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Hippocampus and Human Disease

16.1 Overview

The hippocampus is involved in many disparate disease processes, but only in rare instances is the hippocampus the sole site of pathological damage. It is subject to the same pathologies that can affect other cortical areas, such as tumors, vascular malformations, and cortical dysgenesis; but in addition the hippocampus is also notable for its particular vulnerability to damage as a consequence of ischemia/hypoxia, trauma, and hypoglycemia. There are also instances in which involvement of the hippocampal formation is critical to the manifestation of the disease; foremost among them are Alzheimer's disease and temporal lobe epilepsy, representing approximately 60% of all partial epilepsies. Alzheimer's disease and epilepsy are among the most prevalent of all neurological diseases, with 20 million and 50 million people affected, respectively. Damage to the hippocampus is also the central component of a variety of rare conditions, such as limbic encephalitis and dementia with isolated hippocampal sclerosis. In addition, involvement of the hippocampus is being increasingly recognized in schizophrenia, another common neuropsychiatric disorder.

Acute encephalitis due to herpes simplex virus shows a predilection for limbic structures, and infection can result in selective damage to the hippocampus, amygdala, and associated structures, resulting in acute limbic encephalitis. Subacute limbic encephalitis has also been described in which the pathology more specifically affects the limbic system (Corsellis et al., 1968). The clinical presentation is characterized by behavioral and psychiatric problems (usually aggression and depression), disorientation, short-term memory deficits, hallucinations, seizures, and sleep disturbances (Corsellis et al., 1968; Gultekin et al., 2000). The pathological finding is aggregation of lymphocytes around blood vessels (perivascular lymphocytic cuffing), neuronal cell loss, and gliosis particularly affecting the hippocampus, dentate gyrus, amygdala, cingulate, and parahippocampal structures. There is also often coincidental involvement of the brain stem and cerebellum. Limbic encephalitis can occur in response to certain cancers such as small-cell lung carcinomas, lymphomas, thymomas, and testicular tumors, as an immune-mediated syndrome (one of a number of so-called paraneoplastic syndromes). A similar syndrome has, however, been described in association with Wernicke's encephalopathy, systemic lupus erythematosus, and herpes simplex encephalitis. In these cases, there is a strong association with anti-neuronal antibodies directed against intracellular antigens, but the pathological role of these antibodies remains uncertain. Treatment of the underlying malignancy can alleviate the symptoms.

Hippocampal sclerosis has been observed in a proportion of elderly patients presenting with cognitive impairment. In one study (Dickson et al., 1994), hippocampal sclerosis was observed in 26% of demented patients over the age of 80 years and in 16% of all patients aged over 80. In all cases there was neuronal loss and gliosis affecting CA1, the subiculum, and dentate granule cells, with additional neuronal loss in the entohinal cortex in a proportion of cases. However, concomitant pathology, such as ischemic vascular damage or Alzheimer pathology, was noted in most of the cases in this study; "pure" hippocampal sclerosis is much rarer, affecting only 0.4% of patients with dementia (Ala et al., 2000). These rare instances of pure hippocampal sclerosis are not associated with any increase in risk factors for cerebrovascular disease, and in none of the cases was there a history of a hypoxic episode preceding the onset of cognitive impairment. The relation between hippocampal sclerosis, as a rare cause of dementia in the elderly, and mesial temporal sclerosis, as a substrate for epilepsy affecting a younger age group, remains undetermined, but it is possible that the two diseases arise as a consequence of differing etiologies, with pure hippocampal sclerosis occurring as a consequence of a primary degenerative process rather than secondary to a systemic insult such as hypoxia or fever.

Schizophrenia is thought to involve primarily the prefrontal cortex (Grossberg, 2000), but there is accumulating evidence

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for involvement of mesial temporal lobe structures in its pathophysiology. Indeed, there is evidence accumulating that schizophrenia may be a result of fronto-hippocampal integration. Some patients with temporal lobe epilepsy exhibit a psychosis indistinguishable from schizophrenia. Structural neuroimaging studies have shown a subtle but definite reduction in hippocampal volume (Nelson et al., 1998), which in some cases is present early in the disease; and functional imaging studies using magnetic resonance spectroscopy have documented a reduction in levels of the metabolite *N*-acetyl-D-aspartate, a marker of neuronal viability, in patients with schizophrenia (Maier et al., 1995). Neuropathological studies indicate that the loss of hippocampal volume correlates with a reduction in the size of hippocampal neurons rather than neuronal loss (Arnold et al., 1995). A reduction in neuronal density in certain hippocampal regions, with the CA2 interneurons particularly affected, is also observed in schizophrenia, as well as in manic depression (Benes et al., 1998). Loss of synaptic proteins in the hippocampus (Eastwood and Harrison, 1995) and abnormal MAP2 expression in subicular neuron dendrites also indicate abnormalities of connectivity in patients with schizophrenia (Cotter et al., 2000). The lack of demonstrable gliosis in those with schizophrenia has been argued to support a neurodevelopmental rather than degenerative disease process; the demonstration in schizophrenia of cytoarchitectural abnormalities of pre-alpha cells in the entorhinal cortex and of abnormal orientation of hippocampal pyramidal neurons (Conrad et al., 1991) also support a maldevelopmental disorder.

