

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 April 2010 (15.04.2010)

PCT

(10) International Publication Number
WO 2010/042603 A1

- (51) International Patent Classification:
A01N 43/90 (2006.01) A61K 31/519 (2006.01)
- (21) International Application Number:
PCT/US2009/059819
- (22) International Filing Date:
7 October 2009 (07.10.2009)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/103,768 8 October 2008 (08.10.2008) US
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

[Continued on next page]

(54) Title: AMYLOID AND DEPRESSION

(57) Abstract: The present invention relates to a method of treating a depressive disorder by inhibiting or preventing the accumulation of an amyloid beta protein in the brain. Further, this invention relates to a method wherein the accumulation of an amyloid beta protein is inhibited or prevented by administration of one or more of: a beta-secretase inhibitor, a gamma-secretase inhibitor, a cholesterol lowering drug, a muscarinic agonist, an anticholinesterase inhibitor, an anti-inflammatory drug, an antidepressant, a selective serotonin reuptake inhibitor, a serotonin agonist, a gamma-aminobutyric acid agonist, an anticonvulsant or a vaccine.

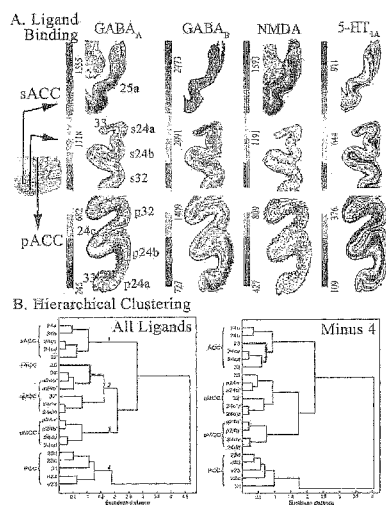


FIGURE 25.15

WO 2010/042603 A1

Published:

— *with international search report (Art. 21(3))*

AMYLOID AND DEPRESSION

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims priority to U.S. Provisional Application No. 61/103,768, filed October 08, 2008 and which is assigned to the same assignee as this application. The aforementioned provisional application is hereby incorporated herein in its entirety by reference.

TECHNICAL FIELD OF THE INVENTION

10 The present invention relates to a method of treating a depressive disorder that prevents the accumulation of an amyloid beta protein in the brain.

BACKGROUND OF THE INVENTION

 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");
15 Aretaeus of Cappadocia (ca. 150 AD) described manic-depression (bipolar disorder), 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
20 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
25 health problem worldwide by the World Health Organization (Murray and Lopez, 1996). Although effective treatments exist for decades now, the disability induced by mood disorders is comparable to 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)
30 classify mood disorders into unipolar (major depression and dysthymia) and bipolar (bipolar disorder 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
5 schizophrenia, personality disorder, or neurosis. The term 'mood' which refers to the internal tone was chosen today as more suitable in comparison to '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
10 chronic. Thus, the presence of structural abnormalities is possible.

The psychological approach (Gabbard, 1995, Agras and 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
15 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
20 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
25 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
30 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 and Dehaene, 1993, Lewis and

Oeth, 1995, Brown et al., 1994, Kling et al., 1995, Cooper and Martin, 1995, Copolov and 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
5 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 a., 1967). Recently is has been shown that higher
10 preoperative rates of glucose metabolism in subgenual anterior cingulate cortex (sACC) was associated with better postoperative 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.

15 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 bipolar disorder (BD) are not
20 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:

25 Early efforts to synthesize theories and empirical data suggested that psychological and biological etiological factors converge in deficits in the diencephalic substrates of pleasure and reward (Akiskal and 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
30 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, 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). The sACC also 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. The details of this circuitry are considered in Chapter 5.

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 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 layer I and layer II. Since 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.

5

SUMMARY OF THE INVENTION

The present invention relates to a method of treating a depressive disorder comprising inhibiting or preventing the accumulation of an amyloid beta protein in the brain.

BRIEF DESCRIPTION OF THE FIGURES

Figure 25.1. Cingulate morphological substrates of depression. A. Network organization from 5HTT gene to behavioral output. B. Amygdalocingulate connections (Vogt and 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.

Figure 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.

Figure 25.3. Intraneuronal A β 42 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.

Figure 25.4. Intraneuronal A β 42 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 μ m).

5 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 PCC of same case as A-C.

E. Layer V neurons lightly labeled in a young 56 year-old Case 11.

10 F. Heavy A β 42 labeling in sACC layer V of a 63 year old. G. Heavy labeling of layer IIIc pyramids in PCC.

Figure 25.5. Case 13 is unique among 9 because this individual had both intraneuronal A β 42 in ACC and diffuse plaques in 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 A β 42 at the asterisk suggesting a potential seeding site of diffuse plaques.

Figure 25.6. Comparison of ACC control case thionin (2, 4) with young (B. Case 7) and old (D. Case 9) BD cases including the A β 42 of which none were in controls. Some A β 42-positive neurons in Case 7 are emphasized with asterisks and the details shown in Figure 25.4. The second pattern of A β 42 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 sparsity are emphasized with arrows.

Figure 25.7. Comparison of A β 42 deposition in ACC and PCC in two BD (9, 10) and two MD (14, 15) cases. The only case with higher ACC plaques is #9 who was 75 at death. In contrast, higher A β 42 plaques 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.

Figure 25.8. Thionin-stained superficial layers in BD 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*.

Figure 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 sparsity and are likely places of spotty neuron losses. In no instance is a complete layer lost or uniformly involved.

5 Figure 25.10. Thionin-stained, superficial layers of ACC in MD 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.

10 Figure 25.11. Thionin-stained, deep layers of ACC in MD 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.

15 Figure 25.12. Thionin-stained sections of dPCC in two control (2, 5) and three BD and 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 500X 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.

Figure 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.

20 Figure 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 to 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).

30 Figure 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 cosegregation of sACC with aMCC (All Ligands) and following removal

of four pivotal receptors (Minus 4) that characterize this regions 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).

5

DETAILED DESCRIPTION OF THE INVENTION

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 functional 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 to 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 coregistered 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. Uytendhoef et al. (1983) reported hypoperfusion restricted to the frontal areas only and frontal hypometabolism was also reported by others (Delvenne and 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 hypermetabolism. 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 nonpsychotic 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 can not 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 evidenced 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 grey matter reduction of 39% in the same area. There are also studies, however, that do not report volumetric changes in cingulate cortex of BPD patients (Lopez-Larson et al., 2002, Brambilla et al., 2002, Zipursky et al., 1997) or report global but not localized grey matter reduction (Lim et al., 1999).

Drevets et al. (1997) reported a decreased metabolism in the sACC bilaterally during depression and maybe 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 grey 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 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.

EXAMPLES

EXAMPLE 1

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 to 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). Since 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 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 to 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 familiar 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 progression throughout life, and the extent to which it is associated with superficial layer neuron loss and shrinkage.

EXAMPLE 2

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.

5 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 to control subjects (Bouras et al., 2001, Thomas et al., 2002, Thomas et al., 2004). Cotter et al. (2002) found no evidence for differences in glial density or neuronal
10 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 control subjects and modestly increased in white matter compared with unipolar patients. These findings are consistent
15 with the presence of an inflammatory response in the ACC of BD; 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 to normal controls; while changes in the gray matter were not significant. Also, the reduction in glial
20 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
25 changes in neuron densities. Glial proliferation could be indicative of an inflammatory response and the sACC may be selectively vulnerable to such insults.

EXAMPLE 3

Normal Cytology of Subgenual Anterior Cingulate Cortex:

In addition to the above enumerated sources of variability associated with populations
30 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 coregistering 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., 2008). 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 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.

EXAMPLE 4

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, (Chap 33), dementia with Lewy bodies (Chap 32), and Alzheimer’s disease (Chap. 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.

EXAMPLE 5

Table 25.1: Case Material

| Control Cases | Gender | Age (Death) | Brain Weight (gm) | 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 haemorrhagic 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 |

Overview of Methods:

Section thickness and sampling strategies are pivotal to the outcomes. As a rule, published studies use sections 5- μm to 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 dPCC. These blocks, including those from control cases, 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 Alzheimer's disease (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 aggregates of degeneration (vs 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.

Since 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 (A β 42) labeled samples from 9 of the 10 depression cases. Since 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 A β 42 and thionin images coregistered. This was done for area s24b of all sACC samples in control and depression cases, although there was no expression of A β 42 in the control cases. The thionin-stained sections were analyzed at a final magnification of about 500x 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 A β 42 deposition uncovered three striking findings: 1. A β 42 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. A β 42 was deposited in two patterns. In the youngest cases A β 42 was entirely intraneuronal, mainly in large neurons in layer V but also with significant but variable staining in outer layer III and layer 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; i.e., 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.

EXAMPLE 6

5 Age Preference:

The intraneuronal pattern occurred only in young cases regardless of diagnostic group with an average age at death \pm 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 A β 42. Since 9 of 10 cases had A β 42 expression and it appears in two patterns with an age dependence, this phenomena must be considered 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 A β 42 and its potential for neurotoxicity, however, requires that it be considered as an important player in depression.

EXAMPLE 7

Intraneuronal A β 42:

Figure 25.3 presents the laminar distribution of intraneuronal A β 42 for Case 7 (63 year old; BD). The adjacent thionin-stained section was coregistered to the A β 42 section and it shows that A β 42-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 layer 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 A β 42 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 A β 42 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).

