, the greatest termination in ACC is to inner layers I and II. As layer III pyramidal neurons have apical dendrites and tufts in layers I and II, they are likely relevant to this interaction as well and this plays an important part in histopathological hypotheses discussed below and the case review that follows. Indeed, a study of the correlated atrophy in the amygdala and ACC by Pezawas *et al.* (2005; Fig. 25.1C) showed a pair of sites with correlated atrophy in aMCC and pACC. This is strong evidence that atrophy and possibly neuron losses in layers II and IIIab of ACC may be linked to interactions with the amygdala. Finally, layer Va pyramidal neurons could participate via apical dendrites but neurons in layers IIIc and VI would be less affected.

Goals of This Chapter

The previous chapter considers the role of cingulate cortex in depression as a key node in a network of vulnerable structures and the impact of various therapeutic approaches on cingulate functions and this network. Here we seek to understand the neuropathological substrates of depression in cingulate cortex. The perspectives of such an effort are critically dependent on the

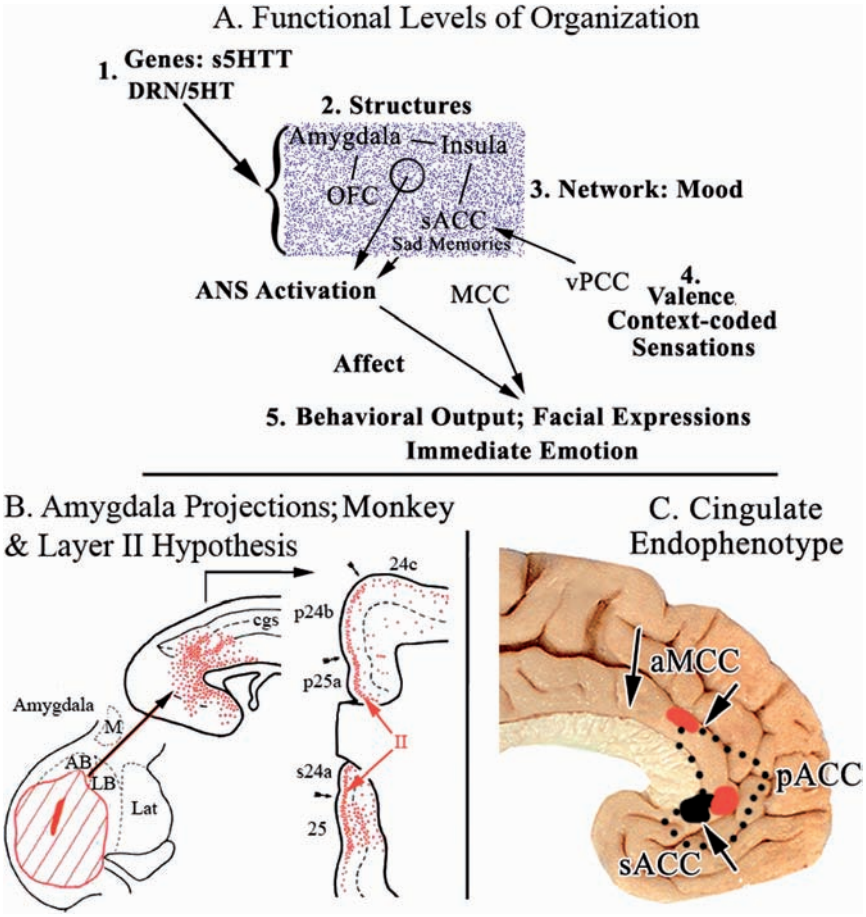

Fig. 25.1 Cingulate morphological substrates of depression. A. Network organization from 5HTT gene to behavioral output. B. Amygdalocingulate projections (Vogt & Pandya, 1987) that support the layer II/IIIab hypothesis. C. Plots of cingulate atrophy (dotted line) and correlated atrophy with that in the amygdala (red; Pezawas *et al*., 2005) plotted onto the medial surface of a control case. The most significant volumetric reduction is shown in black and the subregion borders have arrows. ANS, autonomic nervous system; AB, accessory basal; Lat, lateral; LB, laterobasal; M, medial.

sample population (major or bipolar depression), the prodromal status of cingulate cortex which is not known in the postmortem cases, the stage of the disease, age of onset and at death, and treatment status. The permutations of these possibilities are extraordinary and the scale of this problem extends beyond a single chapter. Thus, we seek to systematize the literature around ACC as one of the pivotal structures that is vulnerable to depression and a major impediment to this endeavor is the differences among studies in methods and nomenclatures. The four-region neurobiological model in control and depressed cases is used to review the literature and 10 postmortem cases of depression. Thick sections were used to analyze ACC and PCC to assure that changes in cytoarchitecture could be identified. The specific goals are as follows:

1 Review the literature concerning the role of the cingulate cortex in BD and MD to generate hypotheses for exploring postmortem tissues.

2 Review the normal architecture of subgenual ACC in thick secions.

3 Evaluate the neuropathology of cingulate cortex in 5 BD, 5 MD, and 5 control cases for:

a) Two laminar/cellular patterns of amyloid-β42 (Aβ42) deposition in ACC and PCC comprised intraneuronal deposition and as plaques in 9 of 10 depression cases.

b) Evaluate the age-dependency and progressive deposition of Aβ42, a unique 74-year-old MD case showing the two patterns simultaneously and the likely transition from one to the other, and suggest links to neuron losses.

c) Report the laminar patterns of neuron loss in 10 depression cases in ACC and PCC.

d) Evaluate neuron shrinkage to determine if it is a unique and separable event from other characteristics of the tissue or whether it is a preliminary step to neurodegeneration.

e) Report laminar changes in glial activity and possible links to neuron loss.

4 Consider drug therapies in the context of laminar patterns of age-linked neurodegeneration including blocking amyloid secretase enzymes, alteration of cholesterol levels, amyloid vaccination, anti-inflammatory therapies, and inhibition of acetylcholinesterase.

Overview

As this chapter considers a number of new leads to the cingulate changes associated with depression, a preliminary overview of some pivotal observations is useful. Although genetic risks indicate an important cingulate endophenotype early in life, depression is also a neurodegenerative disorder as shown in all cases analyzed here. Sites of neuron shrinkage are easily demonstrated in all cases and likely portend neurodegeneration. Glial alterations are also present in all cases and may be involved in degenerative and/or inflammatory processes. Finally, there are clear aging dimensions to neuron loss and it appears that Aβ42 is deposited and may contribute substantially thereto, although other mechanisms are likely. Whether or not this represents a core pathology remains to be determined. BD has a prominent loss of neurons in layers II and III, and MD has losses mainly but not exclusively in the deep layers. Interestingly, these subgroups can also be differentiated with neuron loss in PCC. In terms of possible pharmacological treatments, early neurodegeneration may be compensated for by drugs acting on the serotonin system; however, this process becomes a hurdle over which current drug therapeutics may not be able to jump late in life and other strategies such as those that block the synthesis, buildup or release of Aβ42 may be required.

Cingulate Histopathological Hypotheses

Given the constraints in assessing cingulate neuropathology in depression noted above, what might be sought in postmortem tissue? The literature is considered here in the context of anatomical hypotheses that can be applied to postmortem cases. Localization issues define specific hypotheses and may relate in a significant manner to symptoms of depression. At the outset it should be realized that each postmortem case presents an aggregate pathology that may not accurately reflect particular symptom episodes or early damage. Although changes in sACC would be considered diagnostic of neuropathological changes in depression given the large functional neuroimaging literature on this region, there are many reasons to expect damage in PCC as well. A selective vulnerability of neurons in particular layers in vulnerable regions are also important with layer II receiving amygdala afferents and layer V projecting to the striatum and other brainstem motor areas. These layers could be important to mood and internal activation and motor drive that are altered in depression. Neuron shrinkage has been widely reported as occurring in cingulate cortex and these changes could be associated with volumetric changes reported in the structural imaging literature but it is not clear to what extent these changes are related to neuron loss. Changes in neuron size, number, and glial proliferation associated with inflammatory changes could all occur simultaneously and are assessed in the present case review.

Regional involvements and hypotheses

Localization of structural changes associated with depression derive mainly from volumetric and glucose metabolic studies and they emphasize the significance of reductions of both measures in ACC. Decreased left sACC glucose metabolism in familial MD and familial BD was reported and it could be partly explained by a 48% reduction in cortical volume (Drevets *et al.*, 1997). This reduction is present at first hospitalization for familial depressed patients but not in non-familial cases (Hirayasu *et al.*, 1999). Other studies confirm these findings, but report a volume reduction of 19% in adolescent and middle-aged females with depression in comparison with controls (Botteron *et al.*, 2002).

Carriers of the short allele of a functional 5′ promoter polymorphism of the 5HTT gene have increased anxiety-related temperamental traits, increased amygdala reactivity and elevated risk of depression. In these patients, morphometric analyses showed reduced gray matter volume in short-allele carriers in the areas 24, a24′, and 32 of the ACC and in the amygdala (Pezawas *et al.*, 2005). Figure 25.1C shows the distribution of volumetrically reduced cortex from this very important study (within dotted lines co-registered to a control postmortem case; most significant reductions shown in solid black). This study showed correlations between volumetric changes in the amygdala and cingulate cortex and the two sites with a significant correlation in structural changes are shown in red in the figure and are located mainly in aMCC and pACC.

Most neuroimaging studies in depressive patients report a global brain hypometabolism or hypoperfusion, but also manifest some controversies in terms of localized activity. Mathew *et al.* (1980) reported only left hemisphere hypoperfusion, while Sackheim and Prohovnik (1990) found more global hypoperfusion that was pronounced in frontal areas. Uytdenhoef *et al.* (1983) reported hypoperfusion restricted to the frontal areas only and frontal hypometabolism was also reported by others (Delvenne & Delecluse, 1990). A few studies reported no difference between depressed patients and controls (Brambilla *et al.*, 2002, Anderer *et al.*, 2002, Bremner *et al.*, 2002) and there are studies reporting left

frontal (Drevets & Videen, 1992) or right temporal (Amsterdam & Mozley, 1992) *hyper*metabolism. Bench and Friston (1993) reported a bilateral increase of frontal regional cerebral blood flow (rCBF) in a group of anxious depressive patients. Thus, most studies related the reduced psychomotor activity with a reduced left frontal, ACC, and uncus metabolism or rCBF, while the presence of anxiety was related to an increased metabolism or rCBF of those same areas.

There is strong evidence pointing to the cingulate cortex dysfunction in MD (Videbech, 2000). In unmedicated depressive patients, metabolism was increased, among other structures, in the PCC bilaterally and decreased in the left sACC. After treatment, metabolism significantly further decreased in the left sACC. These changes were largely limited to those subjects who responded to treatment and remained well at 6-month follow-up (Drevets *et al.*, 2002). Decreased activity was reported by Gruber *et al.* (2004) in ACC in BD, Galynker *et al.* (2002) reported a significantly lower rCBF in the right cingulate cortex, Oda *et al.* (2003) in the ACC bilaterally, and Gonul *et al.* (2004) reported that both psychotic and non-psychotic depressed patients showed significantly lower rCBF values in the left ACC. The reduction in metabolic rate might be partially explained by the reduction in volume, but this cannot explain hypermetabolism reported by some studies. It is reasonable to look to the postmortem cases to identify the structural correlates of depression in MD and BD and assess the extent to which it is compatible with enhanced metabolic activity. To the extent there is evidence for neurodegeneration, it must be considered that the hypermetabolism is an early sign of the disease and not a persistent long-term effect.

BD patients during the early stages of the disease may show volume changes of the sACC. Reduction in cortex volume was replicated in three of four studies in patients with familial BD (Hajek *et al.*, 2005). Hirayasu *et al.* (1999) reported a 25% reduction in the volume of the left sACC in patients with familial BD during their first psychotic episode. Kaur *et al.* (2005) added the finding of a significantly smaller PCC bilaterally. Drevets *et al.* (1997) reported a higher gray matter reduction of 39% in the same area. There are also studies, however, that do not report volumetric changes in cingulate cortex of BD patients (Lopez-Larson *et al.*, 2002; Brambilla *et al.*, 2002; Zipursky *et al.*, 1997) or report global but not localized gray matter reduction (Lim *et al.*, 1999).

Drevets *et al.* (1997) reported a decreased metabolism in the sACC bilaterally during depression and may be increased during mania, thus suggesting that the sACC metabolism is state dependent in BD patients. Also, there was a decreased metabolism in the left sACC only in familial BD and familial MD, which could at least partly be explained by a corresponding reduction in cortical volume with magnetic resonance imaging (MRI) with a 39% reduction in mean gray matter volume in same area. Although different PET methods demonstrate consistent abnormalities in the prefrontal, cingulate, and amygdala regions (Kennedy *et al.*, 1997) and reviews suggest that the prefrontal, ACC, and amygdala are consistently implicated in BD and closely associated with mood symptoms, there is a bulk of data suggesting that the metabolic changes in cingulate cortex may relate to specific symptoms or syndromes. It has been reported that the psychomotor-anhedonia symptoms may relate with higher normalized metabolism in the right ACC (Dunn *et al.*, 2002). Also the manic state of BD may be associated with heightened activity in a left frontal cortical–subcortical system that includes the ACC and caudate nucleus (Blumberg *et al.*, 2000b).

In spite of variations among patient populations, most findings in both forms of depression support hypotheses that neuron loss occurs in sACC. The involvement of PCC is less secure and no studies are available of its postmortem composition in depression. The present analysis focuses on ACC and dorsal PCC. Interestingly, although PCC has not been viewed as diagnostic of depression, the present observations suggest that this region may be valuable in differentiating between BD and MD.

Laminar damage and hypotheses

Laminar cortical thicknesses and pyramidal neuron densities were significantly decreased in area p24 and s24 in BD patients. The major changes were in layers III, V, and VI of area s24, whereas patients with major depression were comparable with controls. Immunodot assay showed a significant decrease of both MAP2 and MAP1b proteins in BD but not in patients with MD (Bouras *et al.*, 2001). As the amygdala projects to inner layer I, layer II and layer IIIab pyramids have apical dendrites throughout these layers and the amygdala and ACC experience correlated atrophy in the short-allele 5HTT as discussed above, changes in layer II and upper layer III might reflect interactions with the amygdala. In support of this hypothesis, Chana *et al.* (2003) reported reduced clustering of neurons in layer II of area 24c. Finally, in a meta-analysis, Todtenkopf *et al.* (2005) concluded that there is a bilateral decrease in the density of non-pyramidal neurons in layer II of the ACC of BD patients (31%). They reported no differences in the density of glia with two-dimensional cell counting, but significant reduction in layers III, V, and VI with 3D cell counting. Thus, any study of BD and MD needs to consider neuron sizes and loss in layers II–VI.