The human hippocampus is the beneficiary of a generous arterial supply originating from a number of major arteries, including the anterior choroidal artery and branches of the posterior cerebral artery (Erdem et al., 1993). Despite this, the vascular supply to the hippocampus may be interrupted as a result of embolic disease, as well as by prolonged anoxic insults. Ischemic damage to the hippocampus can occur in isolation or as part of a more widespread cerebrovascular disease process. In addition, animal experiments have shown that hippocampal damage, particularly affecting the CA1 subfield, can occur as a result of chronic nonembolic vascular insufficiency (de la Torre et al., 1992). Despite the known vulnerability of CA1 to ischemic damage (an observation dating back to the observations made by Sommer in 1898) and evidence from animal studies that the hippocampus is particularly sensitive to ischemic insult (Schmidt-Kastner and Freund, 1991), there exist in the clinical literature very few cases in which selective damage to the hippocampus has been observed. Hypoglycemia results in a pattern of damage different from that of ischemia, as it causes necrosis of predominantly the dentate granule cells (Auer and Siesjo, 1988); the clinical significance of such damage is not clear. Lastly, mesial temporal structures are particularly vulnerable to traumatic brain injury; this is partly because of their location in the middle cranial fossa, leaving them susceptible to contusion and vascular injury, but it may also be due to direct excitotoxic effects (Tate and Bigler, 2000).

The characterization of hippocampal involvement in human disease has been of great value to neuroscientists and clinicians. For instance, the unilateral nature of hippocampal sclerosis in temporal lobe epilepsy has provided the opportunity to analyze the individual functions of the left and right hippocampi. Also, the identification of hippocampal sclerosis by high-resolution magnetic resonance imaging (MRI) in epilepsy patients has stimulated the use of curative epilepsy neurosurgery. The hippocampal atrophy that occurs as a result of the pathological changes of Alzheimer's disease can be detected and quantified using in vivo neuroimaging techniques. As a biomarker of disease, the presence of hippocampal atrophy provides important corroborative information at the time of the clinical diagnosis, and the demonstration of progressive hippocampal volume loss is valuable for tracking disease progression. Finally, case studies documenting the nature of cognitive impairments in those rare patients with selective hippocampal pathology have provided important insights into the functions of the human hippocampus (see Chapter 13).

This chapter focuses on two disorders in which the role of the hippocampus has been extensively investigated: Alzheimer's disease and temporal lobe epilepsy. Although in Alzheimer's disease the disease process results eventually in widespread destruction of the cerebral cortex, the damage in the earliest stages of disease is restricted to the entorhinal cortex and the hippocampus, and the memory impairment that results from this disruption of the hippocampal formation represents one of the common characteristics of Alzheimer's disease. In temporal lobe epilepsy, the pathological damage is often restricted to the hippocampus in the form of hippocampal sclerosis. However, unlike Alzheimer's disease, in which the hippocampal damage is secondary to the underlying pathological process, the hippocampus in temporal lobe epilepsy is not only sensitive to damage by seizure activity but can also act as the substrate for epileptic seizure generation.

16.2 Mesial Temporal Lobe Epilepsy and Hippocampal Sclerosis

16.2.1 Introduction

Epilepsy is the propensity to have seizures and is one of the most common serious neurological conditions, affecting 0.4% to 1.0% of the world's population (Sander and Shorvon, 1996). There are approximately 20 to 70/100,000 new cases per year, and the lifetime chance of seizures is 3% to 5% (Sander and Shorvon, 1996). Seizure types can be divided into partial seizures, arising from one part of the brain, and generalized seizures, arising simultaneously throughout the cortex; respectively, these constitute approximately 40% and 50% of seizures in newly diagnosed epilepsy (10% of seizures are unclassifiable) (Sander and Shorvon, 1996). Epilepsy itself can be divided into a number of syndromes determined by seizure type, electroencephalographic (EEG) abnormalities and concomitant neurological deficits. Although all epilepsies are the result of an underlying brain abnormality (e.g., tumor or scar tissue in partial epilepsies, and a metabolic or genetic basis in generalized epilepsies), a convincing cause is identified in only approximately 30% of patients with epilepsy (Sander and Shorvon, 1996). The clinical manifestation of a seizure depends not only on where the seizure starts but also on the speed and pattern of seizure spread. Differing epilepsy syndromes have different pathophysiologies and mechanisms; in this chapter we are concerned solely with temporal lobe epilepsy.

Temporal lobe epilepsy represents approximately 60% of all partial epilepsies. The commonest neuropathological lesion identified in temporal lobectomy series in patients with mesial temporal lobe epilepsy (TLE) is hippocampal sclerosis, or Ammon's horn sclerosis, which is seen in approximately half of the cases (Bruton, 1988). Other major pathologies can be grouped under "lesion-associated TLE" and include vascular malformations, malformations of cortical development, and glioneuronal tumors (Wolf et al., 1993). Of those patients with drug-resistant epilepsy, hippocampal sclerosis is the commonest aetiology.