5 A key Transition: Case 13:

In view of the fact that the intraneuronal and plaque patterns of A β 42 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 A β 42 deposition. Light staining in layer
10 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 A β 42 and might be interpreted as seeding diffuse
15 plaques; although other mechanisms are possible and likely based on the low number of these profiles and high number of diffuse plaques observed. Thus, the life cycle of A β 42 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.

EXAMPLE 8

20 Plaque A β 42:

As noted in Transition Case 13, a second pattern of A β 42 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 coreregistrations of thionin and A β 42 for Case 9; BD aged 75 at death. The
25 diffuse plaques are labeled throughout layers I-V without preference and it is often in a perivascular location.

A comparison with neuron densities is provided by coregistration with adjacent thionin-stained sections to initiate a consideration of the role that A β 42 may play in neurodegeneration in depression. In case 7 patches of neuron losses can be seen in layers III
30 and V and these are layers that contain intraneuronal A β 42 suggesting a possible link to neuron death. However, layer VI also shows profound neuron losses and no intraneuronal A β 42 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 layer II-V; the layers with heavy diffuse plaques. Although this neuron loss could be generated by extracellular A β 42, 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 A β 42 is toxic to layer VI
5 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 A β 42 deposition is associated with additional mechanisms of neurodegeneration. According to this model, neuron losses may build progressively as the pattern of A β 42 shifts.

10

Plaque A β 42 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
15 possible mechanisms of neurodegeneration. A reduction in glucose metabolism was observed in unipolar subjects that were antidepressant drug responses (Mayberg et al., 2000; previous chapter) and volumetric reductions in PCC have been reported early in BD (Kaur et al., 2005). Having already observed intraneuronal A β 42 in PCC and plaques in the transitional Case 13, the search for this protein in the PCC in older individuals will likely
20 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 subject, the likely hypothesis is that the aggregate density of diffuse and mature plaques would be greatest in ACC as shown for Case
25 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
30 plaque in ACC suggesting that in the eighth decade, depressed patients experience a terminal aggregation of A β 42 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 A β 42 plaques.

TREATMENTS

5 Amyloid- β Peptides in Depression: An Aging Dimension:

Amyloid- β peptides are critical for neuron viability (Plant et al., 2003) and A β 42 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 Alzheimer's disease, i.e., mild cognitive impairment, accumulates
10 intraneuronal A β 42 (Gouras et al., 2000). It is not yet known why neurons in adult cases of depression sequester intraneuronal A β 42 as reported here but it may also contribute to neuron death as also shown in Alzheimer's disease (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.

15 The two laminar patterns of A β 42 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 A β 42 in cingulate cortex in cases of
20 depression. First, to what extent is intraneuronal deposition toxic and may account for at least part of neurodegeneration observed in cingulate cortex? Two, since autonomic dysregulation has been observed in Alzheimer's disease 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?

25 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
30 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 A β 42 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.

5 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 A β 42 during early adulthood which continues until the neurons are quite dark with the A β 42 antibody reaction. By the mid-70s the first extracellular, diffuse plaques appear in sACC and the intraneuronal deposits

10 lighten/are reduced. Aggressive extrusion of A β 42 by some pyramidal neurons could lead to seeding of diffuse plaques. Indeed, it has been proposed that GM1 ganglioside-bound A β may initiate A β 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

15 entire cingulate cortex is damaged to some extent by A β 42 deposition.

Table 25.2: Hypothetical Amyloid Progression

1. Late adolescence-early adult; intraneuronal A β 42 layers II-V of entire cingulate cortex
2. 6th decade; intense and disruptive A β 42 deposition
3. 7th decade; transition to diffuse plaques includes lightening of intraneuronal expression and aggressive extrusion contribute to seeding of diffuse plaques in sulcal cortex
4. 8th decade; extensive mature plaques in ACC and more diffuse plaques in PCC

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 A β 42-positive cases is very high (9 of 10). The presence of the neurotoxic A β 42 peptide in the absence of tau neuropathology (no AT8 antibody expression in any case of depression),

20 suggests that an amyloid mechanism of neurodegeneration occurs in BD and MD. The A β 42 peptide is expressed in MCC in mild cognitive impairment (Johnson et al., 2004; Chapter 33) and in PCC in early clinical stages of Alzheimer's disease (AD) during the first few years of

25 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 A β 42 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
5 disease.

The prominent role of amyloid- β peptides in AD and the documented role of A β 42 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
10 amyloid-mediated neurotoxicity have been proposed (Hardy and Selkoe, 2002). As noted by these authors, the build-up of amyloid- β peptides could be reduced by blocking the beta- and gamma-secretases that are responsible for generating the long amyloid- β peptides such as A β 42. Relevance of β -site APP cleaving enzyme 1 is shown in mice deficient in this enzyme that have no A β 42 expression (Luo et al., 2001)

Another approach to blocking amyloid- β peptide buildup is to reduce cholesterol
15 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
20 rodents but there has been no success yet in human vaccination trials. Since this strategy has seen success recently in primates by vaccination of rhesus and vervet monkeys with aggregated A β 42 (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
25 remove A β 42 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 amyloid precursor protein (APP) from the
30 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 Swedish mutant cells, huperazine A stimulates α APP release and diminishes amyloid- β generation (Peng et al., 2006). Huperazine 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 A β 42 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 2500 μm x height for superficial and deep layers about 5000 μm x 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 A β 42 deposition with coregistration to thionin-stained sections. This does not mean we consider all or even part of neuron loss a matter of A β 42 toxicity, although this is a new hypothesis generated by the present sample. Case 12 had no evidence of A β 42 immunoreactivity yet there was clear evidence of neuron loss as discussed below. Since this patient died at the age of 68, they may have been at a transition point that involved a loss of intraneuronal A β 42 and a failure or delay in plaque formation. Alternatively, no A β 42 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 A β 42 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 year 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 to 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 sparsity 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 A β 42 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.

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 24.3 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 issues that must be resolved with a rigorous counting strategy. The shaded values are also meant to draw the eye
5 to the higher levels of neuron loss (about 51-75% loss).

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
10 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
15 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 | |

5 *Does not include estimates of neuron shrinkage; 0 differences from control, 1+, 2+, 3+. No severe losses at 4+ observed. Shading is for 3+ to emphasize layers with greatest loss. Totals are the sum of all pluses. Shaded values in Totals emphasize the case/layer with greatest neuron loss in each subgroup, while in Subgroup Totals emphasize two values ≥ 10 for the two layers with greatest neuron loss.

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., 5 2005). Deposition of A β 42 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 500X and the two controls were placed on either side of each strip from a depression case. A single arrow was 10 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 6 cases were selected for the figure based on changes in area s24b; i.e., they were selected on *a priori* not selected after the analysis shown in Table 25.3 and before photographs were taken or 15 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 20 death.

Figure 25.12 shows that losses in Case 6 were mainly in layers II and Vb. Since 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 25 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 30 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 and 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 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 25.12 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 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.

15 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.

25 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; i.e., 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 μ m-thick sections and this provides for an assessment of differences in laminar cytoarchitecture that are associated with neuron shrinkage. Figure 25.13 shows layer II and the superficial part of layer III of area s24b for two 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. Since 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 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 mm² 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 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 amyloid precursor protein 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 nonsteroidal 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.

10 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.

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 to subjects homozygous for the long 5HTT allele (Hariri et al., 2002). Moreover, electrical stimulation of areas 25 and 32 in the rat excite 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 and 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 and others, 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 and Pandya, 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 of 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. Since 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.

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). 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), 5HT_{1A}. 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 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. Since 5HT_{1A} and anticonvulsant drugs are presently the primary target of treatment for depressed patients, we consider the 5HT_{1A} and GABA_A receptors in detail here.

Serotonergic System:

A review by Blier and Ward (2003) concludes that agonists to the 5HT_{1A} 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 5HT_{1A} 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 5HT_{1A} 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 5HT_{1A} 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, 5HT_{1A} 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 A β 42 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*.

10 GABAergic System:

Recurrent depression may be associated with decreased GABA levels in ACC (Bhagwager et al., 2007) and two 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 anticonvulsants such as GABApentin and valproic acid partially reverse the reduced functional ACC activity in BD. To the extent that these latter drugs enhance the release of GABA stores, the recovery of ACC activity associated with negative affect may be due to elevated GABA_A levels. Also, since 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, Pinc and Nowak, 2005). The former tend to be postsynaptic, 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, highest GABA_A binding is in sACC where there also is 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 structures 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
5 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.

10 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 glutamic 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
15 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:

20 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-III also have intracellular deposits of A β 42 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
25 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 lose their efficacy is the progressive and nonreversible 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
30 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.

Although certain embodiments and examples have been described in the detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the embodiments and examples without departing from the teachings thereof. All such modifications are intended to be encompassed within the below claims of the invention.