The disruption of deep-cingulate layers has been implicated in depression. Chana *et al.* (2003) reported that layers V and VI had neurodegeneration in area 24c; however,

Cotter *et al.* (2001) studied adjacent area 24b in the same cases and reported no changes in neuron density. Other authors reported a reduced neuronal somal size in layer V in the area 24c of the ACC (9%) while neuronal density was increased in the same area (24%; Chana *et al.*, 2003). A decreased clustering of neurons in the area 24c of the ACC bilaterally was seen in BD patients in comparison with MD and controls and neuron somal size was reduced in layer V (16%) and neuronal density was *increased* in layer VI in BD (63%; Chana *et al.*, 2003). Also, there was no change in the number or size of neurons in area s24. A reduction in glial number of 41% was statistically significant only in familial BD bilaterally. Ongür *et al.* (1998) reported reduced glial number in familial unipolar depressive patients. Thus, layers V and VI appear to be involved in many cases, although these observations do not always agree among studies and different cingulate areas. The deep-layer neurodegeneration hypothesis is viable, needs to be considered in terms of progression throughout life, and the extent to which it is associated with superficial layer neuron loss and shrinkage.

Glia and inflammatory responses

Ongür *et al.* (1998) reported no change in the number or size of neurons in area s24, but did find a 24% reduction in glia in familial MD. Glial loss was also reported by Cotter *et al.* (2001; 22%) and an associated change in neuronal size of 23% in layer VI bilaterally in area p24b compared with controls. The magnitude of glial loss was similar in another study that reported a 19% reduction of oligodendroglial cells and suggested this could contribute to the atrophy of frontal neurons (Uranova *et al.*, 2004). There are studies of glial fibrillary acidic protein that are negative with MD comparable with controls (Bouras *et al.*, 2001; Thomas *et al.*, 2002, 2004). Cotter *et al.* (2002) found no evidence for differences in glial density or neuronal size in BD compared with controls; on the contrary, they reported a reduction in glial density and neuron size in layer VI bilaterally in the area 24b in MD (Cotter *et al.*, 2001).

Intercellular adhesion molecule-1 immunoreactivity in the gray and white matter of the area 24 in BD patients was increased compared with controls and modestly increased in white matter compared with unipolar patients. These findings are consistent with the presence of an inflammatory response in the ACC; they were found bilaterally and were independent from age, medication history, suicide, or family history (Thomas *et al.*, 2004). In the white matter of ACC, area 24b bilaterally, Webster *et al.* (2005) found decreased levels (32%) of glial fibrillary acidic protein mRNA in BD patients in comparison with normal controls; while changes in the gray matter were not significant. Also, the reduction in glial number (41%) was statistically significant only in familial BD bilaterally and the same study reported reduced glial number in familial unipolar depressive patients (Ongür *et al.*, 1998).

The lack of a linkage between neurodegeneration and glial densities is surprising. Changes in glial densities are hypothesized here but they are expected to have some link with changes in neuron densities. Glial proliferation could be indicative of an inflammatory response and the sACC may be selectively vulnerable to such insults.

Normal Cytology of Subgenual Anterior Cingulate Cortex

In addition to the above enumerated sources of variability associated with populations of depression, postmortem studies are impeded by a lack of consistent criteria for cytoarchitectural studies to define sACC. Many investigators consider area 25 to be equivalent to sACC, which it is not, because sACC is also comprised of parts of areas 33, 24, and 32 (Palomero-Gallagher *et al.*, 2008a). Two studies of the cytoarchitecture of human sACC (Chu *et al.*, 1997; Ongür *et al.*, 2003) report substantially different criteria for subgenual areas than those used by Brodmann (1909). In addition to Brodmann's text and limited illustrations, one can identify his histological criteria for designating each area by co-registering a digital photograph of the surface of a postmortem/histological case to his original map. Taking a microphotograph in the centroid of each area provides a cytological standard for criteria with modern immunohistochemistry that can then be modified according to the many cytologies apparent within each of Brodmann's broad areas (Vogt *et al.*, 2004; Palomero-Gallagher *et al.*, 2008a).

Chu *et al.* (1997) stated that area 25 has a dysgranular layer IV, while Brodmann stated it is agranular in both the monkey (Vogt *et al.*, 1987) and human (Vogt *et al.*, 2004; Palomero-Gallagher *et al.*, 2008a). Although area 25 is a large part of sACC, the border of sACC with pACC is determined by areas 32 and 24 that lie rostral to area 25. The border between these subregions is based on a thick layer II and a thin and undifferentiated layer III in both areas. Obviously, area 32 differs from area 24 in the presence of a dysgranular layer IV in the former area as defined by Brodmann. In another study, Ongür *et al.* (2003) state that area 32ac is agranular and provided no supporting histological evidence for this claim, although Brodmann stated it is granular. Indeed, we have shown this area is dysgranular not agranular (Vogt *et al.*, 1995, 2004; Palomero-Gallagher *et al.*, 2008a,b). To the extent that area '32pl' of Ongür *et al.* is agranular, they have observed a second division of area 25 rather than a division of area 32. The Ongür study did not identify

an area 33 and the designation of area 32 as agranular led them to identify part of area 32 as area 10m. In view of the many contradictions in cytoarchitectural criteria and nomenclatures introduced for sACC, there is no basis for a rigorous neuropathological assessment of sACC in depression. Rather, beginning with a rational nomenclature and criteria based on Brodmann principles serves as the basis of a logical neuropathology of sACC.

Figure 25.2 shows examples of subgenual area 24b (s24b) stained with both thionin and neuron-specific nuclear binding protein (NeuN). This area has a thinner layer III than its dorsal counterpart in pACC and it is not differentiated with only medium-sized pyramids (Palomero-Gallagher *et al.*, 2008a). There is a layer II that is dense and the neurons can form large and irregular clumps. Layer Va is dense as is true for all cingulate areas, while a neuron-dense layer VI makes

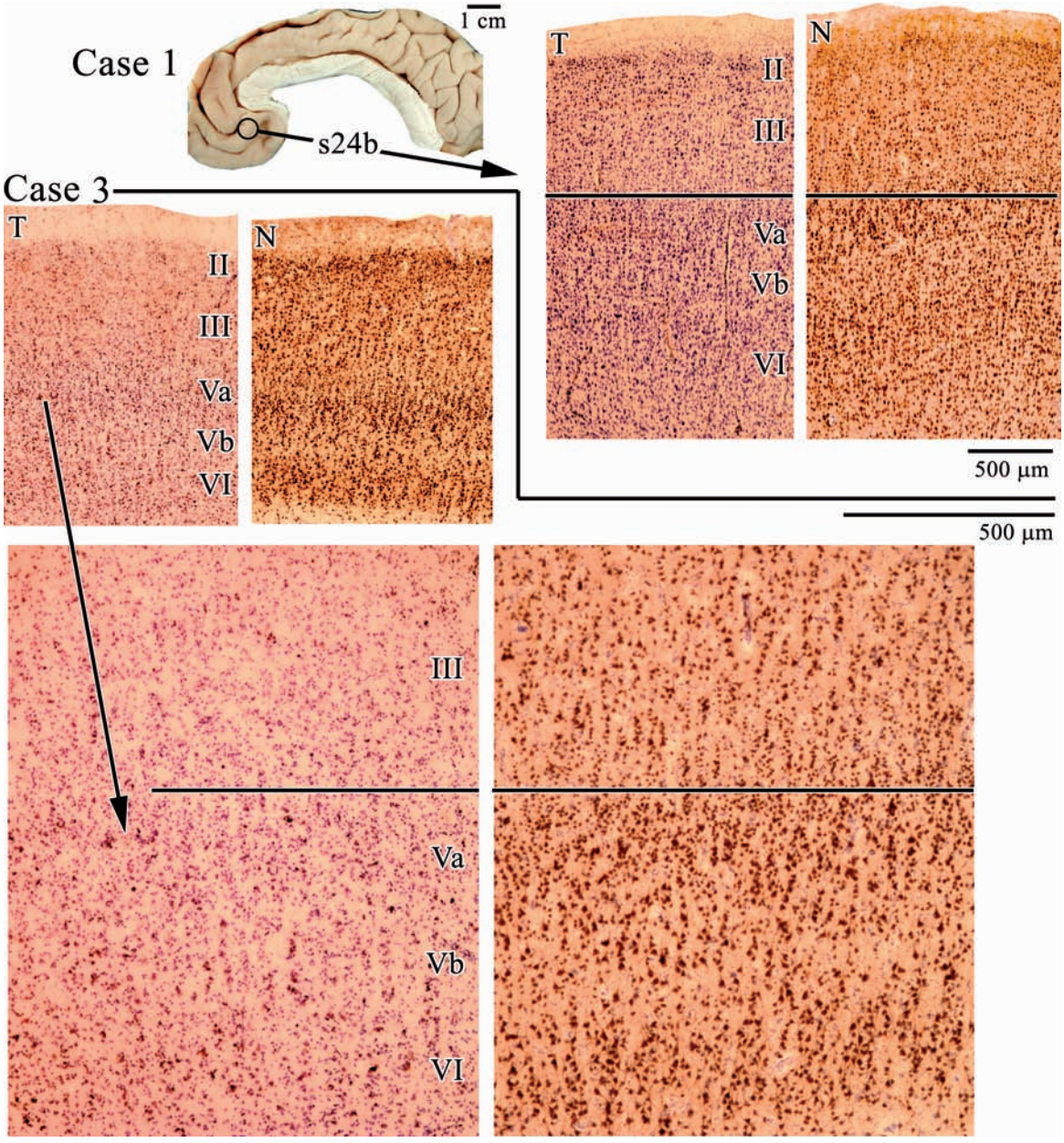

Fig. 25.2 Structure of area s24b in two control cases stained with thionin (T) and NeuN (N). Layer Va is densely packed with large neurons and, although layer Vb is relatively neuron spare; note large pyramids in this layer magnified for Case 3. Layer III is particularly thin in subgenual areas.

differentiation of layer Vb possible; although there are many large neurons in layer Vb itself as shown with the higher magnification in Figure 25.2.

Survey of Cingulate Cortex in Postmortem Depression Cases

Depression presents a particularly baffling problem for postmortem assessments; genetic/prodromal syndrome, fluctuating clinical course in BD, no clear disease markers, and a literature that is often contradictory. Indeed, studies from the same research team on the same samples but adjacent cortices can produce conflicting results. This either means there are highly variable and selective regional vulnerabilities or differences in how methods are applied. Although there is evidence of neurodegeneration in depression, the rate and course of progression is not known and the aggregate degeneration may provide a critical barrier to therapeutic interventions. Finally, the methods are pivotal, as are expectations for each and the specific hypotheses raised before setting out a particular anatomical research strategy. The logic of this assessment follows:

1 Define precisely the structure of control ACC as above.

2 Evaluate markers for diseases with cingulate vulnerabilities as in other chapters including amyloid-β 42 (Aβ42) for mild cognitive impairment (Chapter 33), dementia with Lewy bodies (Chapter 32), and Alzheimer's disease (AD; Chapter 35).

3 Qualitatively evaluate thionin-stained sections for changes in neuron densities and sizes by layer in ACC and extend this analysis to dorsal PCC.

4 Evaluate glia in terms of neuron loss and/or inflammatory response.

5 Evaluate neuron shrinkage in the context of laminar changes in neuron densities.

Cingulate tissues

Five postmortem cases for control, BD, and MD were used to analyze cingulate cortex. The status of each case is summarized in Table 25.1.

Overview of methods

Section thickness and sampling strategies are pivotal to the outcomes. As a rule, published studies use sections 5–10 µm thick. However, the thinner the section is the more difficult it is to visually assess laminar changes in neuron densities. Indeed, below 20 µm and even at 30 µm thickness it is difficult to reliably identify laminar architecture in the human cortex. This makes it quite difficult to define precise regions-of-interest when a layer is the target. If there is a focus of pathology in only one or two layers, a stereology sampling strategy that does not define the region-of-interest in terms of laminae can easily conclude there is no change in neuron or glial density because what changes have occurred in a single layer are lost in counts of many layers.

One-cm-thick blocks were taken from all sACC and dorsal posterior cingulate cortex (dPCC). These blocks, including those from controls, were all cut at a thickness of 50 µm to begin a qualitative review of the laminar architecture in samples from each subregion. Interestingly, there are no pictures in the literature showing the laminar changes in neuron density in depression compared with control cases. What does the ACC look like in BD where there is a 41% reduction in glial numbers but no changes in neuron density as reported by Ongür *et al.* (1998)? The glial analysis in the latter study was performed independent of layers and no photographic documentation was provided. Our experience with laminar patterns of neurodegeneration in AD (Vogt *et al.*, 1990, 1998) shows that we can qualitatively identify losses of 15–20% or greater. Of course, to the extent changes are focused on a layer and they involve

TABLE 25.1 Case material

Control cases	Gender	Age (death)	Brain weight (g)	Cause of death
#1	F	52	1210	Lung cancer
#2	M	61	1179	Lung cancer
#3	M	70	1410	Lung cancer
#4	F	75	1173	Liver cancer
#5	F	80	1078	Liver cancer
Bipolar disorder				
#6	F	32	n.d.	Suicide/subarach. & intravent. haematoma
#7	M	63	1270	Myocardial infarction
#8	F	68	1248	Post-ulcer G.I. bleeding
#9	F	75	1225	Cardiac insufficiency
#10	F	86	1140	Myocardial infarction
Major depression				
#11	F	56	1240	Lung hemorrhagic infarct
#12	M	68	1530	Cardiac insufficiency
#13	M	74	n.d.	Pulmonary embolism
#14	F	80	1100	Cardiac insufficiency
#15	F	82	1230	Bronchopneumonia

aggregates of degeneration (versus a homogeneous one spread throughout the entire cortical thickness), they are readily detected with this survey approach as seen below where all cases had photodocumented evidence of neuron loss.

As there are no disease markers unique to BD or MD, we surveyed the tissue for markers used to identify other disease presuming all would be negative. All sections sampled from all cingulate blocks immunoreacted for antibodies to α-synuclein (see Chapter 32) and phosphorylated tau (AT8; Chapters 33 and 35) were negative in spite of positive disease-control staining. Surprisingly, amyloid-β 42 (Aβ42) labeled samples from 9 of the 10 depression cases. As the laminar distribution of this antibody is shown in those cases, we begin with them in assessing layers of vulnerability. Sections from one-in-six series through all blocks were stained with thionin. Examples of area s24b were digitally photographed, imported into Photoshop and the Aβ42 and thionin images co-registered. This was done for area s24b of all sACC samples in control and depression cases, although there was no expression of Aβ42 in the control cases. The thionin-stained sections were analyzed at a final magnification of about 500× and a pair of young/old controls placed on either side of each depression case to assure that changes in neuron densities could be assessed in the framework of aging.

Two Laminar and Neuronal Patterns of Age-linked Amyloid-β 42 Peptide Deposition

An assessment of Aβ42 deposition uncovered three striking findings: (1) Aβ42 was observed in 9 of 10 depression cases (5 with BD and 4 of 5 with MD; no evidence of it in Case 12). (2) Aβ42 was deposited in two patterns. In the youngest cases, Aβ42 was entirely intraneuronal, mainly in large neurons in layer V, and also with significant but variable staining in outer layers III and II. In older cases, regardless of diagnosis, dense extracellular plaques were in layers I–Va with no evidence of intraneuronal deposition in any layers including layers III and V. In addition, Case #13 was a 74-year-old male who had a transitional pattern; that is, light intraneuronal deposition in ACC and the first signs of diffuse amyloid plaques in PCC. This is an important case as it confirms that these two patterns are topographically dissociable even in the same cases and it shows an approximate age at which the transition to diffuse amyloid plaques occurs. (3) As there was no expression of phosphorylated tau (AT8 antibody) or α-synuclein, these cases do not represent typical forms of diseases associated with aging such as Alzheimer's and diffuse Lewy body diseases.

Age preference

The intraneuronal pattern occurred only in young cases regardless of diagnostic group with an average age at death±SEM of 54.8 ± 8.0 (n = 4, Cases 6, 7, 8, 11). The plaque deposition occurred only in old cases with an average age at death of 80.8 ± 2.3 years (n = 4; Cases 9, 10, 14, 15). The transitional Case 13 was 74 years old at death and is not included in these latter calculations and Case 12 had no Aβ42. As 9 of 10 cases had Aβ42 expression and it appears in two patterns with an age dependence, this phenomena must be considered as an important part of the age-dependent progression of depression regardless of diagnostic category. Whether or not this is a unique feature of the analyzed cases or is a core pathology remains to be determined. The high rate of Aβ42 and its potential for neurotoxicity, however, requires that it be considered as an important player in depression.

Intraneuronal Aβ42

Figure 25.3 presents the laminar distribution of intraneuronal Aβ42 for Case 7 (63 years old; BD). The adjacent thionin-stained section was co-registered to the Aβ42 section and it shows that Aβ42-expressing neurons in layer Vb are large and form aggregates as noted at the asterisks at two levels of magnification (A and B). Expression in upper layer III is in small pyramidal neurons that are irregularly dispersed throughout this layer; sometimes extending into layer II but not usually. This suggests that layers II, III, and V are vulnerable to neuron loss and this can be assessed by comparing thionin-stained sections in control and depression cases as done below for all cases. It is an interesting fact that the youngest Case 6 (32 suicide death) had intraneuronal Aβ42 throughout her entire cingulate cortex

The details of neuron morphology with intraneuronal deposits are shown in Figure 25.4. In terms of neuron types there are large neurons in layers Va and Vb; the somata of which often lean toward other pyramids to contribute apical dendrites to clusters that pass through layer V and into layer III (Fig. 25.4; #6 ACC V). The Aβ42 deposits in the somata and apical dendrites are both solid throughout as well as in granules as shown in the magnification pullout in Figure 25.4A. Besides labeling of medium and large pyramids, there are spindle neurons with two primary and vertically oriented dendrites in layer V. The arrow in the third photograph in Figure 25.4 points to the basal process of one of these neurons. These are projection cells and are unique to rostral limbic cortices including the ACC (Nimchinsky *et al.*, 1995).

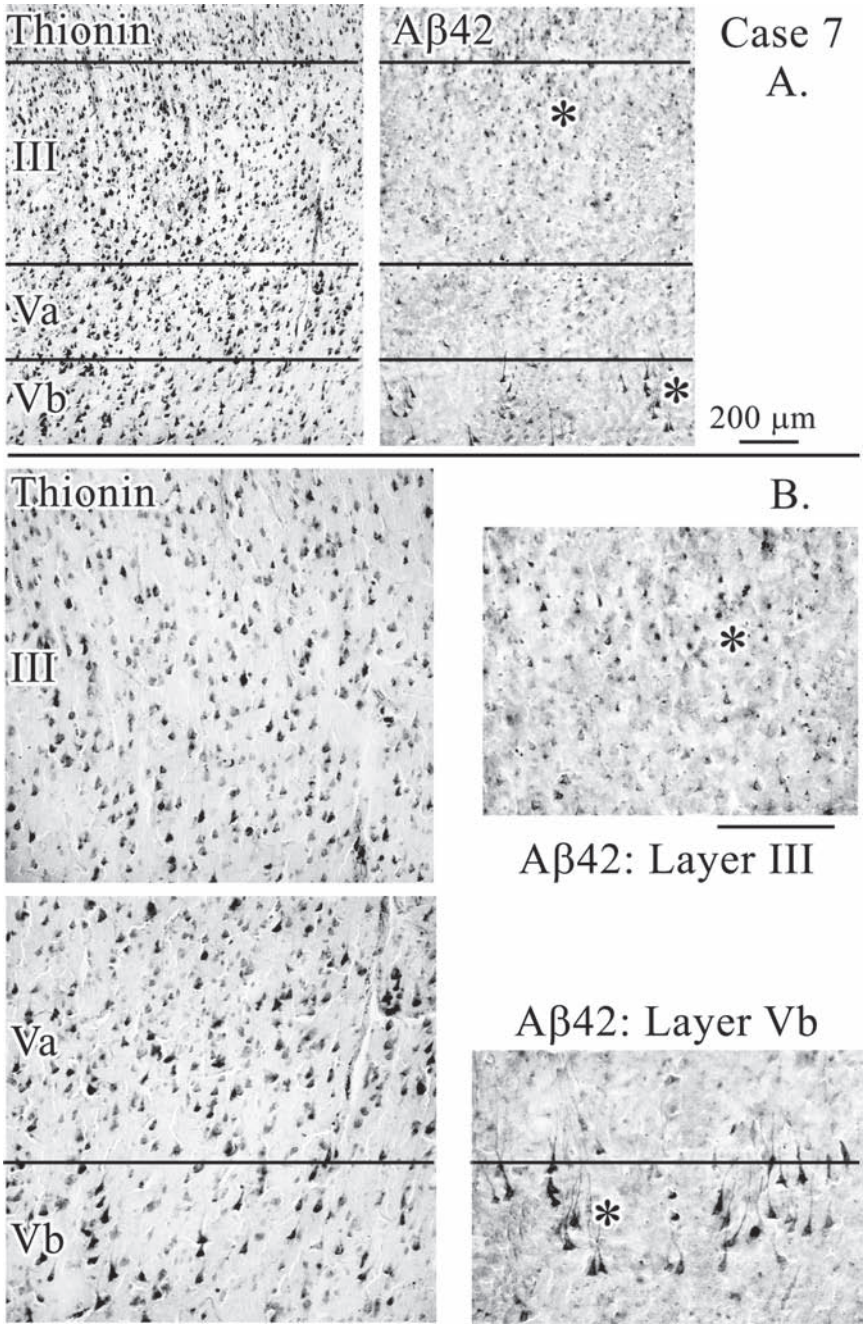

Fig. 25.3 Intraneuronal Aβ42 in area s24b of Case 7 at two magnifications (A and B). The layers in this and subsequent figures are aligned at the III/Va boundary and the asterisks in A emphasize the cell groups that are magnified in B. Two large clusters of neurons are shown in layer Vb.

A key transition: Case 13

In view of the fact that the intraneuronal and plaque patterns of Aβ42 deposition have an age dependence, Case 13 is of special note because both patterns are present therein and the patient died at age 74. It appears that the early 70s is the key point of transition between the two patterns. Figure 25.5 shows the features of Aβ42 deposition. Light staining in layer V neurons suggests this is a late stage of the intraneuronal pattern. In contrast, the posterior cingulate gyrus has dispersed-diffuse plaques on the gyral surface and a substantial increase in them on the ventral bank of the cingulate sulcus. Interestingly, a single pyramid (asterisk) had a severe perisomatic build-up of Aβ42 and might be interpreted as seeding diffuse plaques; although other mechanisms are possible and likely based on the low number of these profiles and high

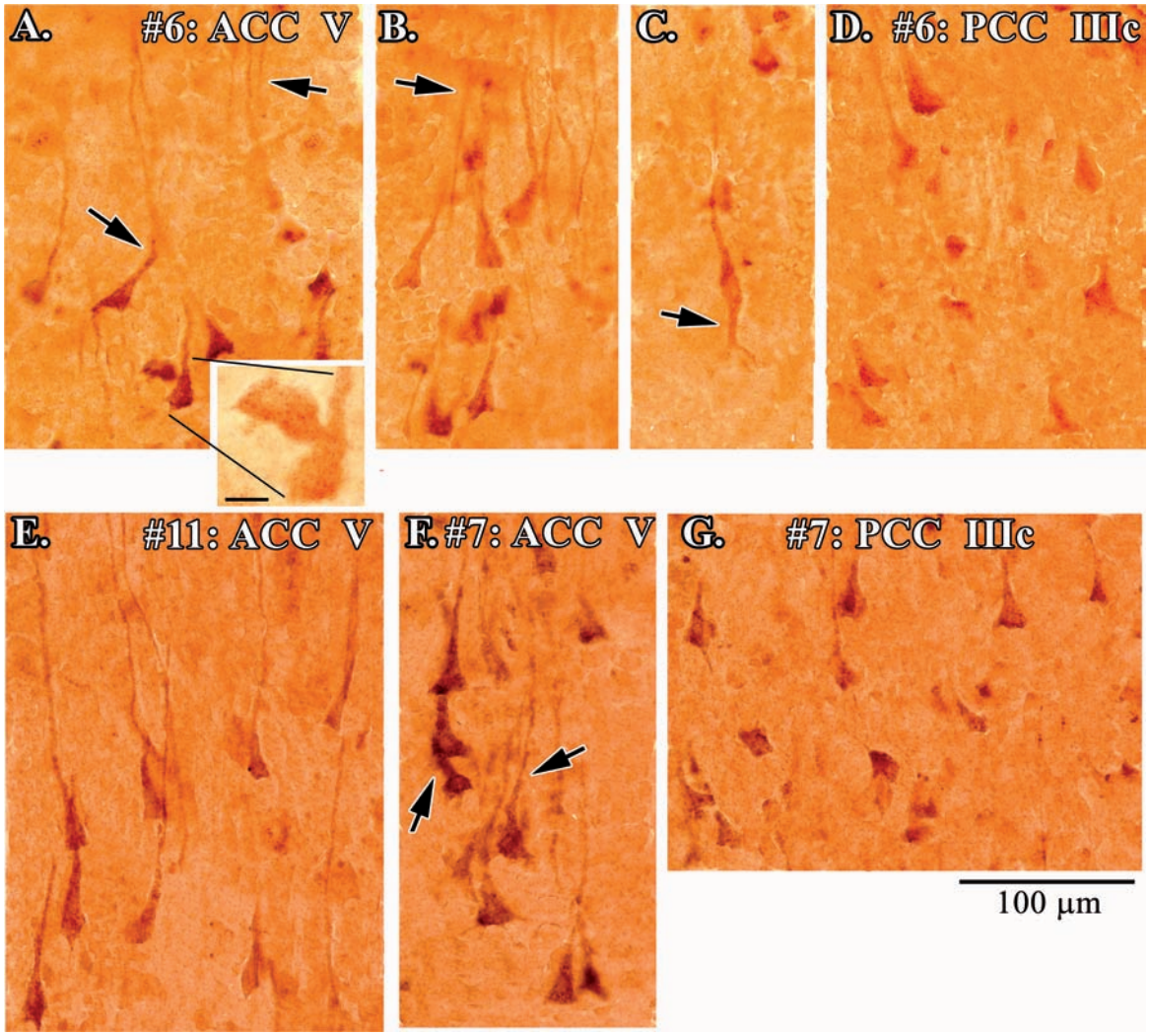

Fig. 25.4 Intraneuronal Aβ42 deposition. A. Leaning pyramidal neuron (arrow at apical dendrite and a cluster of apical dendrites); pullout magnification of two neurons with granular deposits in somata (scale, 10 µm). B. Aggregate of layer V pyramids and another dendritic cluster (arrow). C. Spindle neuron with an arrow at the primary, descending basal process. D. Large layer IIIc pyramids in posterior cingulate cortex (PCC) of same case as A-C. E. Layer V neurons lightly labeled in a young 56-year-old Case 11. F. Heavy Aβ42 labeling in sACC layer V of a 63-year-old. G. Heavy labeling of layer IIIc pyramids in PCC.

number of diffuse plaques observed. Thus, the life cycle of Aβ42 appears to involve a reduction in the intraneuronal build-up with seeding and subsequent deposition of diffuse extraneuronal plaques. With further aging as noted below, the diffuse plaques often condense into mature plaques with a central core.

Plaque Aβ42

As noted in Transition Case 13, a second pattern of Aβ42 staining is the formation of plaques in individuals that died at or over 75 years of age. These plaques were either diffuse in the 70s or also mature and containing a dense core when the patient was in their 80s. Figure 25.6 shows co-registrations of thionin and Aβ42 for Case 9; BD aged 75 at death. The diffuse plaques are labeled throughout the layers I–V without preference and it is often in a perivascular location.