5 Abbreviations:

Amygdala nuclei

| | | |
|----|--------------|---|
| | AB | accessory basal |
| | Lat | lateral |
| | LB | lateral basal |
| 10 | M | medial |
| | ANS | autonomic nervous system |
| | APP | amyloid precursor protein |
| | 5HT | serotonin |
| | 5HTT | serotonin transporter |
| 15 | A β 42 | amyloid- β 42 peptide |
| | ACC, p, s | anterior cingulate cortex; pregenual, subgenual |
| | AD | Alzheimer's disease |
| | aMCC | anterior midcingulate cortex |
| | APP | amyloid precursor protein |
| 20 | BD | bipolar disorder |
| | GABA | γ -aminobutyric acid |
| | MCC | midcingulate cortex |
| | MD | major depressive disorder |
| | MRI | magnetic resonance imaging |
| 25 | NeuN | neuron-specific nuclear binding protein |
| | NMDA | N-methyl-D-aspartate |
| | PCC; d, v | posterior cingulate cortex; dorsal, ventral parts |
| | rCBF | regional cerebral blood flow |

SSRI

selective serotonin reuptake inhibitor

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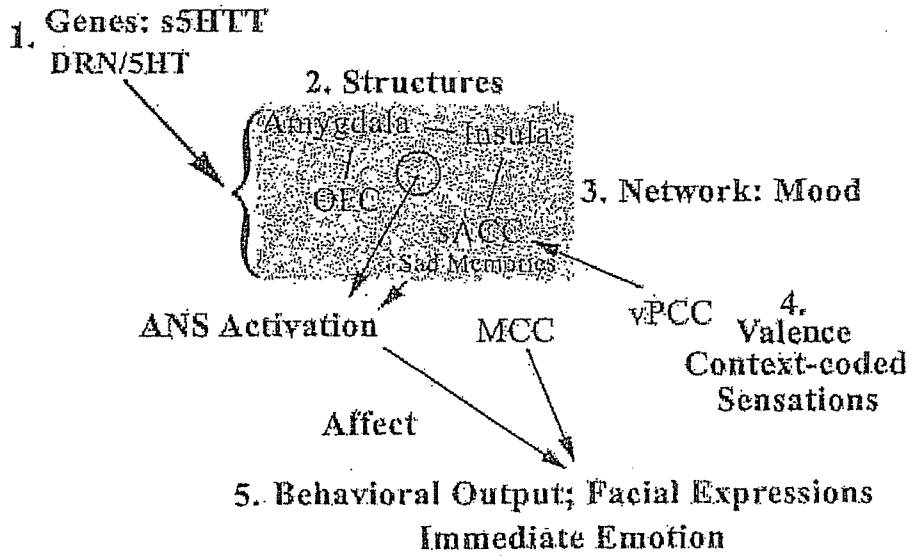
Zipursky RB, Seeman MV, Bury A, Langevin R, Wortzman G, Katz R (1997) Deficits in gray matter volume are present in schizophrenia but not bipolar disorder. *Schizophr Res* 26:85-92.

WHAT IS CLAIMED IS:

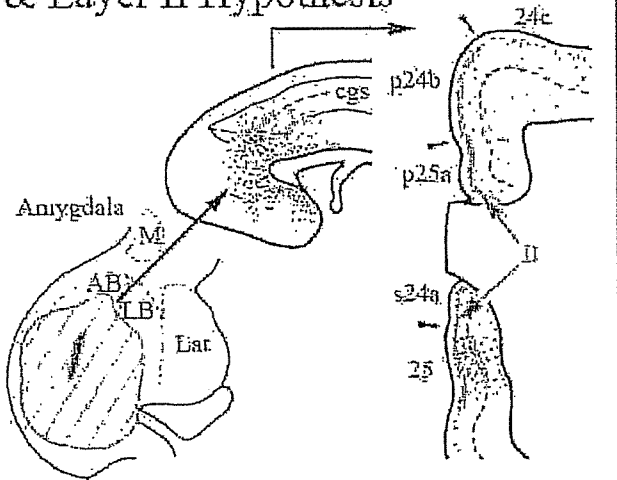
1. A method of treating a depressive disorder comprising inhibiting or preventing the accumulation of an amyloid beta protein in the brain.

2. The method of claim 1, wherein the accumulation of an amyloid beta protein is
5 inhibited or prevented by administration of one or more of: a beta-secretase inhibitor, a
gamma-secretase inhibitor, a cholesterol lowering drug, a muscarinic agonist, an
anticholinesterase inhibitor, an anti-inflammatory drug, an antidepressant, a selective
serotonin reuptake inhibitor, a serotonin agonist, a gamma-aminobutyric acid agonist, an
anticonvulsant or a vaccine.