A comparison with neuron densities is provided by co-registration with adjacent thionin-stained sections to initiate a consideration of the role that Aβ42 may play in neurodegeneration in depression. In case 7, patches of neuron losses can be seen in layers III and V and these are layers that contain intraneuronal Aβ42 suggesting a possible link to neuron death. However, layer VI also

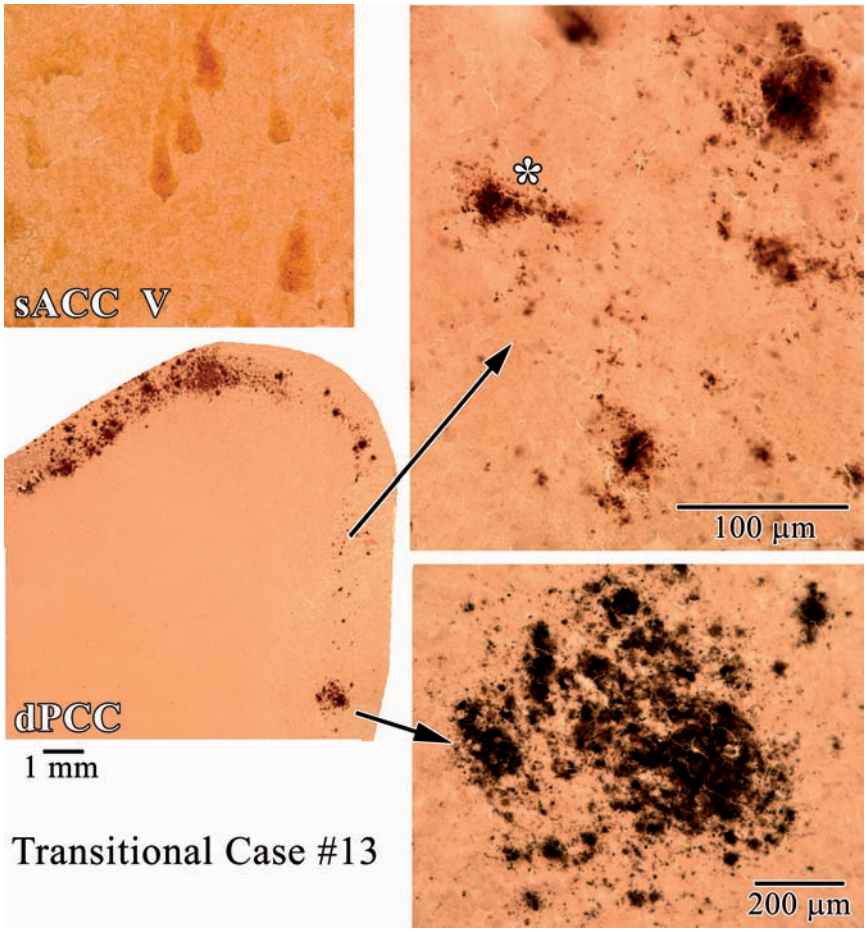

Fig. 25.5 Case 13 is unique among 9 because this individual had both intraneuronal Aβ42 in ACC and diffuse plaques in posterior cingulate cortex (PCC). The dense deposition on the ventral bank of the cingulate sulcus can be seen at low magnification (dPCC) and it is not matched on the gyral surface where two higher magnification photographs show the composition of a large diffuse plaque (bottom of two photographs) and the architecture of smaller plaques in between the latter and the dorsal bank of the cingulate gyrus (top of two). Of particular note is the large pyramidal neuron that is heavily decorated with granular deposits of Aβ42 at the asterisk suggesting a potential seeding site of diffuse plaques.

shows profound neuron losses and no intraneuronal Aβ42 has been observed in this layer suggesting death associated with another mechanism at this stage of the disease.

Case 9 in Figure 25.6 has extensive neuron loss in layers II–V; the layers with heavy diffuse plaques. Although this neuron loss could be generated by extracellular Aβ42, there is also extensive neurodegeneration in layer VI where virtually no plaques occur except for a few mature ones. It is possible in this instance that extracellular Aβ42 is toxic to layer VI neurons as they frequently have apical dendrites that project through layer V.

Thus, the laminar co-distribution of intraneuronal and extracellular plaques provides one or more sources of neuron death. It is also possible that the shift in the pattern of Aβ42 deposition is associated with additional mechanisms of neurodegeneration. According to this model, neuron losses may build progressively as the pattern of Aβ42 shifts.

Plaque Aβ42 Deposition in Posterior Cingulate Cortex

Although less frequently analyzed and discussed, the PCC has been implicated in depression and this suggests a wide cingulate involvement and the potential to select among possible mechanisms of neurodegeneration. A reduction in glucose metabolism was observed in unipolar subjects that were antidepressant drug responsive (Mayberg *et al.*, 2000; Chapter 24) and volumetric reductions in PCC have been reported early in BD (Kaur *et al.*, 2005). Having already observed intraneuronal Aβ42 in PCC and plaques in the transitional Case 13, the search for this protein in the PCC in older individuals will likely prove useful.

Figure 25.7 provides a comparison of ACC and PCC for the two oldest cases with BD and MD. If one expects that sACC is a diagnostic region for depression as might be concluded from the extensive literature on the

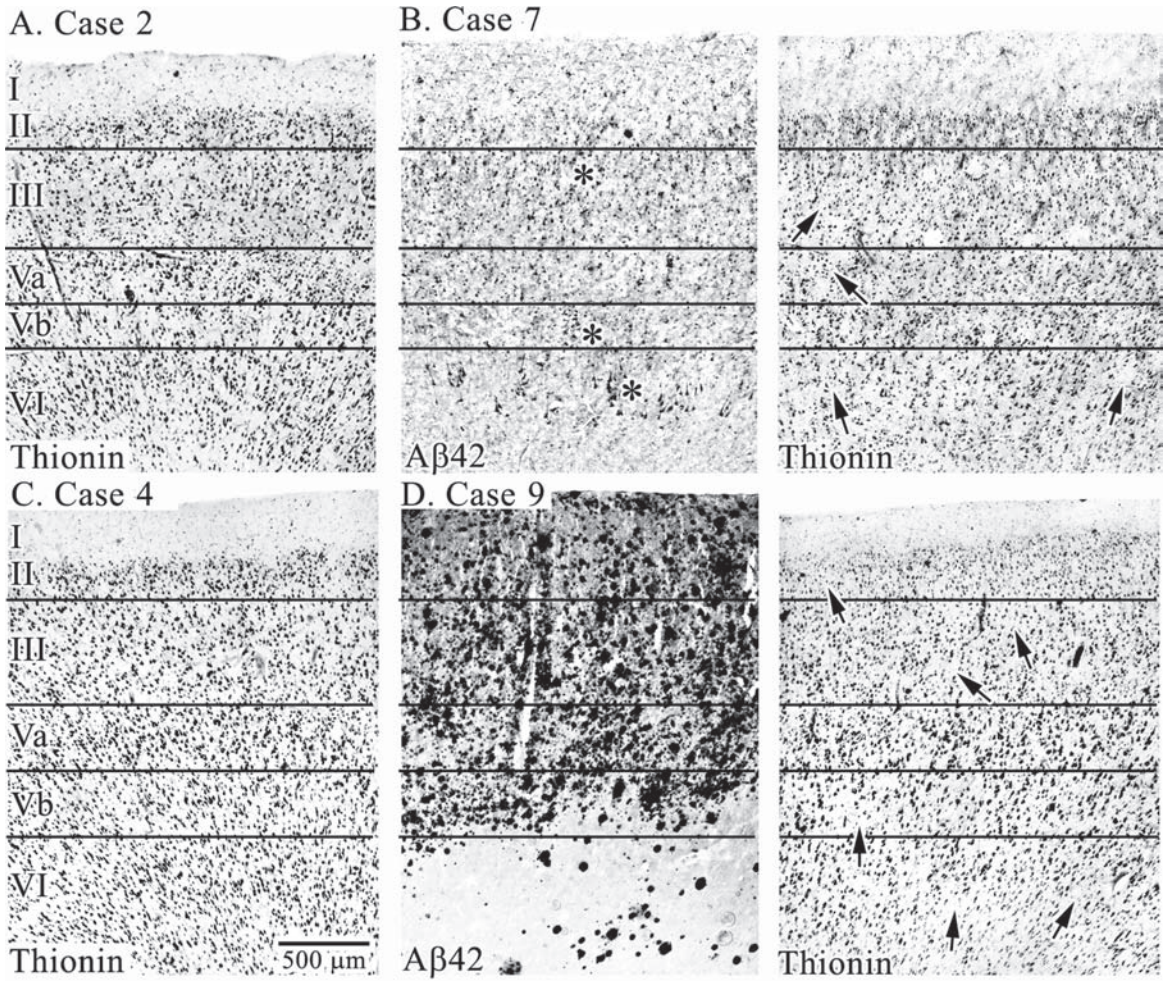

Fig. 25.6 Comparison of anterior cingulate cortex (ACC) control case thionin (2, 4) with young (B. Case 7) and old (D. Case 9) BD cases including the Aβ42 of which none were in controls. Some Aβ42-positive neurons in Case 7 are emphasized with asterisks and the details shown in Figure 25.4. The second pattern of Aβ42 is shown for Case 9 with diffuse plaques extending throughout layers I–V. Neither BD case appears to have normal cytoarchitecture. Some areas of neuron sparcity are emphasized with arrows.

subject, the likely hypothesis is that the aggregate density of diffuse and mature plaques would be greatest in ACC as shown for Case 9. Surprisingly, this is the only case for which that occurred. Cases 10, 14, and 15 all had richer deposits of plaques in dPCC than in ACC. The possible reason for this is that Case 9 was only 75 at death, while the other three cases were in their eighth decade. This would suggest that plaques in ACC are at a later stage as demonstrated with a higher density of mature/dense core plaques. This is indeed true. In each instance there is very little diffuse plaque in ACC suggesting that in the eighth decade, depressed patients experience a terminal aggregation of Aβ42 into dense core plaques.

Case 10 dPCC (Fig. 25.7, low magnification insert) shows an enhanced deposition in the dorsal bank of the cingulate gyrus, suggesting once again as with Case 13, that the earliest diffuse plaque formation has a regional selectivity in PCC. Thus, the hypothesis that plaque deposition begins in ACC and progresses to PCC is confirmed by the forms and densities of Aβ42 plaques.

Amyloid-β Peptides in Depression: An Aging Dimension

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). Moreover, human cortex that is vulnerable to AD, that is, mild cognitive impairment, accumulates intraneuronal Aβ42 (Gouras *et al.*, 2000). It is not yet known why neurons in adult cases of depression sequester intraneuronal Aβ42 as reported

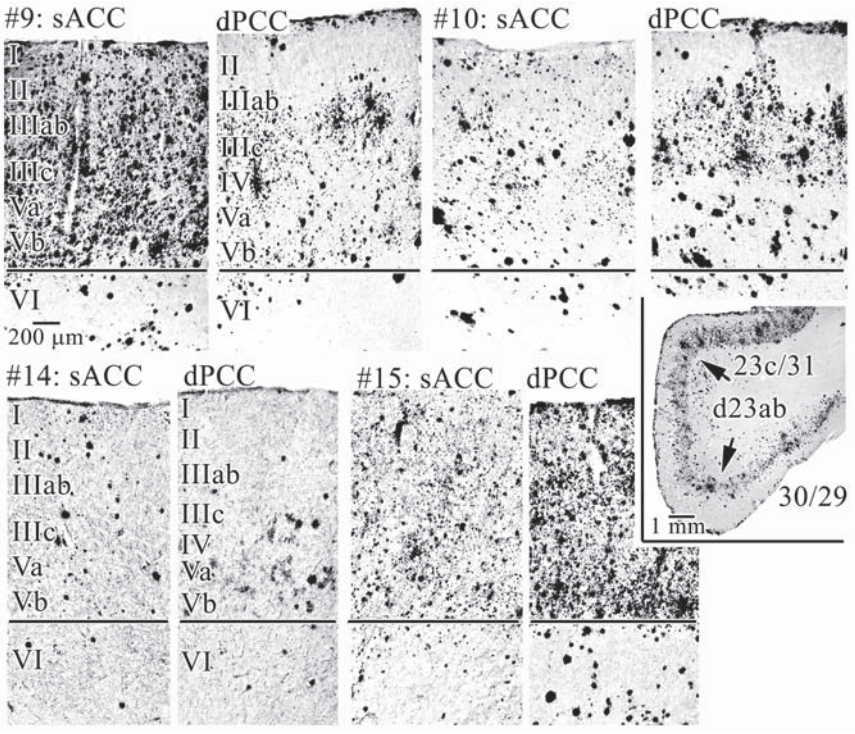

Fig. 25.7 Comparison of Aβ42 deposition in anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC) in two bipolar disorder (9, 10) and two major depression (14, 15) cases. The only case with higher ACC plaques is #9 who was 75 at death. In contrast, higher Aβ42 plaque densities were observed in the other three cases in the eighth decade. Evidence that ACC is further along in progression is the proportionately more mature plaques in ACC than in PCC.

here but it may also contribute to neuron death as also shown in AD (Sheng *et al.*, 1998; Parvathy *et al.*, 2001). As no fibrillar tau was observed in depression, however, this damage may not be linked directly to Alzheimer neuropathology.

The two laminar patterns of Aβ42 deposition can be used as landmarks to assess laminar patterns in neurodegeneration; even though they are not diagnostic of a particular clinical subgroup. Indeed, their presence in both BD and MD at similar ages suggests an underlying age-linked phenomenon that may indicate common stages of disease progression. Two important questions arise from the presence of Aβ42 in cingulate cortex in cases of depression. First, to what extent is intraneuronal deposition toxic and may account for at least part of neurodegeneration observed in cingulate cortex? Second, as autonomic dysregulation has been observed in AD and its markers have been observed in sACC (Chu *et al.*, 1997), to what extent is the presence of this peptide reflective of age-linked neurodegeneration?

The appearance of a unique conformation of tau (recognized with the antibody MC1) is also an early event in AD (Weaver *et al.*, 2000). Although the MC1 antibody was not assessed in the present cases, no case expressed the early phosphorylation of tau as shown with the AT8 antibody and there were no neurofibrillary tangles in these cingulate gyri as is true for mild cognitive impairment and AD. Interestingly, however, the sACC is impacted by AD (Chu *et al.*, 1997) and there is evidence of impaired autonomic function in such cases (Ahron-Peretz *et al.*, 1992; Algotsson *et al.*, 1995), although a direct link between this pathology and altered autonomic functions has not been demonstrated.

The description of Aβ42 deposition above included a consideration of age dependency for the two patterns. Although the bimodal distribution of the two patterns is revealing, transitional Case 13 is particularly important because it showed that both patterns can occur in the same brain, they do not overlap and area 74 is a critical time for this shift. From these observations we can propose a time course that is age dependent and appears in both forms of depression. This progression is summarized in Table 25.2. The keys to this progression include a full cingulate deposition of intraneuronal Aβ42 during early adulthood which continues until

TABLE 25.2 Hypothetical Amyloid Progression

1	Late adolescence–early adult; intraneuronal Aβ42 layers II–V of entire cingulate cortex
2	Sixth decade; intense and disruptive Aβ42 deposition
3	Seventh decade; transition to diffuse plaques includes lightening of intraneuronal expression and aggressive extrusion contribute to seeding of diffuse plaques in sulcal cortex
4	decade; extensive mature plaques in ACC and more diffuse plaques in PCC

ACC, anterior cingulate cortex; **PCC,** posterior cingulate cortex.

the neurons are quite dark with the Aβ42 antibody reaction. By the mid-70s the first extracellular, diffuse plaques appear in sACC and the intraneuronal deposits lighten/are reduced. Aggressive extrusion of Aβ42 by some pyramidal neurons could lead to seeding of diffuse plaques. Indeed, it has been proposed that GM1 ganglioside-bound Aβ may initiate Aβ aggregation by seeding fibril formation and could lead to plaque deposition (Hayashi *et al*., 2004). As diffuse plaques build up in dorsal PCC those in ACC are forming mature plaques with cores and the diffuse plaques disappear. During the eighth decade the entire cingulate cortex is damaged to some extent by Aβ42 deposition.

Regulating Amyloid Deposition as a Therapeutic Approach to Depression

This is the first report of an amyloid-peptide buildup in depression and the proportion of Aβ42-positive cases is very high (9 of 10). The presence of the neurotoxic Aβ42 peptide in the absence of tau neuropathology (no AT8 antibody expression in any case of depression), suggests that an amyloid mechanism of neurodegeneration occurs in BD and MD. The Aβ42 peptide is expressed in MCC in mild cognitive impairment (Johnson *et al*., 2004; Chapter 33) and in PCC in early clinical stages of AD during the first few years of diagnosis (Vogt *et al*., 1998; Chapter 35). Even in prodromal AD, the tau and amyloid deposition do not overlap in the same layers and areas and generally seem to have differentiable mechanisms (Chapter 33). The lack of cognitive symptoms in the present 10 cases and tau neuropathology indicates these cases of BD and MD do not reflect AD neuropathology. Finally, as Aβ42 was observed at ages 32 and 56 in postmortem cases of depression, this event is certainly a midlife problem and could arise even earlier in the disease.

The prominent role of amyloid-β peptides in AD and the documented role of Aβ42 in disease etiology, provide important strategies for removing these peptides in depression and possibly for interrupting some or most of the neuron death. The amyloid hypothesis is well established as an etiology of neurodegeneration in AD and strategies for clearing or reducing amyloid-mediated neurotoxicity have been proposed (Hardy & Selkoe, 2002). As noted by these authors, the build-up of amyloid-β peptides could be reduced by blocking the β- and γ-secretases that are responsible for generating the long amyloid-β peptides such as Aβ42. Relevance of β-site amyloid precursor protein (APP) cleaving enzyme 1 is shown in mice deficient in this enzyme that have no Aβ42 expression (Luo *et al*., 2001).

Another approach to blocking amyloid-β peptide buildup is to reduce cholesterol levels either through diet and/or with cholesterol lowering drugs. Chronic use of cholesterol lowering drugs such as the statins has been associated with a lower incidence of AD (Wolozin *et al*., 2000) and high cholesterol diets increase amyloid-β pathology in experimental animals (Sparks *et al*., 2002).

Direct removal of amyloid-β peptides has been an important strategy as shown in rodents but there has been no success yet in human vaccination trials. As this strategy has seen success recently in primates by vaccination of rhesus and vervet monkeys with aggregated Aβ42 (Gandy *et al*., 2004; Lemere *et al*., 2004), there may be technical challenges still to be overcome with this approach in humans and these will need to be resolved to remove Aβ42 in depression.

Another strategy for reducing potentially toxic amyloids is by modulation of cholinergic function. Indeed, one of the earliest suggestions of a central mechanism of depression was a cholinergic hypothesis (Janowsky *et al*., 1972). It appears that muscarinic agonists shift enzymatic processing of APP from the amyloidogenic pathway involving β- and γ-secretases to the non-amyloidogenic α-secretase pathway (Lin *et al*., 1999). Huperazine A is an acetylcholinesterase inhibitor that has been reported to improve memory in adult humans (Zangara, 2003). In human embryonic kidney 293 APP Sweedish mutant cells, huparizine A stimulates αAPP release and diminishes amyloid-β generation (Peng *et al*., 2006). Huparizine A alters APP processing in human neuroblastoma SK-N-SH cells via protein kinase C and MAP kinase pathways (Peng *et al*., 2007). Thus, acetylcholinesterase inhibitors, including huparizine A, may be used to shift enzymatic activity toward the non-amyloidogenic pathway to reduce the level of amyloid-β peptides expressed in depression.

Neurodegeneration in Depression

Neuron densities are considered in the context of changes in the normal laminar architecture in 50-μm-thick sections so that changes can be linked to laminar alterations of Aβ42 deposition and neuron shrinkage. With thick sections, each area can be digitally photographed in a 2.5 mm wide strip providing high contrast images for a large sample volume (e.g., width 2,500 μm × height for superficial and deep layers about 5,000×50 μm = 0.625 mm^3). The selection process during photography is meant to reflect the common status of a cortical area and is not biased toward the most or least neuron loss and a wide field of view in each photograph assures a good sample from which to draw conclusions. In the past when drawings of cortical strips were used to assess neuron densities, the perikarya were drawn in three strips that were 160 μm in width (Vogt *et al*., 1990) providing a narrow sampling window when compared with current digital imaging methods. Here the field

of analysis can reach more than ten times the earlier limits at the current 1,200–2,000 μm in width.

Neurodegeneration is also assessed in terms of Aβ42 deposition with co-registration to thionin-stained sections. This does not mean that we consider all or even part of neuron loss a matter of Aβ42 toxicity, although this is a new hypothesis generated by the present sample. Case 12 had no evidence of Aβ42 immunoreactivity yet there was clear evidence of neuron loss as discussed below. As this patient died at the age of 68, they may have been at a transition point that involved a loss of intraneuronal Aβ42 and a failure or delay in plaque formation. Alternatively, no Aβ42 was ever present in this case and neurodegeneration must be explained by an alternative route. Even when this peptide is present, multiple mechanisms of neuron losses are possible.

An overview of cytoarchitectural changes in two BD cases were shown in relation to Aβ42 deposition in Figure 25.6. At this low magnification, damage can be seen in both cases in layers III and V with less in layer II. Here we magnify further superficial and deep layers for BD and MD. All cases of depression had neuron loss in at least one or two layers and the younger adults had least.

Bipolar disorder

Least neuron damage is in Case 6 (32 years old) and Figure 25.8 emphasizes some sites of neuron loss with arrows pointing in layer II to two patches of shrunken neurons above which are areas of reduced neuron densities. In layer III two large circles emphasize neuron shrinkage and possibly some neuron loss. It appears that neurodegeneration is in an early stage in Case 6 when compared with the other cases. Indeed, Case 7 has somewhat more neuron loss in layers II and III, and cases 8 and 9 have profound loss throughout all of layers II and III. Finally, case 10 has significant neuron shrinkage but overall neuron densities in layers II-III appear closer to that in Case 6.

In terms of the deep cortical layers in ACC, Figure 25.9 emphasizes the following observations. Although all neurons do not shrink and there is no layer with complete neuron loss, there are clearly sites of both in most cases. Case 8 had severe neuron loss in layers II and upper III and the deep layers are more preserved and have only some points of neuron sparcity in layer VI. As a rule in these cases, layer Va is least affected with some points of neuron shrinkage but less patent neuron loss. Once again, points of neuron shrinkage increase with age, but the association is not tight as Case 7 also experiences significant shrinkage and neuron loss in the deep layers.

Major depression

The patterns of neurodegeneration in ACC are more restricted in MD than in BD. To begin, the control in Figure 25.10 is an older individual at age 80 and they appear to have fewer overall neurons than does Case 11 of MD. The latter case was only 56 at death and this likely accounts for differences in neuron densities. Case 13 is the transitional case in terms of Aβ42 deposition and it also appears to be relatively normal. Thus, cases 11 and 13 have approximately normal numbers of neurons in the superficial layers. Only Cases 12, 14 and 15 have reduced neuron densities in layers II–III and these differences within MD and the control are not selective for either layer. There was also an observable number of shrunken neurons scattered throughout these layers.

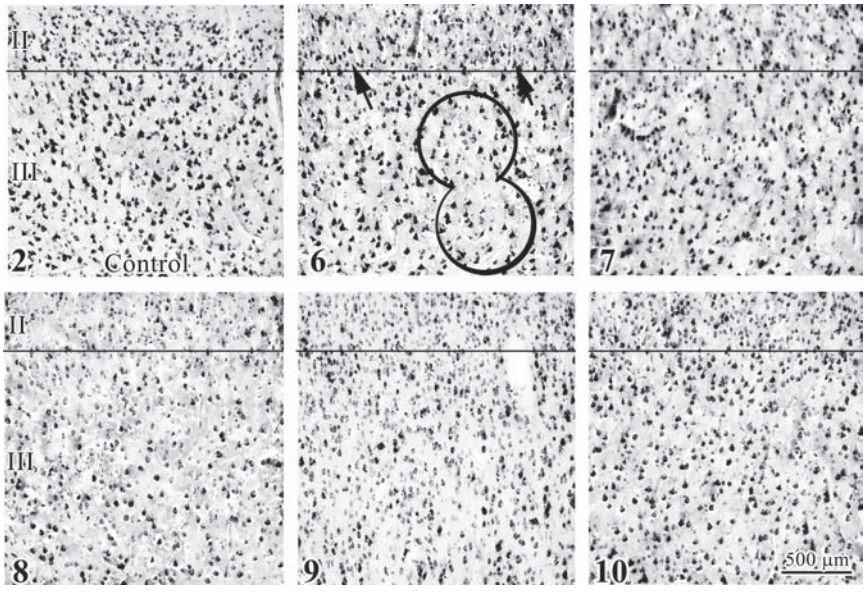

Fig. 25.8 Thionin-stained superficial layers in bipolar disorder and a control. As differences from the control and other cases are subtle, arrows pointing into layer II Case 6 indicate two groups of shrunken neurons above which are areas of clearing indicating neuron loss. Neuron shrinkage and losses are more profound in all other cases, although that in Case 10 appears to reflect mainly shrinkage rather than loss *per se*.

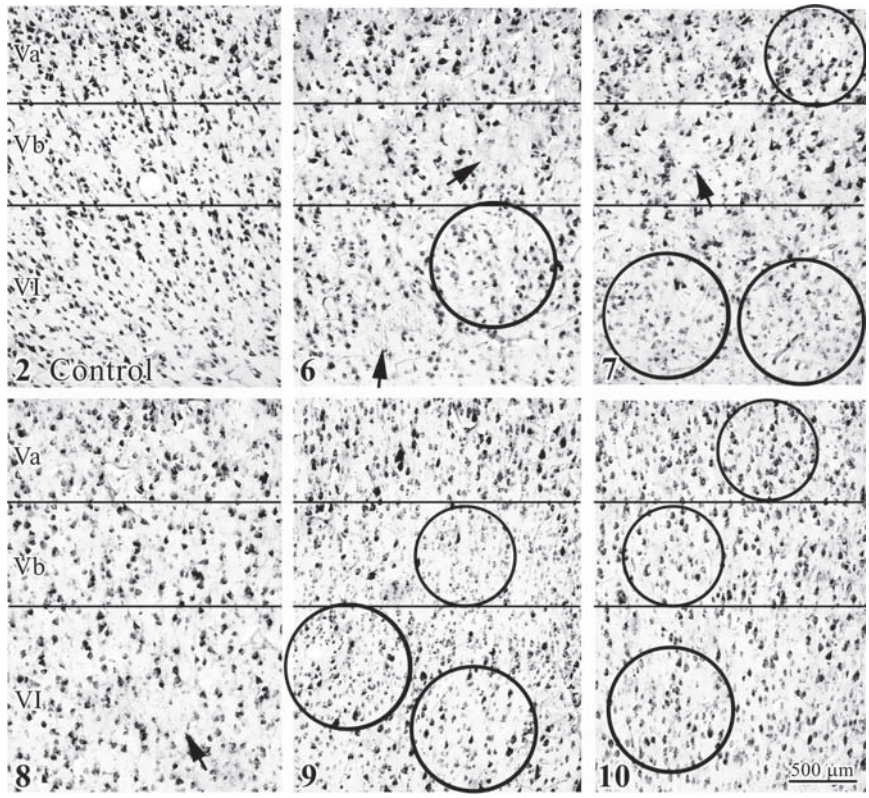

Fig. 25.9 Thionin-stained deep layers in BD and a control case. The circles emphasize sites where neuron shrinkage is profound and there are likely active sites of neurodegeneration. Arrows point to sites of neuron sparcity and are likely places of spotty neuron losses. In no instance is a complete layer lost or uniformly involved.

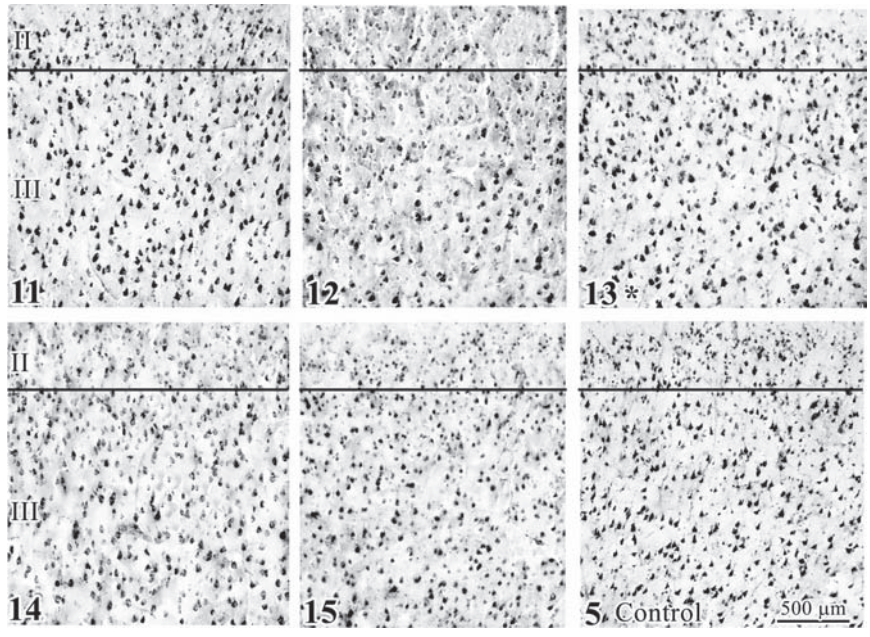

Fig. 25.10 Thionin-stained, superficial layers of anterior cingulate cortex in major depression and a control. Cases 11 and 13 have neuron densities similar to the old control, while Cases 12, 14, and 15 have significant amounts of neuron shrinkage and loss.

The deep layers of ACC have neuron loss that is very apparent in MD as shown in Figure 25.11. The layer with the greatest overall losses is layer Vb; in no instance does it appear to be intact. Next, layer VI is severely involved in Cases 11, 12, 14, and 15. Only Case 13 appears to have an approximately normal density of neurons in layer VI. Finally, layer Va is severely involved in Cases 12, 14, and 15 with lesser involvement in Cases 11 and 13. Overall, deeper layers are more impacted than superficial layers in MD and there seems to be clear age-link as Cases 14 and 15 are the oldest and most impaired.