A. Functional Levels of Organization



B. Amygdala Projections; Monkey & Layer II Hypothesis



C. Cingulate Endophenotype

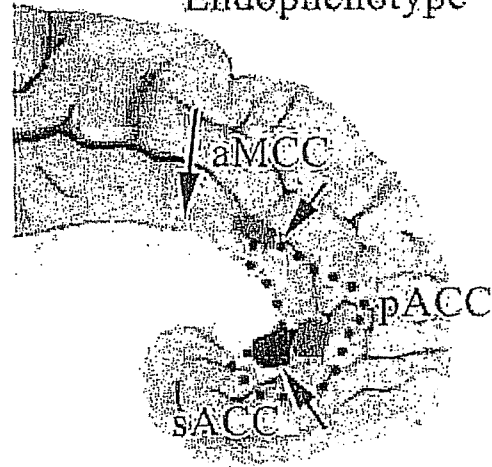


Figure 25.1

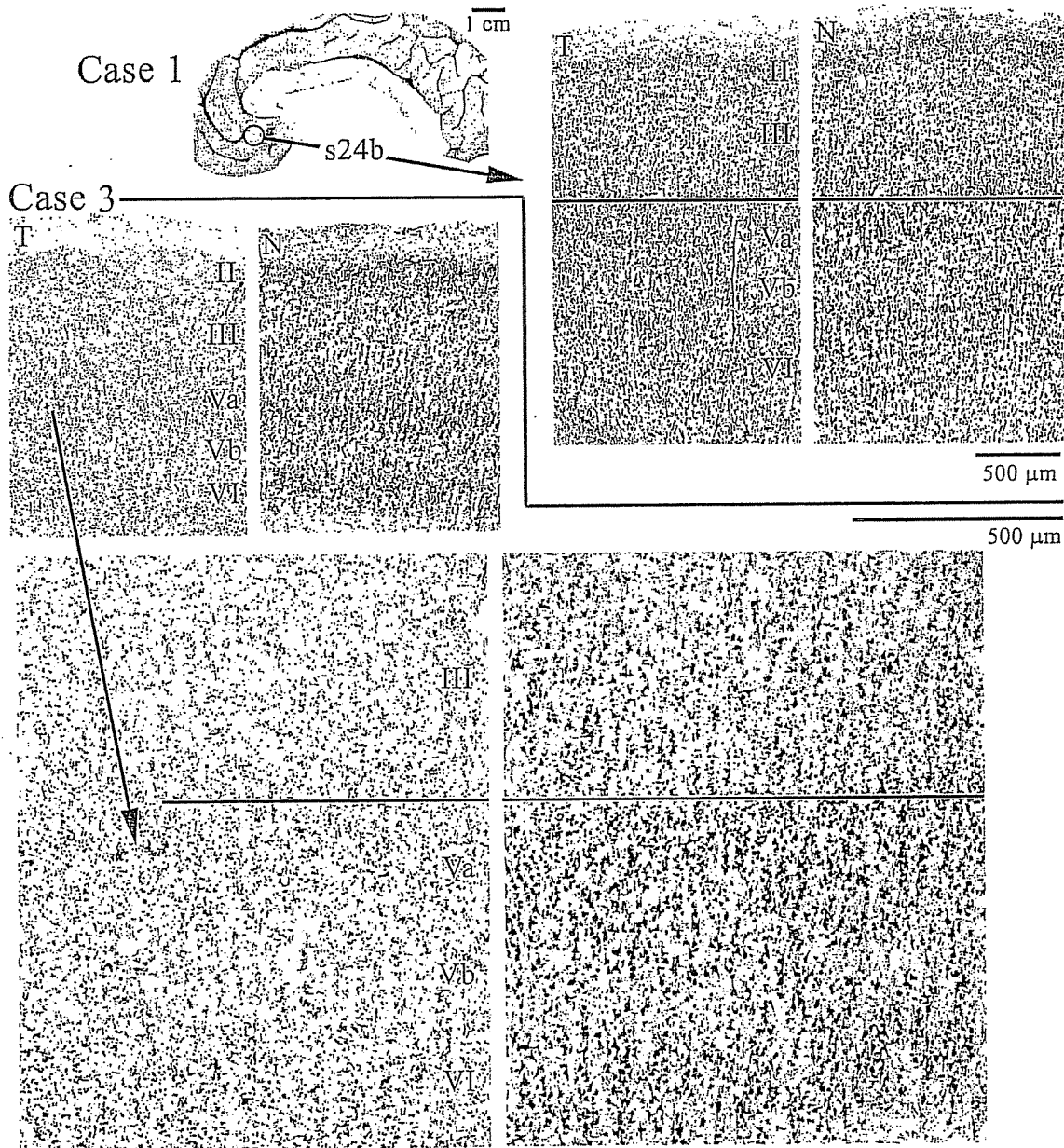


Figure 25.2

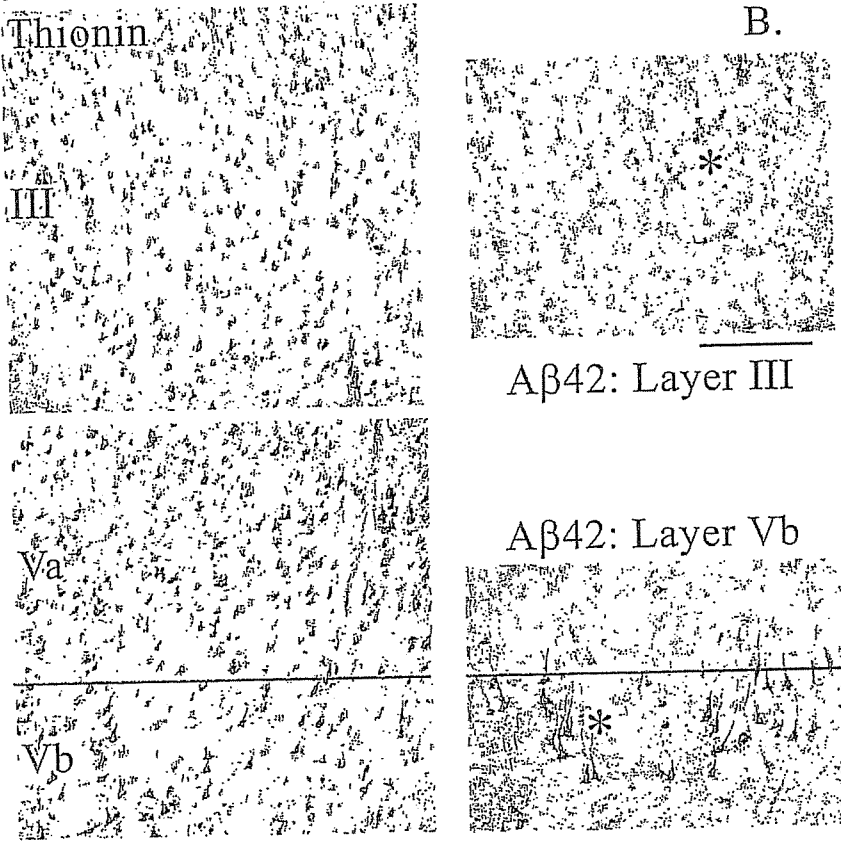
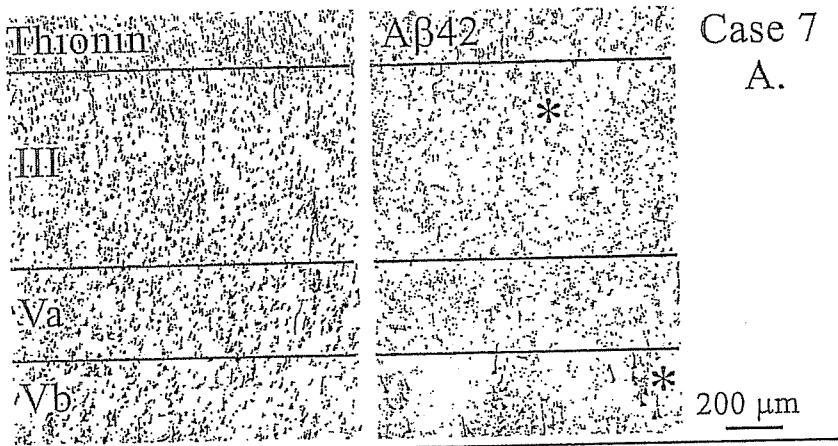


Figure 25.3

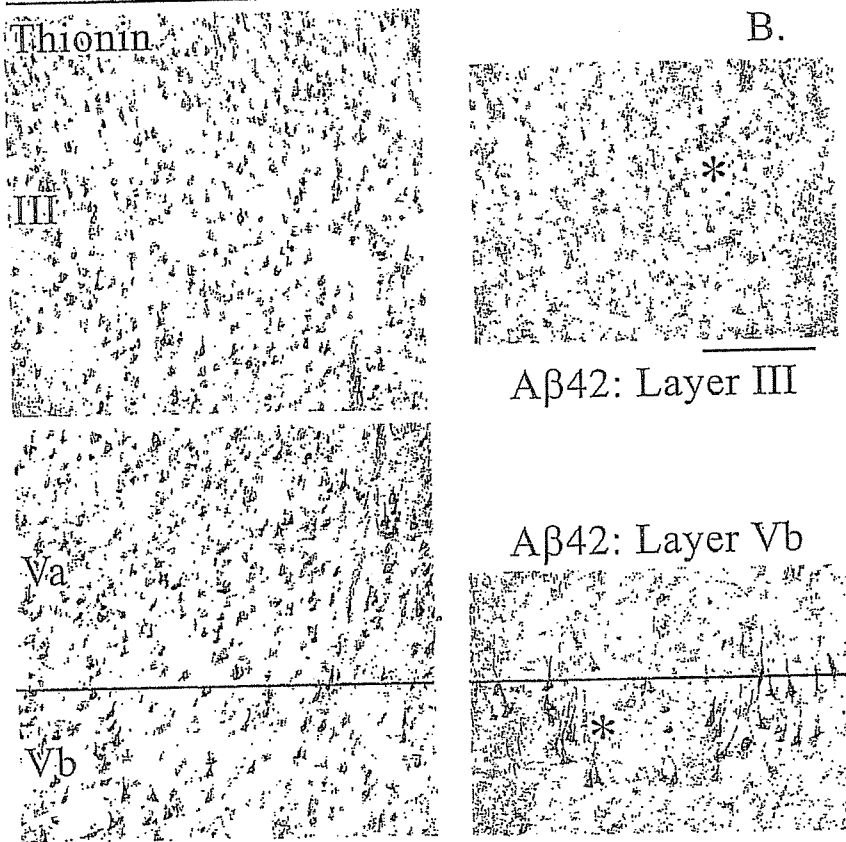
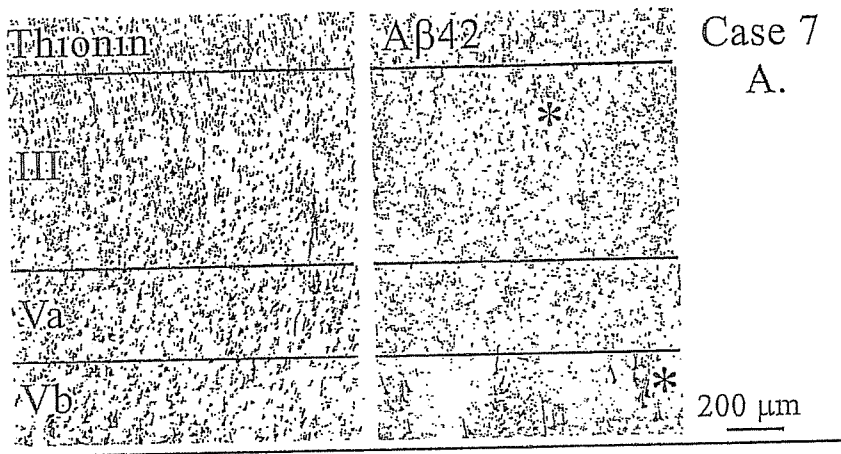