Summary of Neuron Loss by Clinical Subgroup

Table 25.3 summarizes the laminar changes in neuron densities based on qualitative assessment expressed with a 0–4+ scale. No 4+ values were observed in which severe neuron loss equates to essentially no neurons in a layer (i.e., 76–100% loss). The numbers provide an estimate of neuron loss and the columns and rows are summed to provide perspective on cases and layers with greatest loss. Obviously, there are many volumetric

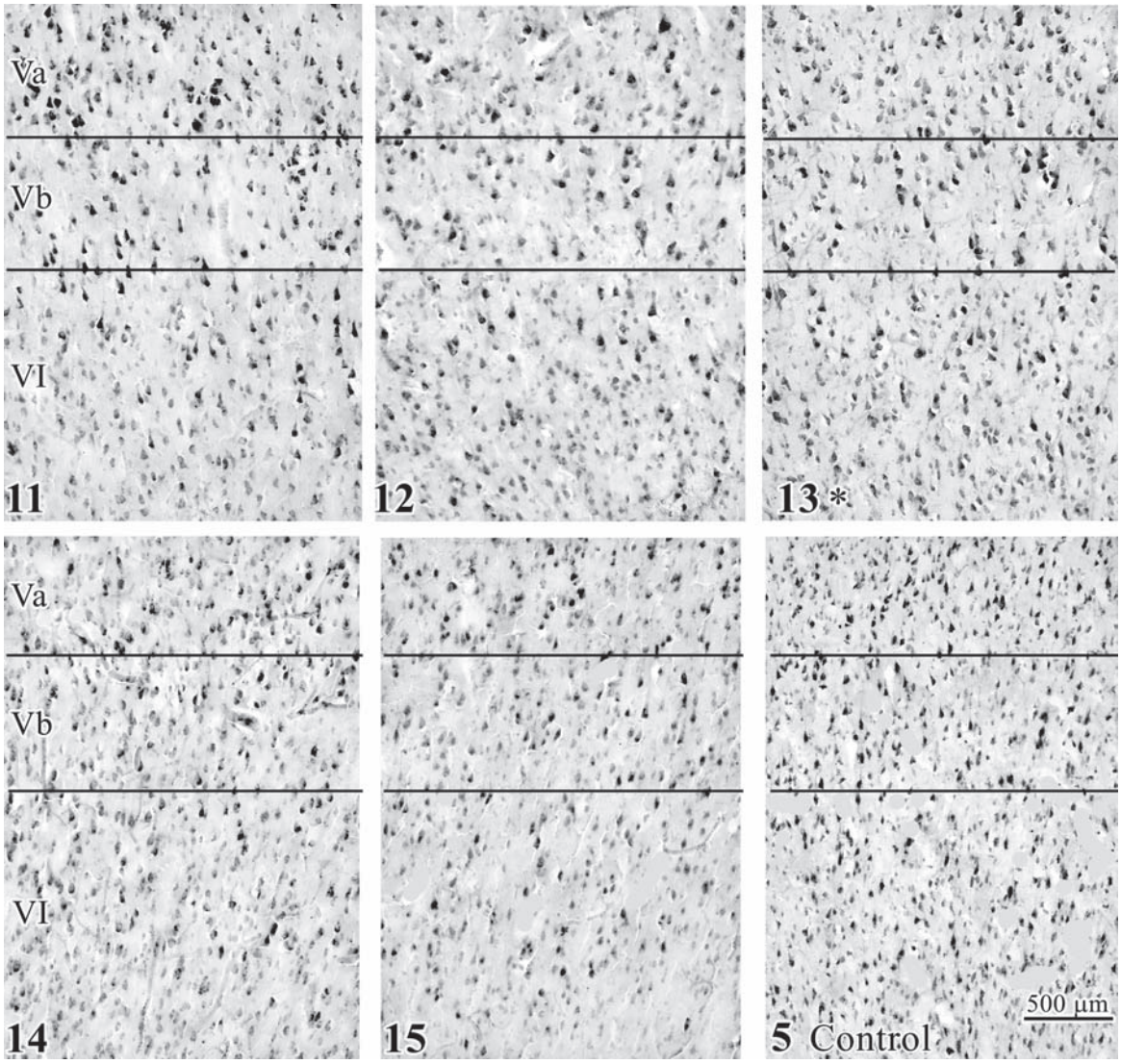

Fig. 25.11 Thionin-stained, deep layers of anterior cingulate cortex in major depression and a control. Greatest overall neuron loss is in layer Vb with no case appearing intact. Layer VI is severely involved in Cases 11, 12, 14, and 15; only Case 13 appears approximately normal. Layer Va is severely involved in Cases 12, 14, and 15 and to a lesser extent in Cases 11 and 13.

issues that must be resolved with a rigorous counting strategy.

As a rule, the older patients had greater neurodegeneration and Table 25.3 provides the age next to each case number. In addition to a generally increasing loss with age, the two cases with greatest overall loss were 75 and 82 years old. Furthermore, losses in both categories of depression followed a similar pattern though layer II of BD had a generally greater loss than other layers and that in MD was greatest in layer V. Thus, neurodegeneration in area s24b occurs in all cases, it appears to have an age link but not all cases fit this profile, and it may have a limited diagnostic value for each subgroup with neuron loss greatest in superficial layers in BD and more pronounced than in MD and vice versa for the deep layers. Interestingly, dorsal PCC provides a substantially different view of neurodegeneration in depression and a potential site for diagnostic differentiation of BD and MD.

TABLE 25.3 Laminar changes in area s24b neuron densities*

Layer		II	III	Va	Vb	VI	Totals
	Case/age						
BP	6/32	++	+	++	++	+	8
	7/63	++	+	+	++	++	11
	8/68	+++	+++	++	0	+	13
	9/75	+++	++	+	+++	++	15
	10/86	+	+	++	0	++	9
MD	11/56	0	0	0	++	++	6
	12/68	+++	+++	+++	+++	+	16
	13/74	+	0	++	++	0	5
	14/80	++	+	++	0	++	10
	15/82	+++	++	+++	++	+++	18
Subgroup totals							
BD		11	8	8	7	8	
MD		9	6	10	9	8	

BD, bipolar disorder; MD, major depression.
*Does not include estimates of neuron shrinkage; 0 differences from control, 1+, 2+, 3+. No severe losses at 4+ observed. Totals are the sum of all pluses.

Neuron Loss in Posterior Cingulate Cortex

The PCC has been implicated in depression with a reduction in glucose metabolism observed in unipolar patients that were antidepressant drug responsive (Mayberg *et al.*, 2000; Chapter 24) and volumetric reductions in PCC have been reported early in BD (Kaur *et al.*, 2005). Deposition of Aβ42 shown here also includes PCC, although at a lower level than in sACC in most cases and neuron loss also occurs in PCC.

Figure 25.12 shows dorsal area 23b in control (young #2 and old #5), BD and MD cases. In this analysis, neuron densities in each layer were assessed at 500× and the two controls were placed on either side of each strip from a depression case. A single arrow was placed at that point in any layer with observable neuron loss to emphasize points of greatest loss; realizing that in most instances neuron losses are widely distributed beyond these points; indeed, losses are scattered throughout all layers that received an arrow. The six cases were selected for the figure based on changes in area s24b; that is, they were selected on *a priori* grounds and not selected after the analysis shown in Table 25.3 and before photographs were taken or analyzed. The sample selection was based on the following observations. BD Case 6 was the youngest age at death and had least neuron losses in s24b; Case 7 had greatest losses in layers II and III; Case 9 had greatest losses in layer Vb; MD Case 11 was the youngest age at death and had lowest overall losses in area s24b; Case 12 had greatest losses in layers II and V, while Case 15 had greatest losses in layers II, V, and VI and was the oldest of all cases at death.

Figure 25.13 shows that losses in Case 6 were mainly in layers II and Vb. As this is the youngest (suicide) case at death, this is the greatest loss in these layers that might be expected. Interestingly, it is greater than that observed in area s24b; compare this case in Figure 25.12 with Figure 25.8 #6. Of all the BD cases, Case 7 had the greatest overall neuron losses in layers II, IIIab, V, and VI; however, also reflecting a greater overall degeneration than noted in area s24b. Case 9 had greatest losses in layers II–IIIab and VI with relatively preserved layers in between. Interestingly, in area s24b, the density of neurons in layers II and IIIab were lower than in PCC. Thus, overall, neuron loss was greater in dPCC than in sACC; rather a surprising finding for a disease that supposedly impacts subgenual cingulate cortex to the greatest degree. It is interesting to note that layer IV of dPCC in BD appears to be essentially intact. Finally, in all instances where there are significant neuron losses, there is a commensurate shrinkage of the remaining neurons. In other words, neuron shrinkage is not an independent and unique finding in either category of clinical depression.

It is striking that every layer in dPCC in every case of MD experiences substantial neuron loss; including layer IV. This is quite different from BD. The youngest MD case at death had limited overall neuron losses in area s24b, while pronounced changes in area d23 were in

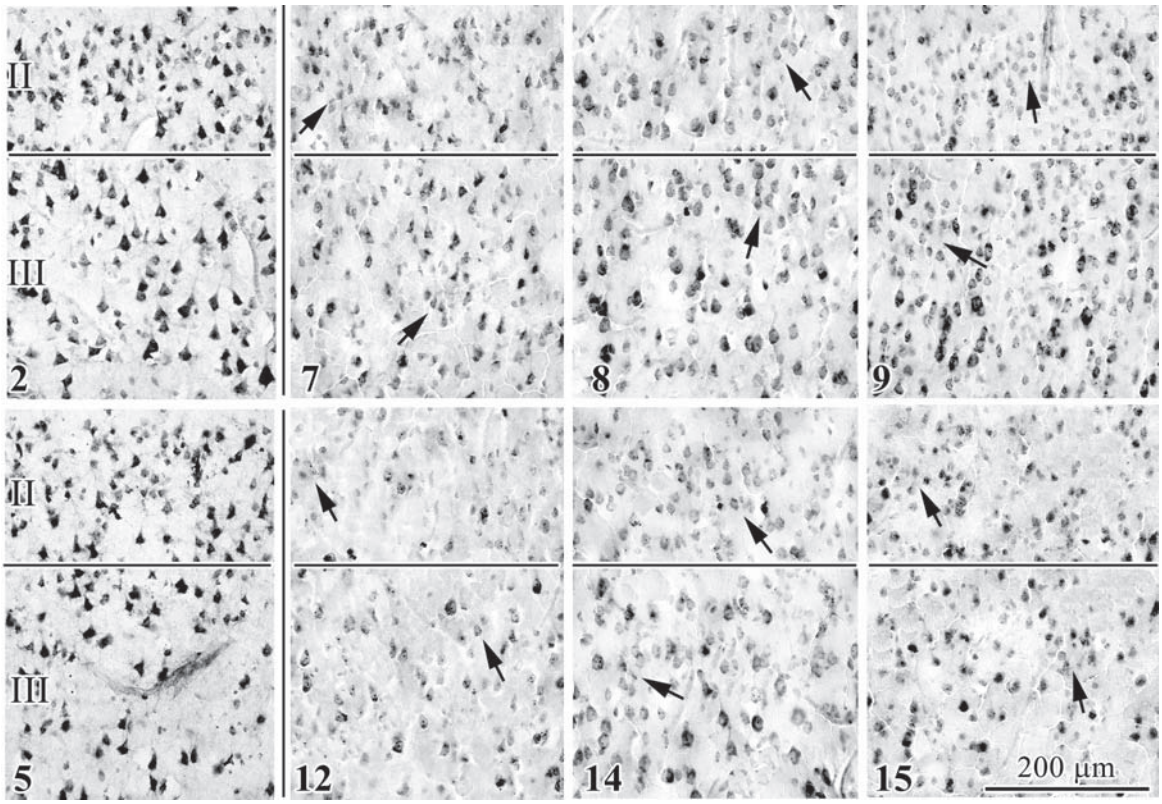

Fig. 25.12 Thionin-stained sections of dorsal posterior cingulate cortex in two control (2, 5) and three bipolar disorder (BD) and major depression (MD) cases selected ***a priori*** as detailed in the text. The arrows are points with greatest overall neuron loss in each layer as assessed at 500× with each cortical strip between the control cases. Extensive laminar neurodegeneration is most pronounced in MD and this subgroup experiences profound losses in layer IV that do not appear in BD; see next figure.

all layers. Indeed, the greatest neurodegeneration of all cases occurred in dPCC in Case 11. Although still 56 at death, this case suggests that PCC is vulnerable in MD early in the disease. The other two MD cases in Figure 25.13 show substantial neuron losses in all layers of area d23.

The conclusions for neuron loss in MD are similar to those for BD in that losses are greatest overall in dorsal posterior than in sACC. However, neuron densities in dPCC can be used to differentiate BD and MD with the latter experiencing the earliest and most extensive neurodegeneration that includes layer IV. One way to provide a diagnostic perspective is to look at overall neurodegeneration and then consider layer IV. If layer IV is substantially impaired and there is an overall laminar reduction in neuron densities, the case is likely one of MD.

Neuron Shrinkage

Neuron shrinkage has been reported in MD with an 18% reduction in layer VI and a similar trend in layer V, but no changes were observed in BD (Cotter *et al.*, 2001). Chana *et al.* (2003) observed reductions in somal sizes in BD (-16%) and MD (-9%) in area 24c in the dorsal cingulate sulcus. In contrast, Benes *et al.* (2000) found no changes in neuron sizes in BD. To the extent that neuron shrinkage is associated with neurodegeneration and there is an age-linked progression in this process; it might be expected that differences in patient ages would influence these interrelated events. The observations above show less neuron loss in BD than in MD and, to the extent this is linked to neuron shrinkage, this is compatible with most other observations on the latter process.

A review of the present cases shows neuron shrinkage occurs in all cases and it is most dramatic in those layers where there are active regions of neurodegeneration; that is, points of clear reductions in neuron densities as marked in the above figures with arrows and circles. Although a stereological study has not yet been performed on these cases, they have been studied in 50-µm-thick sections and this provides for an assessment of differences in laminar cytoarchitecture that are associated with neuron shrinkage. Figure 25.12 shows layer II and the superficial part of layer III of area dPCC for two

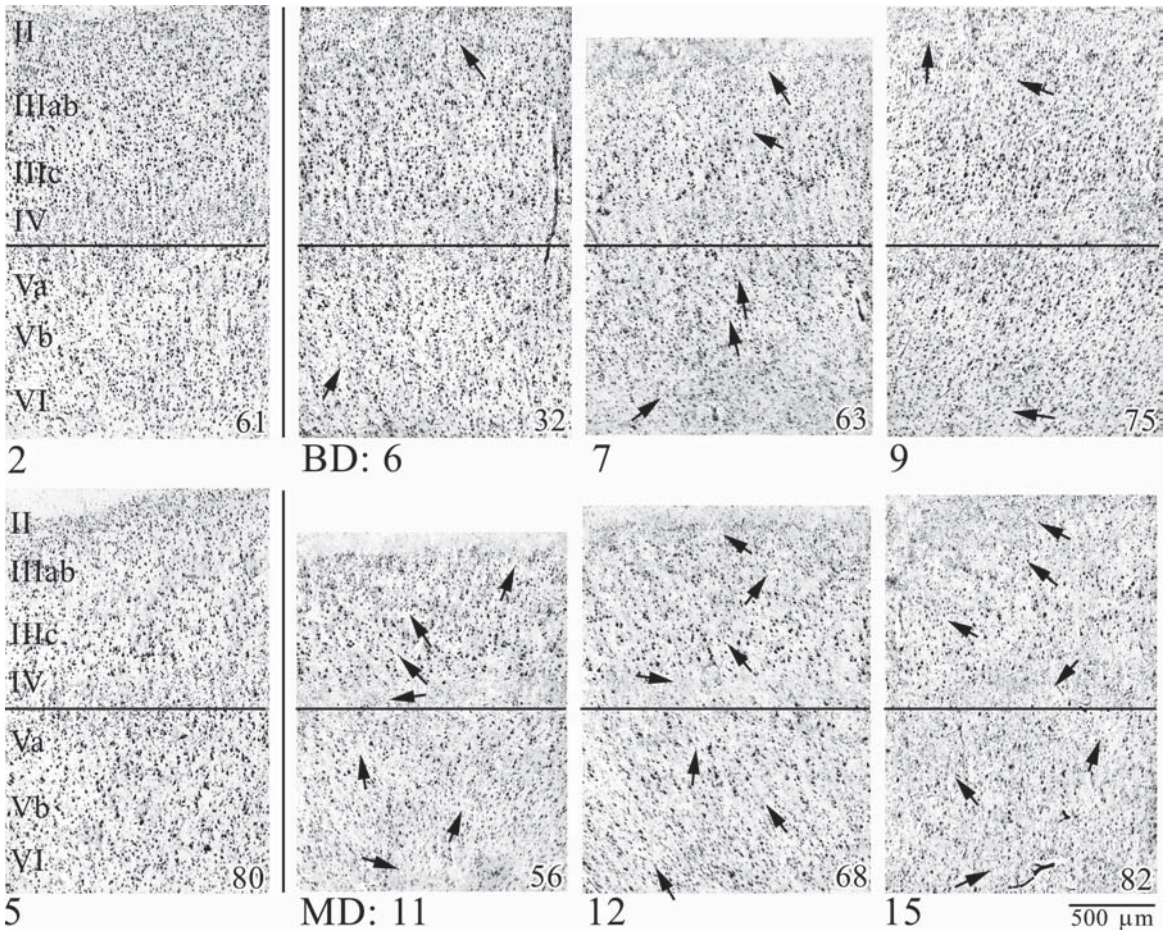

Fig. 25.13 Neuron shrinkage occurs in every case and is most apparent at those sites that have significant neuron loss. Arrows point to examples of neuron shrinkage in each case. Cases 2 and 5 are controls, 7–9 BD, and 12–15 MD.

control and three each of BD and MD cases. One arrow in each layer emphasizes patches of shrunken neurons in each layer. A further magnification is considered below so the structure of shrunken neurons is apparent and it can be linked to sites of active neuropil. Active neuropil are sites where there is an above average density of glia likely including both microglia and phagocytic astroglia. Thus, shrinking is a part of the neurodegenerative process and it is not unique to diagnostic category except the extent to which neuron loss is so linked. Shrinkage in dPCC can be used to differentiate BD and MD but only as a secondary factor associated with neurodegeneration.

Neuron Shrinkage, Glia, and Active Neuropil

In view of the many studies showing a reduced volume and metabolism of sACC in depression reviewed above and in the previous chapter, it is surprising that a report of neuron and glial densities showed only a reduction in glial cells in depression without any changes in neuron densities in either BD or MD (Ongür *et al.*, 1998). In contrast, Bouras *et al.* (2001) showed that BD is associated with significant reductions in the thickness of layers III, V, and VI and reductions in neuron densities in these layers in sACC in BD, although not in MD. A study of area p24b showed deep layer neuron loss with no changes in glial cell densities (Cotter *et al.*, 2001). In another study, an increase in intracellular adhesion molecule-1 expression was reported for area 24 suggesting an inflammatory response (Thomas *et al.*, 2004) and suggesting an increase in glial activity rather than a reduction. Another study showed reduced neuron somal sizes and an increase in neuron densities in area 24c (Chana *et al.*, 2003). Finally, a study of neurons expressing calcium-binding proteins reported a reduction of these neurons in layer II of area s24 that was not

significant after correction for multiple comparisons (Cotter *et al.*, 2002).

Although there may be increases and/or decreases in glial densities depending upon the age of the sample population and the average stage of their disease, the view that depression is primarily associated with a loss of glia (Ongür *et al.*, 1998) is not supported and mechanisms that rely solely on glial changes do not speak to the full body of present observations. Why such a finding might result from an unbiased stereology study is an important question as glial changes have been a dominant theory for much of the past decade. It appears the use of a counting strategy for an entire area without defining laminar boundaries in a cytoarchitecturally bounded area may have generated a misleading perspective on sACC neuropathology. Also, Ongür *et al.* (1998) did not provide photographic documentation of the changes and it is difficult to assess their numbers. Indeed, assessment of the present 10 cases showed substantial neuron atrophy and neurodegeneration in all cases and it can be photographically documented. In addition, there are sites of active neuropil with glial proliferation. As the latter changes are not uniform across an entire area and neuron loss is expressed on a laminar basis, regions-of-interest need to be specified in terms of layers and cingulate areas rather than general references to medial prefrontal cortex.

As observed above, neuron shrinkage is extensive in all layers that have sites of neuron loss in both diagnostic categories. Figure 25.14 compares layers II–III and Va in sACC in two cases of BD with a control that has an intermediate age between the two depression cases. Layer II in both cases has significant neuron loss and shrinkage, while that in the older Case 9 has substantially more shrinkage in layers III and V. It is possible the process is a progressive one with age.

There is no readily apparent increase or decrease in glial densities as each section is surveyed and this will require a quantitative approach. There are areas of active neuropil that have a large number of glial cells; likely both microglia and phagocytic astroglia. One such site is magnified in layer Va in Figure 25.14. In this 15.6 mm^2 area there are 8 glial cells (arrows), three 'normal'-sized pyramids and at least two atrophied neurons (asterisks). This configuration of cells is not frequent and it cannot be considered as a repetitive or laminar-specific entity. For this reason, stereology throughout an area could easily miss such cellular configurations. Certainly, they are associated with an active process that is impacting neuronal integrity and could reflect an inflammatory site in BD. In the older case, evidence for proliferating glia is present as in layer V (arrows), but layer V is greatly damaged in terms of neuron shrinkage and loss and it appears this case is in a later stage of pathology progression. No layer in any depression or control case had a predominance of glial proliferation or loss over changes in neuron sizes and densities already noted.

Regulating Inflammation as a Therapeutic Approach to Depression

Inflammation may not be a secondary component of neurodegeneration in AD and Rogers *et al.* (1996) suggested clinical trials of conventional anti-inflammatory medications could slow the onset and progression of AD. An increase in intracellular adhesion molecule-1 expression was reported for area 24 in depression suggesting an inflammatory response in this disorder as well (Thomas *et al.*, 2004) and the present study showed sites of active neuropil. In these sites there is a buildup in the numbers of glial cells and neuronal shrinkage suggesting there is a link in the inflammatory site between glial proliferation and neurodegeneration. The sACC may be particularly responsive to inflammatory reactions due to its location near to the nasal sinuses and afferent nerves from the olfactory tract.

Inflammation alone could account for neurodegeneration (Rogers *et al.*, 1996). Seabrook *et al.* (2006) showed that minocycline, which is a second generation tetracycline with neuroprotective effects differing from its antibiotic actions, can reduce microglial function in APP transgenic mice. They suggest that given at the appropriate age, minocycline has anti-inflammatory actions. In addition, two clinical studies showed delayed AD onset associated with a history of anti-inflammatory drug use (Breitner *et al.*, 1994) and non-steroidal anti-inflammatory drugs appeared to slow the progression of AD (Rich *et al.*, 1995). Thus, anti-inflammatory medications may provide some relief from neurodegeneration in depression by blocking inflammation and similar clinical trials are justified as the extent of glial proliferation and inflammatory activated proteins are identified in depression.

Circuit Consequences of Neurodegeneration in Depression

Attempts to model the functional impairments in depression must consider the consequences of laminar neuropathological changes in cingulate cortex and their specific impact on particular cortical projections. These differential projections affect the role of cingulate damage in the depression-vulnerable network. Two connection systems are likely important and considered here; interactions with the amygdala and projections to subcortical motor systems. Indeed, it is the disruption of these pathways that mediates at least in part altered mood and akinesia.

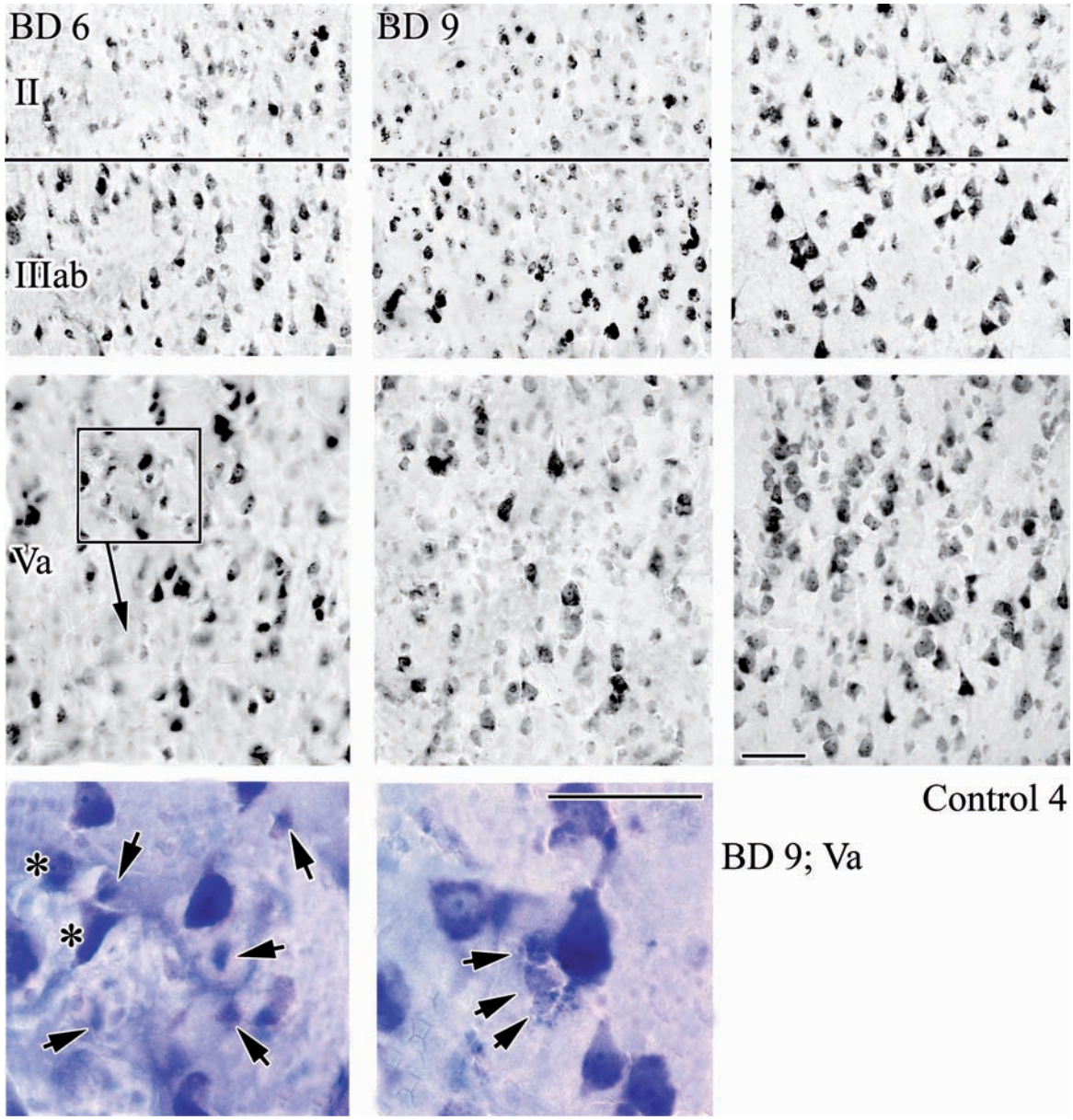

Fig. 25.14 Neuron shrinkage and active neuropil in BD. Young (6) and old (9) BD cases magnified to show neuron shrinkage in layer II of both cases and 'spreading' in the old case into layer III compared with a 74-year-old control (4). Though some shrinking is apparent in case 6, it is much more profound in case 9 where neuron density is also quite high as would be expected with progressive shrinking. Similar events occur in layer V but in some places there are zones of active neuropil where many glia (arrows) appear. Shrunken neurons are apparent in the site shown for case 6 (two asterisks at high magnification) and three normal-sized neurons are in the field. In contrast, case 9 does not have a well-defined layer V and the areas of active neuropil are rare though glia appear to be proliferating (arrows).

Amygdala–ACC interactions via layer II

The amygdala has been implicated in fear and anxiety and may contribute in a profound way to the functions of ACC as discussed above in relation to Figure 25.1B. Individuals with a 5HTT polymorphism have enhanced fear responses in the amygdala compared with subjects homozygous for the long 5HTT allele (Hariri *et al.*, 2002). Moreover, electrical stimulation of areas 25 and 32 in the rat excites neurons in the lateral nucleus of the amygdala and this stimulation blocks neuronal plasticity in the amygdala associated with affective conditioning (Rosenkranz *et al.*, 2003). Chapter 9 reviews the reciprocal functional interactions between the ACC and amygdala. Most importantly, the reciprocal interactions

between these two structures appears to be associated with very early neuropathological damage to both as they have prodromal and correlated atrophy in subjects that are homozygous for the short allele of the 5HTT (Pezawas *et al*., 2005; Fig. 25.1C). Thus, the laminar specificity of amygdala projections in and around layer II of sACC may be prenatally damaged and participate in important ways in mood dysregulation and responses to fearful objects and events throughout life. As such, these vulnerable sites in depression could seed progressive changes associated with neurodegeneration in layers II and III throughout life.