Figure 25.4

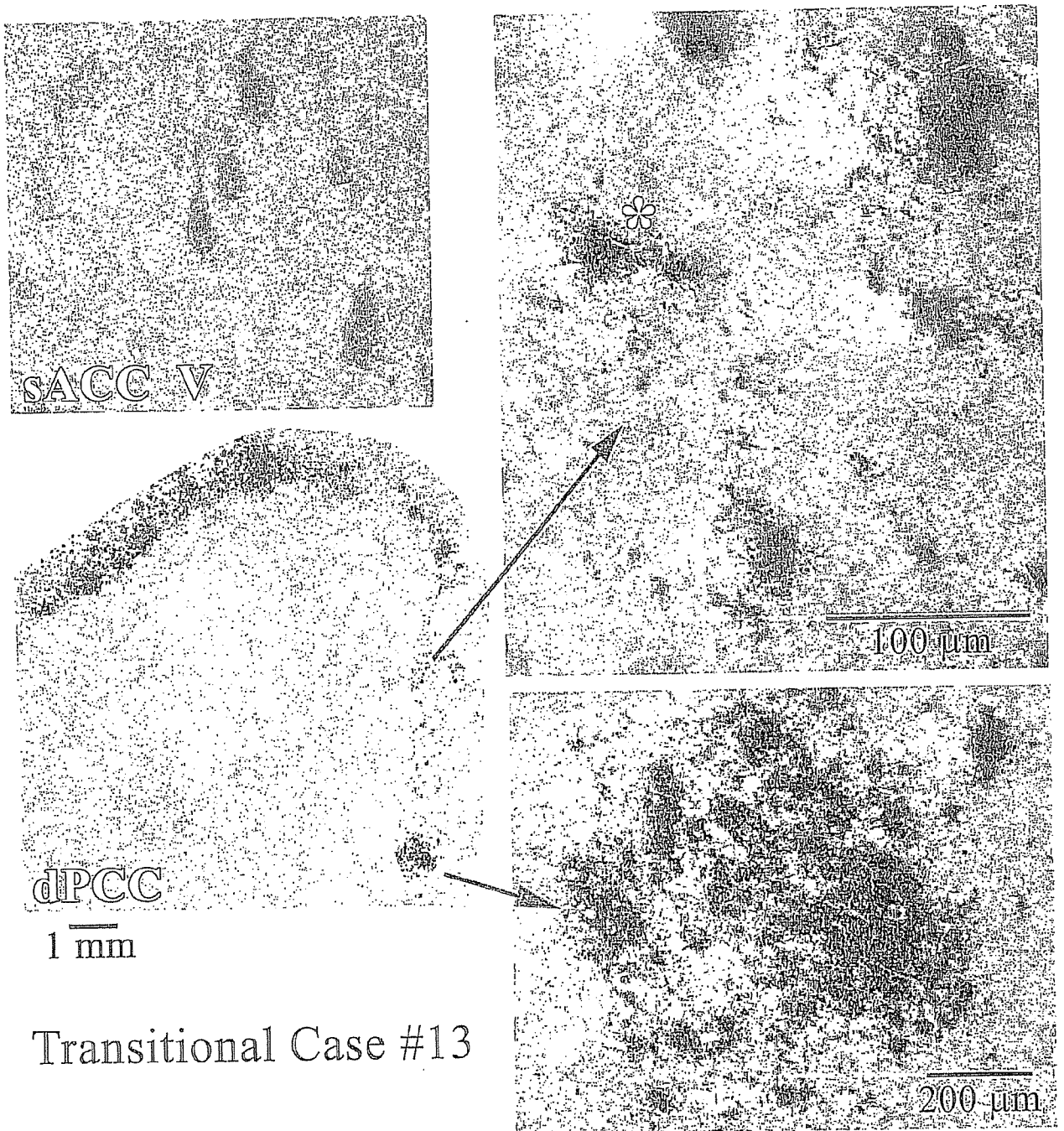


FIGURE 25.5

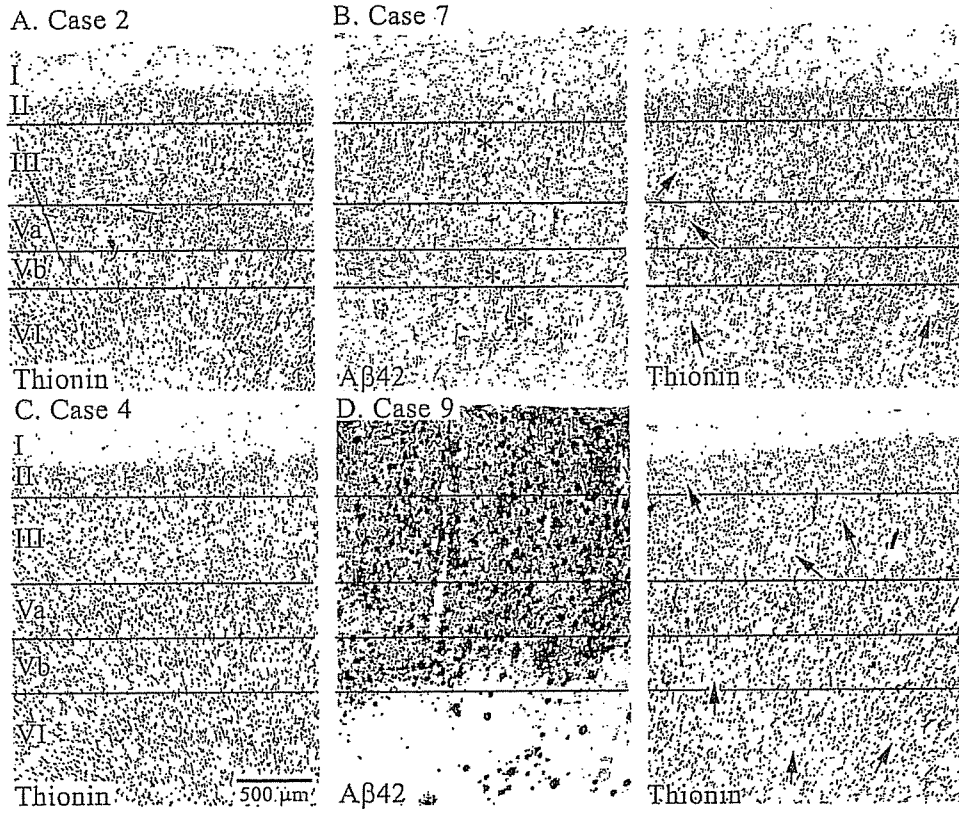


FIGURE 25.6

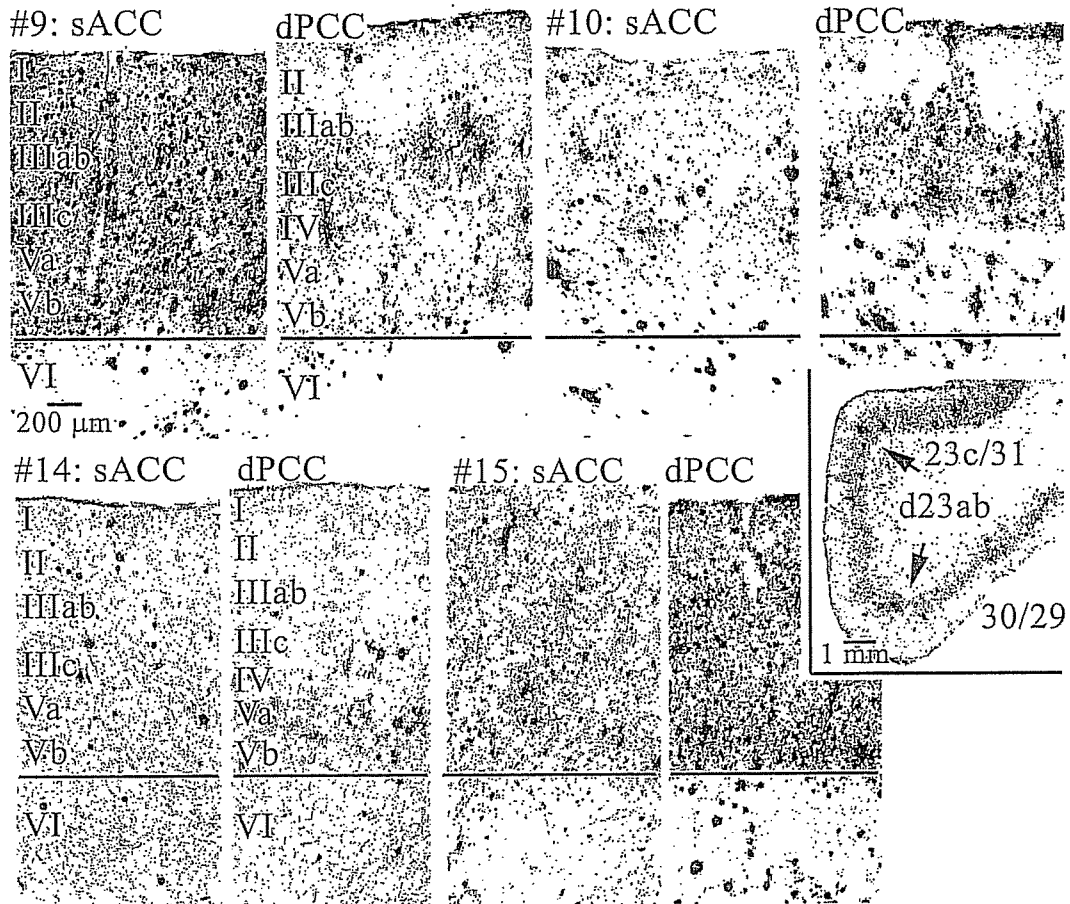


FIGURE 25.7

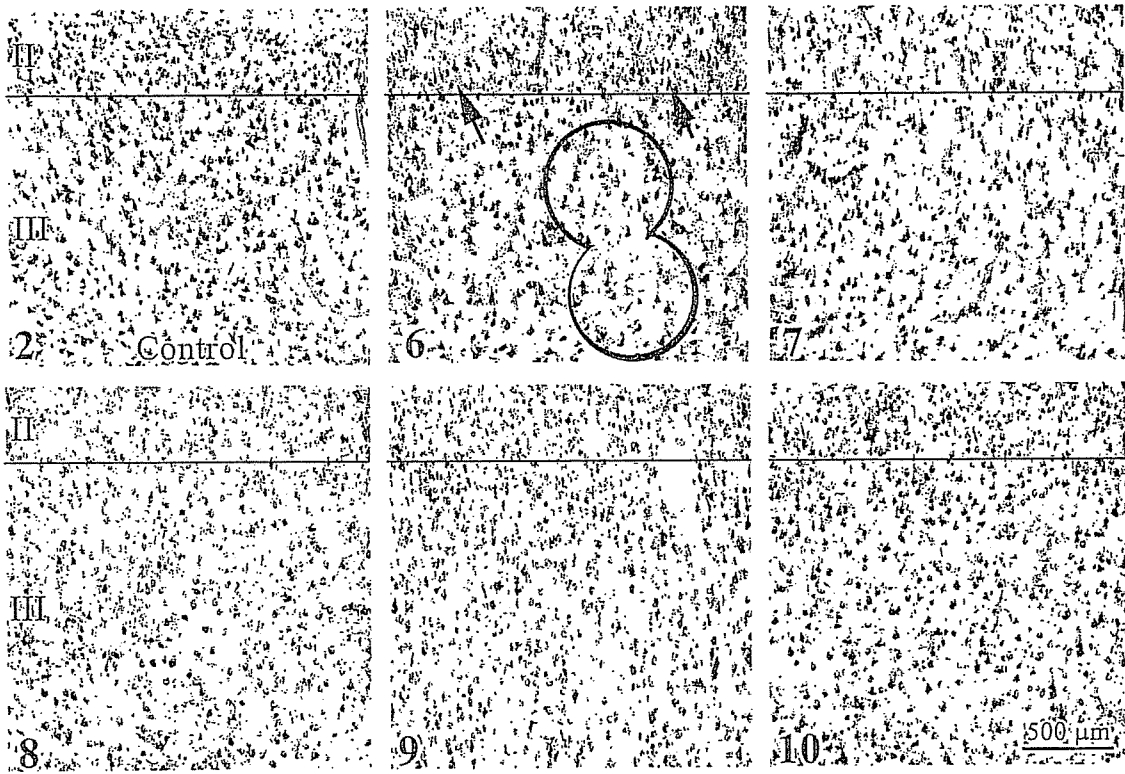


FIGURE 25.8

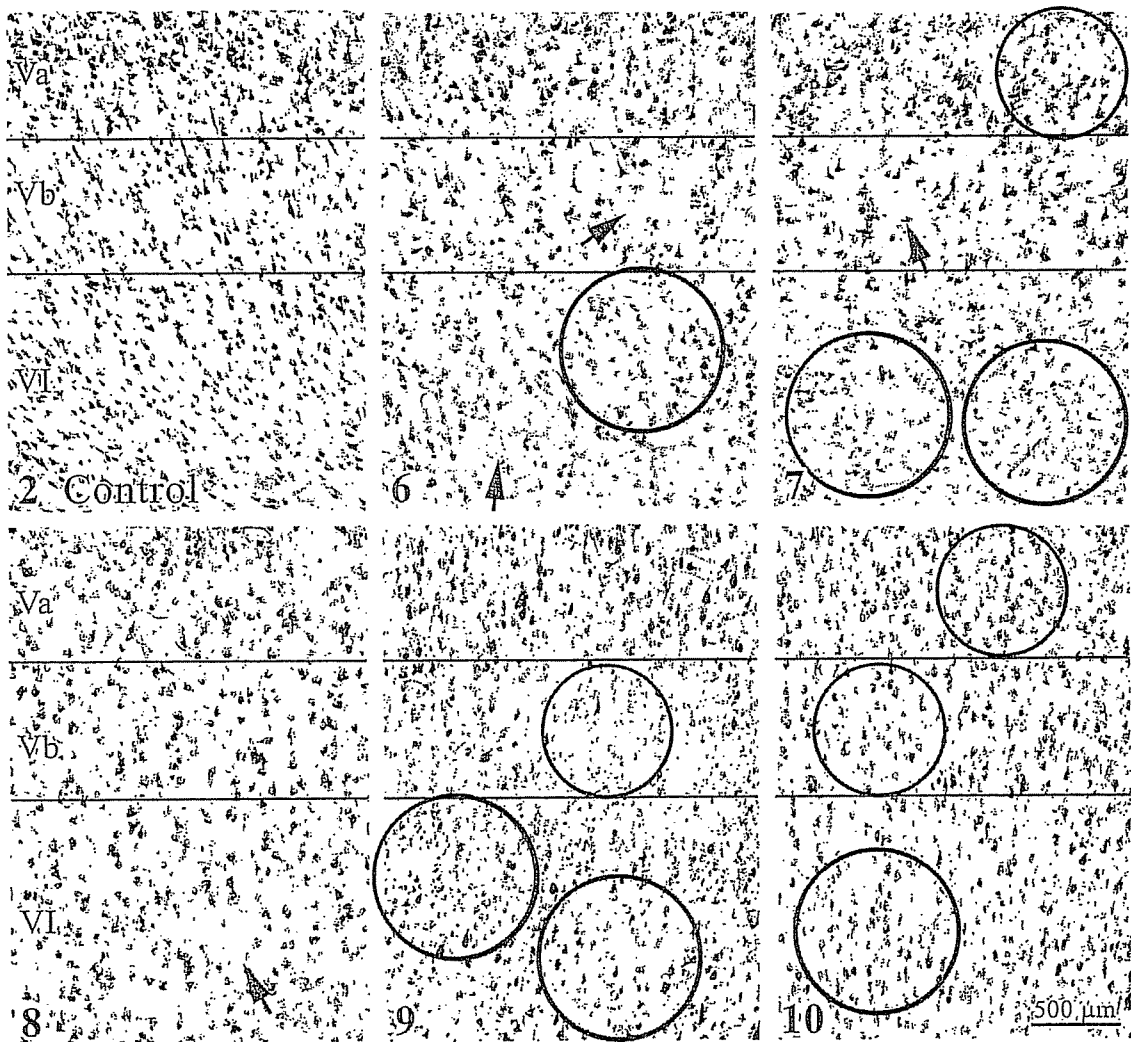


FIGURE 25.9

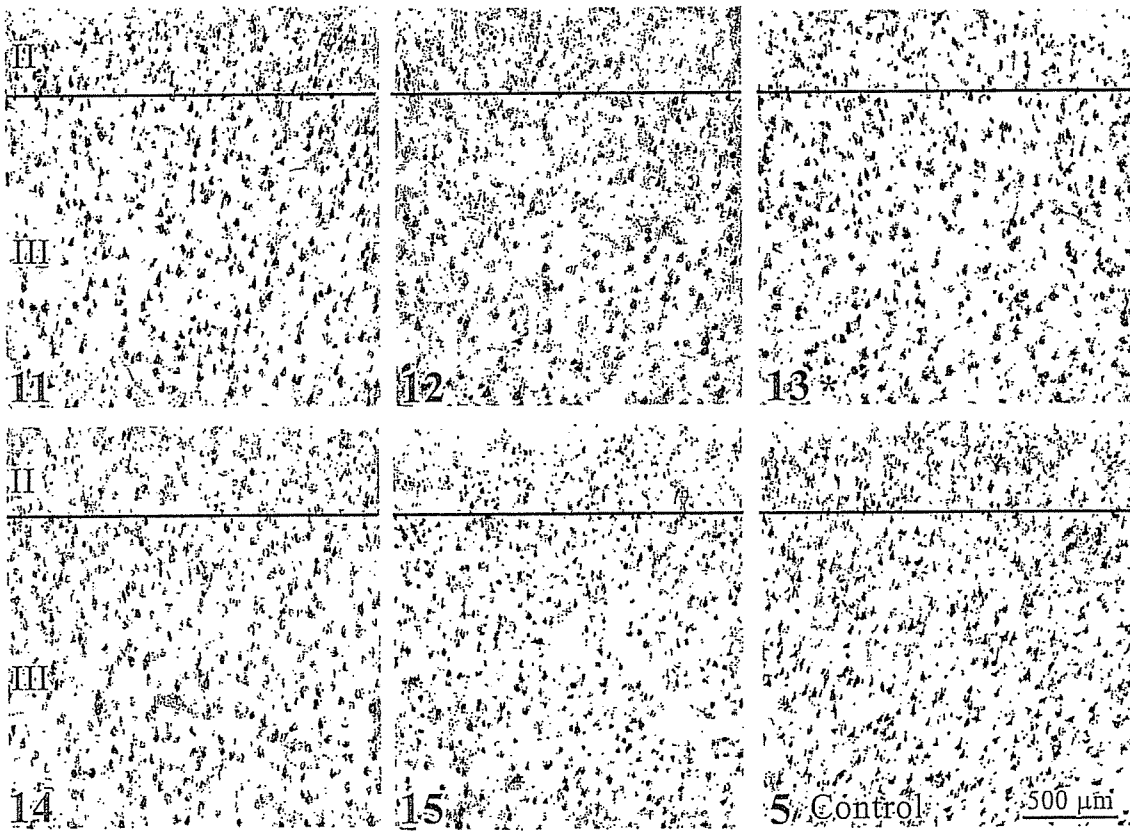


FIGURE 25.10

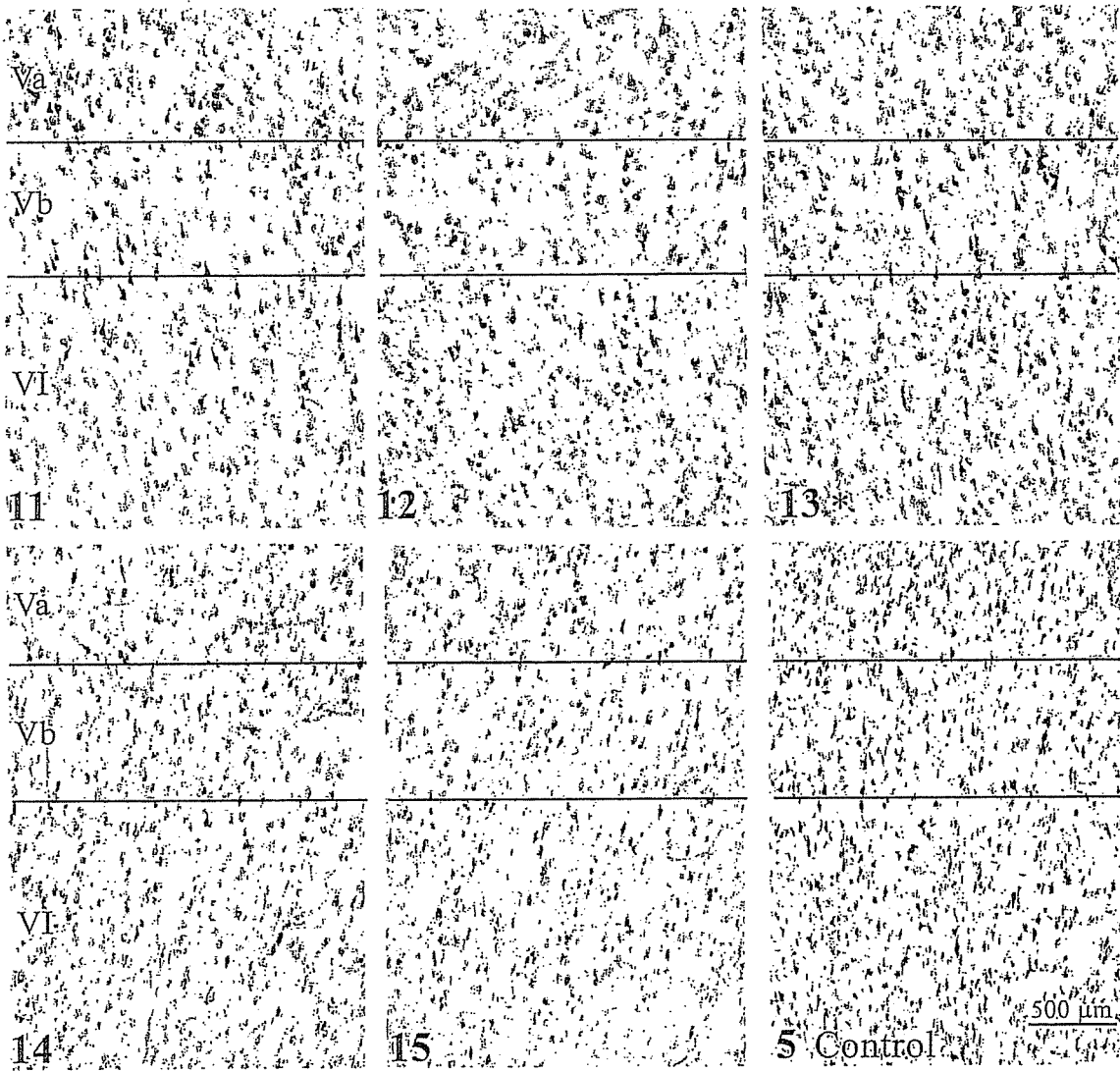


FIGURE 25.11

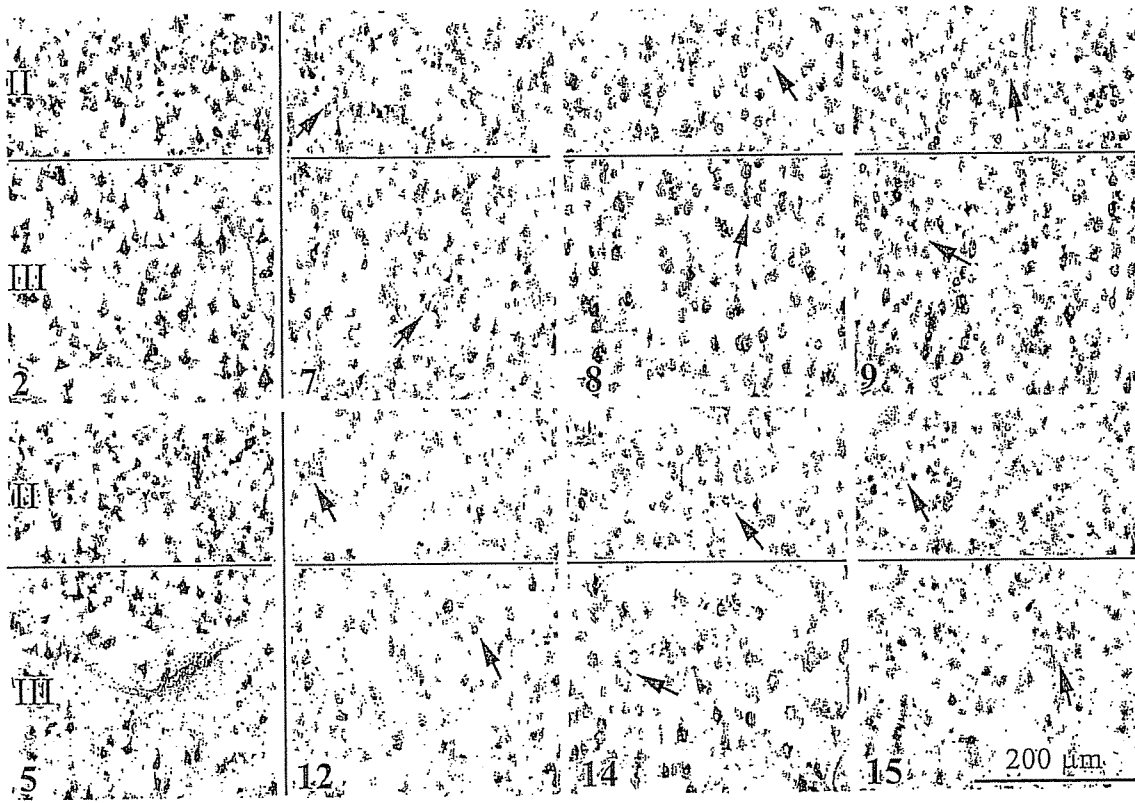


FIGURE 25.12