A characteristic of periallocortical cortex including sACC is the presence of 'extraverted' pyramidal neurons (Sanides & Sanides, 1972). Ramón y Cajal (1922) described large star cells in layer II of entorhinal cortex that had extensive dendritic ramifications throughout layer I as is true of the extraverted pyramidal neurons. A relay from layer II to deeper layers has been proposed in neocortex (Thompson *et al*., 2002) and may also be the case for ACC. Layer III provides the primary outflow to other cortical areas including dorsolateral prefrontal, orbitofrontal, rostral superior temporal sulcal cortex, and ventral PCC (Vogt *et al*., 1987). Moreover, the relative importance of amygdala afferents in sACC and the layer II projection to layer III is likely to be much greater than is the case for pACC where layer III is more expanded and the relative significance of amygdala input is reduced.

Thus, the impact of progressive neuron loss in layers II and III, particularly in BD, reported here and in previous studies by Benes *et al*. (2001) and others discussed above likely disrupts interactions with the amygdala as well as intracortical circuits to layer III and the subsequent outflow of such information to other cortical regions in the depression-vulnerable network. Identifying the nature of the neurodegenerative seed initiated in layer II of sACC will be pivotal to stopping the progressive impairment of corticocortical outputs from this subregion and associated mood disorders.

Neurodegeneration in layer V: Impact should be on subcortical motor systems

Although early ACC stroke cases associated with akinetic mutism were large (Barris and Schuman, 1953), the view that ACC mediates motor activation including speech has been confirmed with human functional imaging and experimental animal studies and its general relevance to the role of ACC in depression cannot be overstated. Indeed, the ACC and MCC have the most extensive projections into autonomic and skeletomotor systems of any cortical region and these are reviewed in Chapter 15 as is the circuit basis for the cingulate vocalization function. As many of the neurons in layer V project to motor systems, neuron loss in layer V of ACC and MCC, particularly in MD, are expected to inactivate or dysregulate motor output. As with akinetic mutism, the syndrome does not result in an inability to move but rather in an overall paucity of volitional movement and speech. There are likely two substrates for altered responsivity to emotional objects and events. These are reduced access to sensory cortices by impaired processing through PCC and reduced output from layer V into subcortical motor systems.

Chapter 13 reviews the pathways that link PCC to sensory afferents and sensory-spatial orientation. The argument is presented that all sensory modalities have access to vPCC via specific connections from the occipital and parietal lobes and these drive vPCC when the content (valence) and context have relevance to particular internal states. This access is gated by a reciprocal connection with sACC that guides valence based on previous experience. To the extent that PCC has such information and it suffers neurodegeneration, this may provide one of the mechanisms by which neuronal activity in sACC is reduced in depression and could mediate anhedonia and reduced mood regulation.

Failure to activate a rich pattern of subcortical motor projections may also contribute to depressive symptoms and treatment options could be progressively impaired as neurodegeneration proceeds in layer V; particularly in MD. Figure 15.13 in Chapter 15 shows the outputs of ACC and MCC into subcortical motor structures and the citations supporting each connection are detailed in Chapters 15 and 28. These include projections to the striatum and nucleus accumbens, hypothalamus and periaqueductal gray, the red and pontine nuclei, the monoamine raphe and locus coeruleus nuclei, and the spinal cord.

Drug Therapeutics in the Context of Cingulate Pathology

The previous chapter considers therapeutic responses of depressed patients to SSRIs, while here we consider the localization of two transmitter systems in ACC and some of the expectations for drug success given the present observations of cingulate neuropathology. The sACC is unique in terms of its transmitter receptor binding as shown with laminar differences and densities in binding and polar coordinate plots (Palomero-Gallagher *et al*., 2008b; Chapter 2). Figure 25.15 provides sample coronal sections through the ACC to show the distribution of ligands that are responsible for the unique receptor profile of this region: $GABA_A$, $GABA_B$, N-methyl-D-aspartate (NMDA), and 5HT1A. In addition to the coronal sections, this figure shows the hierarchical clustering analyses of 15 neurotransmitter receptors for postmortem cases with and without these particular four receptors in the analysis. The red highlight shows that with the four

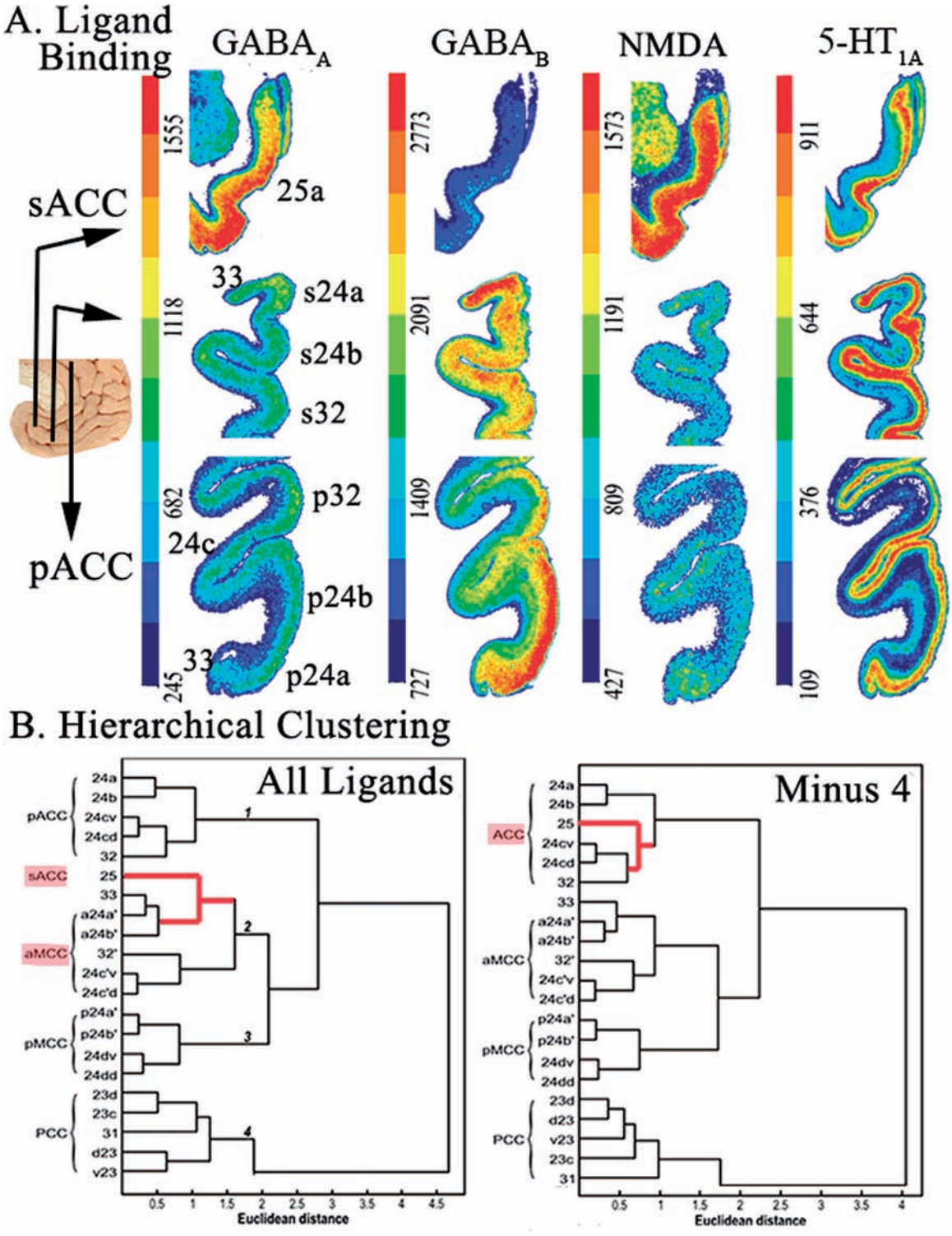

Fig. 25.15 Receptor distributions of ACC targets that are effective in treating depressive symptoms. A. Coronal sections showing laminar patterns of binding in parts of ACC indicated with arrows from the medial surface. B. Hierarchical clustering with 15 receptors showing co-segregation of sACC with aMCC (All Ligands) and following removal of four pivotal receptors (Minus 4) that characterize this region's unique binding patterns and showing the clustering with other parts of ACC. These four receptors have been implicated in therapeutic responsive in MD (GABA and 5-HT1A) and neurodegenerative processes (NMDA).

receptors sACC area 25 co-segregates with aMCC areas (All Ligands), while without them (minus 4) it co-segregates with the ACC. Thus, these four receptors make a primary contribution to the unique receptor binding properties of the sACC. As 5HTergic and anticonvulsant drugs are presently the primary target of treatment for depressed patients, we consider the 5HT1A and $GABA_A$ receptors in detail here.

Serotonergic system

A review by Blier and Ward (2003) concludes that agonists to the 5HT1A receptor are valuable and efficacious for therapy of MD and the large STAR*D analysis reported by Trivedi *et al.* (2006) shows that the SSRI citalopram can generate a 47% response rate in outpatients with MD. Although response rates to SSRIs can be somewhat lower in other studies, the pivotal link between symptom response and the cingulate 5HT system is quite convincing. Figure 25.15 shows that 5HT1A binding is highest in pACC and in layers II–III throughout this subregion, while layers V and VI have a very low density of binding. One of the highest levels of citalopram binding to the 5HTT is in pACC as shown in a postmortem study by Mantere *et al.* (2002) and these findings have been verified by Varnäs *et al.* (2004) who showed a high density of both 5HT1A and 5HTT in the superficial layers of ACC. Interestingly, patients with MD that had not received SSRIs have a lower 5HTT binding potential in many parts of the limbic system including ACC (Parsey *et al.*, 2006). Thus, one of the primary sites of actions of SSRIs and 5HT1A agonists is in the superficial layers of ACC.

Review of Table 25.3 shows that layers II and V have greatest neuron loss. To the extent that layer V pyramidal neurons have apical tuft dendrites in layer I, 5HTergic afferents to layers I–III could modulate the functions of remaining neurons in layers II–V and this might be a primary site of action of 5HT drugs. Another aspect of the present analysis is that Aβ42 builds up with age and neurodegeneration has a link to age as shown in Table 25.3 with the exception of just two cases. In the context of aging, it is interesting that 5HT1A receptor binding potential does not appear to change with age in ACC (Parsey *et al.*, 2002). This latter finding must eventually meet with the problem of neurodegeneration in the superficial layers and might be explained by up-regulation of receptors as adjacent neurons degenerate. Indeed, it may be critical to determine what the threshold number of remaining neurons needs to be to provide therapeutic sites for drug actions rather than assessing the level of neuron loss *per se*.

GABAergic system

Recurrent depression may be associated with decreased GABA levels in ACC (Bhagwager *et al.*, 2007) and 2 months of SSRI treatment returns it to normal levels in occipital cortex (Sanacora *et al.*, 2002). It is well known that sad memories are stored in sACC (Chapters 1, 11, 14, 24, 26) and patients with BD have a reduced activation of ACC to facial emotions (Blumberg *et al.*, 2005). The latter study also showed that treatment with anti-convulsants such as GABApentin and valproic acid partially reverse the redced functional ACC activity in BD. To the extent that these latter drugs enhnace the release of GABA stores, the recovery of ACC activity associated with negative affect may be due to elevated $GABA_A$ levels. Also, as $GABA_B$ receptors have a presynaptic localization and can regulate GABA release, ligands for this receptor may have efficacy in treating depression.

The $GABA_A$ receptor is a ligand-gated chloride channel while, $GABA_B$ receptors control potassium or calcium conductances via a G-protein-mediated reduction in adenylyl cyclase activity (review, Pilc and Nowak, 2005). The former tend to be posynaptic, while the latter are mostly presynaptic and regulate GABA release. Preclinical studies are underway to develop GABAergic compounds that have antidepressant actions, however, a critical issue in the human brain is that these two receptors have profoundly different distributions in ACC. As shown in Figure 25.15, the highest $GABA_A$ binding is in sACC where there also is the lowest $GABA_B$ binding (Chapter 2). In contrast, $GABA_B$ binding is very high in pACC. One of the difficulties in identifying therapeutic drugs, therefore, is the dissociation of these receptors in ACC rather than their coexpression in particular stuctures as generally assumed in experimental animal studies. Moreover, the efficacy of drugs acting at $GABA_A$ receptors and benzodiazepine regulatory binding actions are likely associated with sACC and could occur in isolation of $GABA_B$ actions. Thus, each compound with an action on the GABAergic system could produce different levels of relief for symptoms of depression and anxiety and there is little reason to expect an exact overlap of symptom relief when targeting the ACC.

It is unlikely that any transmitter system operates independently and chronic administration of antidepressant drugs that regulate catecholamine synthesis, binding and/or uptake in rats increase $GABA_B$ binding in frontal cortex (Pratt and Bowery, 1993). Although no compounds are currently available for clinical use, some $GABA_B$ antagonists in animal models suggest value as treatments for depression (Pilc and Nowak, 2005). The mechanism of differential actions of $GABA_A$ and $GABA_B$ ligands lies at least in part in their differential distribution in sACC.

In terms of ACC neuropathology, Figure 25.15 raises interesting associations with ligand binding. $GABA_A$ binding is very high and almost uniform by layer. The localization of GABAergic terminals with gluatamic

acid decarboxylase-65 immunoreactivity showed that these terminals are high and evenly distributed throughout area 24 (Benes *et al*., 2000) confirming the ligand binding observations. Benes *et al*. (2000) also showed that the density of these terminals in BD was reduced in all layers bilaterally, but these differences were most significant in layers II (28%) and III (37%). Also there were no differences in the size of cell bodies but there was a 27% reduction in the density of non-pyramidal neurons in layer II of the ACC in BD and the density of glial cells was normal (Benes *et al*., 2001).

Progressive neurodegeneration: A hurdle for drug therapeutics

Layers II and III are the prominent sites of SSRI actions based on receptor localization and greatest neuron loss early in BD is in layers II and III. Neurons in layers II and III also have intracellular deposits of Aβ42 early in depression. These observations together suggest that impaired neuronal function in layers II and III may be improved to some extent by SSRI therapy. However, neurodegeneration appears to progress in depression and is not limited to these two layers. In MD, the greatest neuron loss appears to be in layer V and progressive destruction of layer V substantially deafferents subcortical motor systems. One of the reasons that SSRI therapies might loose their efficacy is the progressive and non-reversible loss of neurons throughout sACC and even in PCC. Furthermore, it is possible that the efficacy of electrical stimulation in the cingulum bundle below the sACC (Mayberg *et al*., 2005) results in significant activation of the remaining outputs to motor systems. In view of the pivotal role of age-related changes in amyloid-β peptides and neurodegeneration, a more complex series of therapeutics may be needed to provide a comprehensive treatment of depression.

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