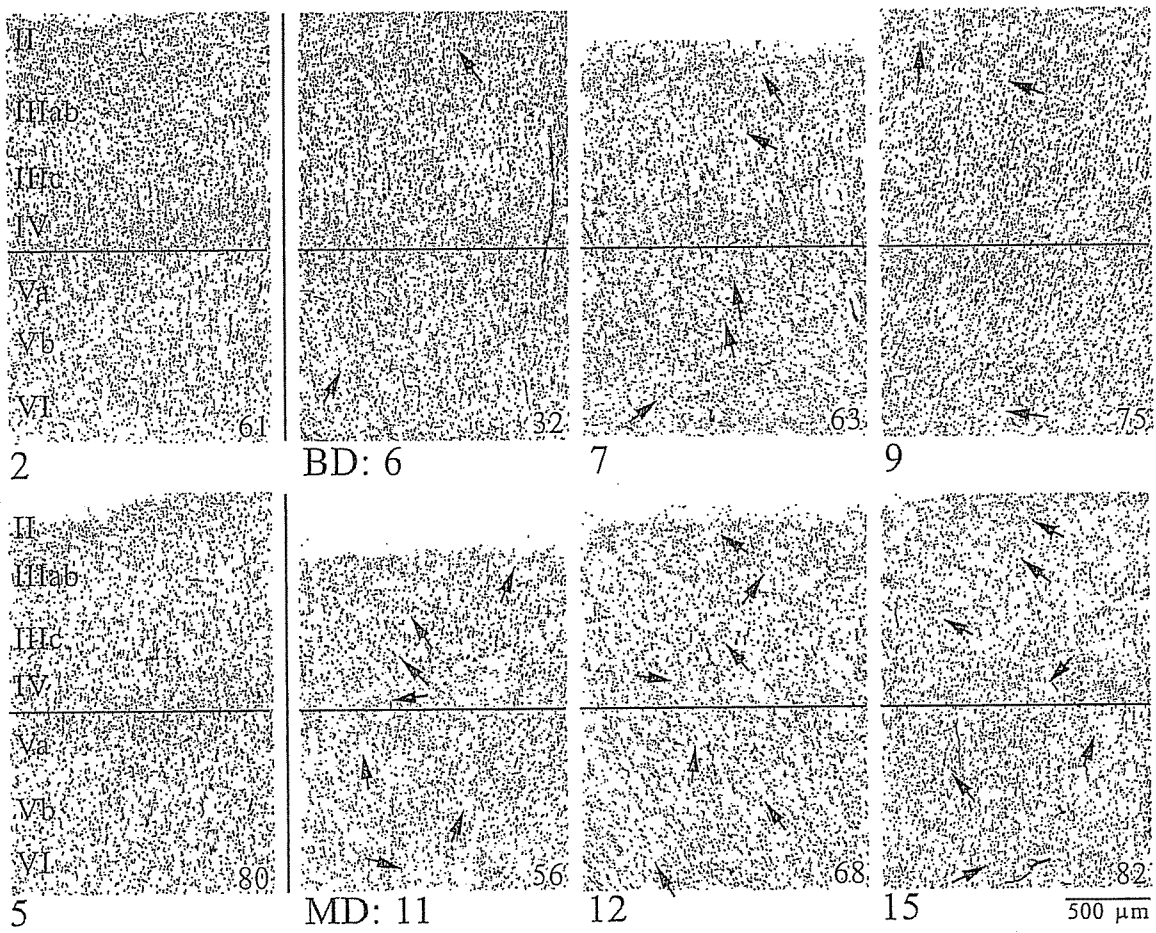


FIGURE 25.13

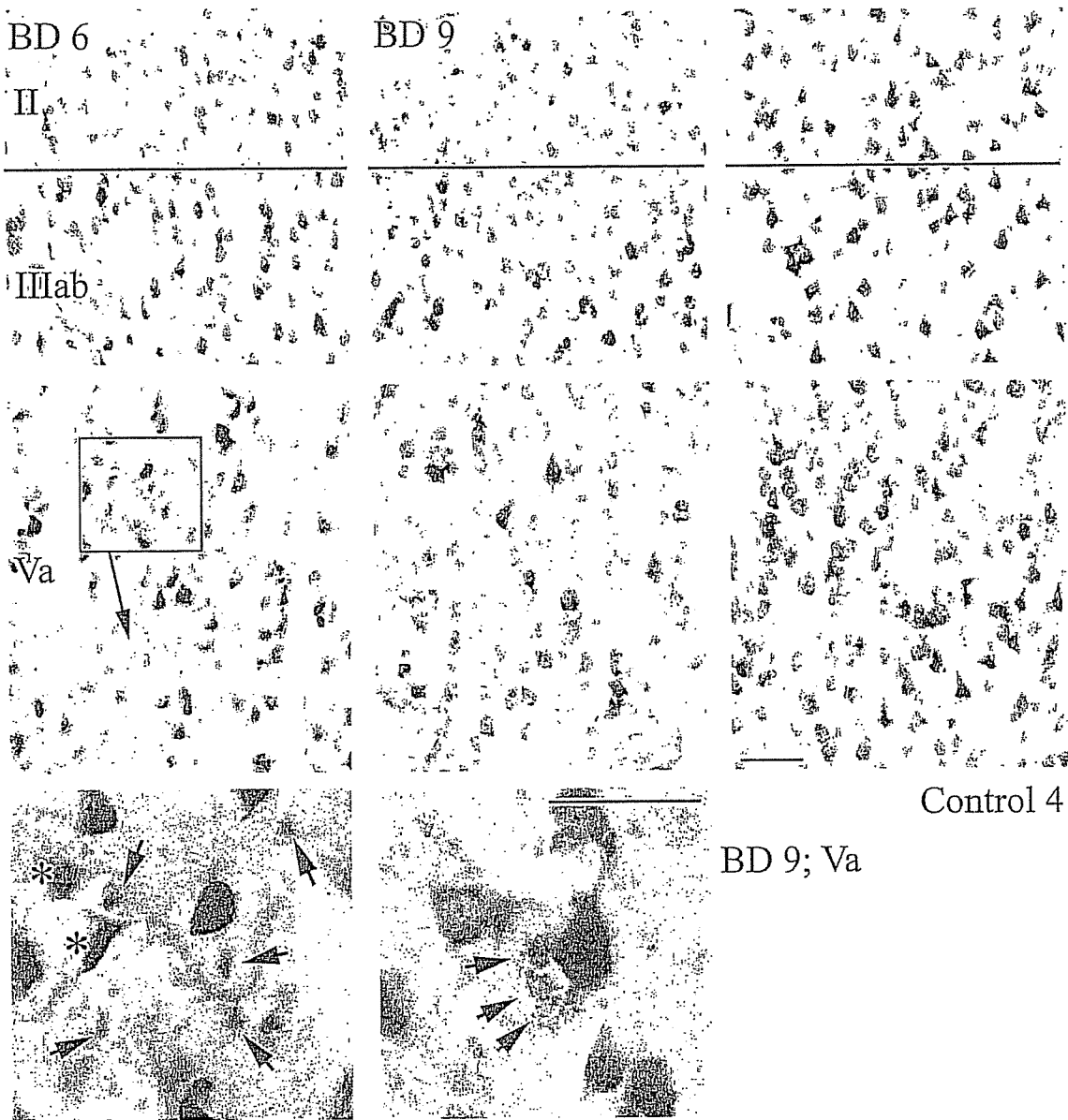
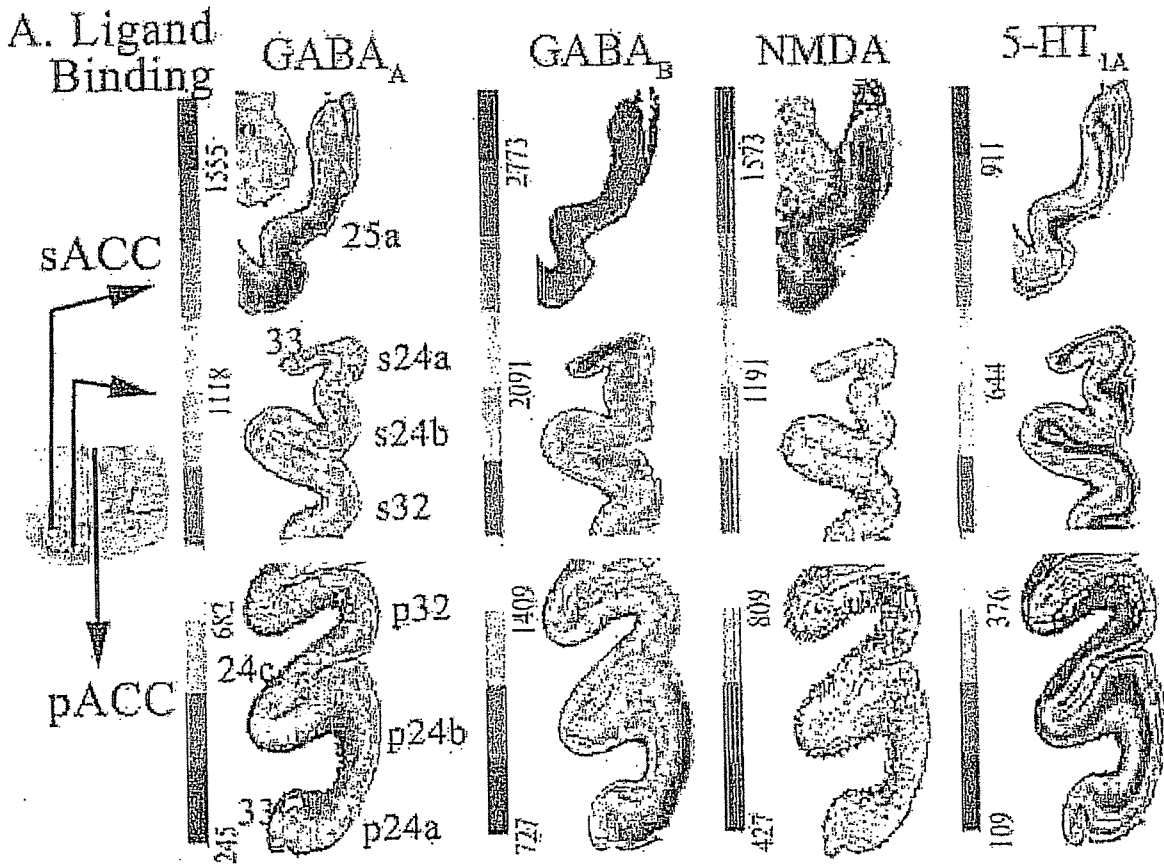


FIGURE 25.14



B. Hierarchical Clustering

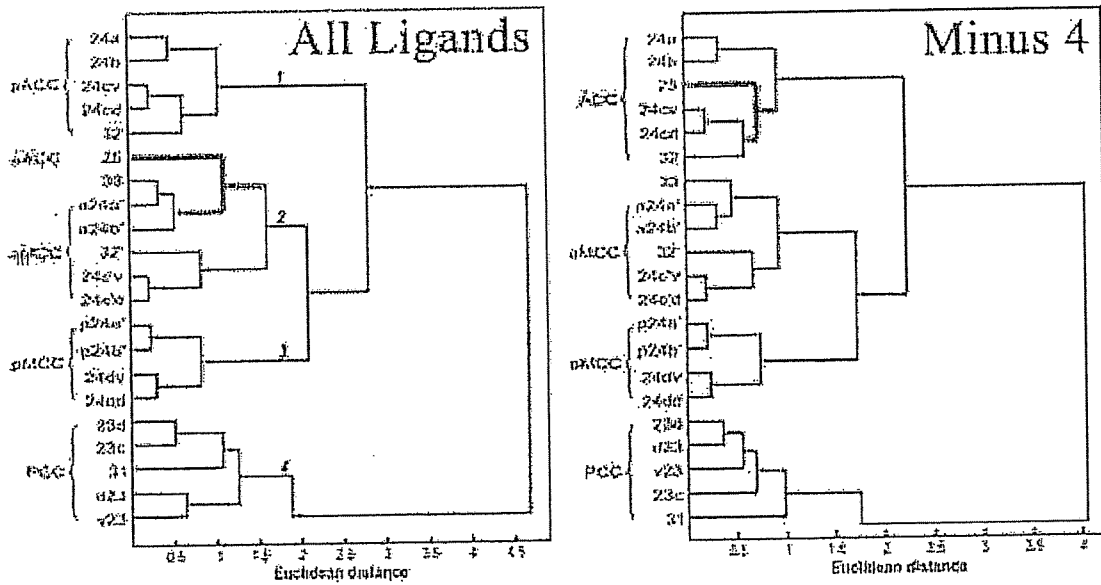


FIGURE 25.15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/59819

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 43/90; A61K 31/519 (2009.01)

USPC - 514/259.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC: 514/259.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC: 424/400; 514/2, 278, 300 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST (PGPB, USPT, EPAB, JPAB) Google, Google Patent Search

depressive, depression, mood, disorder, disease, condition, amyloid beta, Abeta, A-beta, brain, aggregation, accumulation, aggregate, accumulate, inclusions, beta-secretase inhibitor, gamma-secretase inhibitor, cholesterol lowering drug, muscarinic agonist,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| X | US 2006/0135403 A1 to (GERVAIS et al.) 22 June 2006 (22.06.2006) abstract; para [0004], [0006], [0008], [0016], [0022], [0031], [0040], [0043]-[0044], [0112], [0244], [0258] | 1-2 |

Further documents are listed in the continuation of Box C.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

24 November 2009 (24.11.2009)

Date of mailing of the international search report

09 DEC 2009

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