In 1825, Bouchet and Cazauvielh presented their findings on 18 autopsied patients in a thesis that attempted to establish the relation between epilepsy, "l'épilepsie," and insanity, "l'aliénation mentale" (Bouchet and Cazauvielh, 1825). They noted that in five cases where there were changes in the cornu ammonis four were characterized by induration and one had softening. Sommer (1880) further described in detail the neuropathological finding of hippocampal sclerosis in the brains of patients with chronic epilepsy. He noted gliosis and pyramidal cell loss in predominantly the CA1 region of the hippocampus, and he proposed that these lesions were the cause of the epilepsy. That same year, Pfleger described hemorrhagic lesions in the mesial temporal lobe of a patient dying in status epilepticus and concluded that neuronal necrosis was the result of impaired blood flow or metabolic disturbances that occurred during the seizure (Pfleger, 1880). Since that time the debate as to whether hippocampal sclerosis is the cause or result of epilepsy has continued.

Three lines of evidence indicate that seizures originate in the sclerosed hippocampus. First, hippocampal sclerosis is closely associated with a particular seizure semiology, the psychomotor seizure—a seizure type first recognized by John Hughlings Jackson. Second, EEG evidence points to seizure onset in the sclerosed hippocampus. Lastly, surgical resection of the sclerosed hippocampus results in seizure remission.

16.2.2 Clinical Features

The typical history of a patient with hippocampal sclerosis is contained in Figure 16–1, see color insert. There is often an antecedent history of an insult (usually febrile seizures) followed by a gap before seizures begin many years later. These seizures often prove resistant to treatment. There is an increased co-morbidity including psychiatric problems (depression, psychosis), increased mortality and neuropsychological deficits that relate to the side of the hippocampal sclerosis: verbal memory deficits with dominant (usually left) temporal lobe involvement and nonverbal memory deficits with nondominant lobe involvement.

Seizure Semiology

Mesial temporal lobe seizures usually take the form of complex partial seizures, in which consciousness is disturbed, and less commonly simple partial seizures, in which consciousness is preserved (Walker and Shorvon, 1997). The seizure usually has a gradual evolution over 1 to 2 minutes (substantially longer than extratemporal seizures) and lasts longer (2–10 minutes) than complex partial seizures originating in extratemporal sites. The commonest warning (often termed aura, literally "breeze") is that of a rising sensation from the stomach. Other gastrointestinal auras can occur, especially nausea, stomach rumbling, and belching. Auras can also consist of olfactory-gustatory hallucinations, autonomic symptoms, affective symptoms, disturbances of memory, or visual hallucinations and illusions (especially with seizures involving the temporal neocortex). Autonomic symptoms include changes in heart rate and blood pressure, pallor or flushing of the face, pupillary dilatation, and piloerection. Affective symptoms typically take the form of fear (the most common and often extremely intense), depression, anger, and irritability. Euphoria and erotic thoughts have also been described. Dreamy states and feelings of depersonalization commonly occur. Déjà vu, déjà entendu, and other abnormalities of memory such as recollections of childhood or even former lives can also be present with this form of epilepsy.

After the aura and in the early stages, motor arrest and absence are prominent. Typically, this is followed by marked automatisms. The automatisms of mesiobasal TLE can be prolonged and are characteristically oroalimentary (e.g., lipsmacking, chewing) and/or gestural (e.g., fidgeting, undressing, walking). Typically, the automatisms are more marked ipsilaterally and may be associated with contralateral posturing. There may be some apparent responsiveness, and "conscious behavior" can occur during nondominant temporal lobe seizures. During the seizure, speech with recognizable words lateralizes the focus to the nondominant temporal lobe. Secondary generalization is less common than in extratemporal lobe epilepsy. Postictal confusion is typical, and postictal dysphasia can occur following dominant temporal lobe seizures. Postictal headache and postictal psychosis have also been described. There is profound amnesia for the absence and automatism (Walker and Shorvon, 1997).

EEG Characteristics

Scalp EEG recordings usually demonstrate interictal epileptiform abnormalities (spikes, sharp waves) over the mid/anterior temporal region, but it is common for these epileptiform

A

A 32-year old man presented with refractory partial epilepsy. He had a prolonged febrile seizure at the age of 18 months, and spontaneous seizures began at the age of 8 years. These seizures have continued despite trying all available antiepileptic drugs. The seizures take the form of simple partial seizures in which he perceives a sense of fear rising from his stomach, and complex partial seizures in which he has an aura as above and then loses touch with his surroundings. During the complex partial seizure he is described as making chewing movements, his right hand is postured and he fiddles with his clothes with his left hand. The seizure lasts a couple of minutes, and he is then confused and dysphasic afterward for approximately 5 minutes.

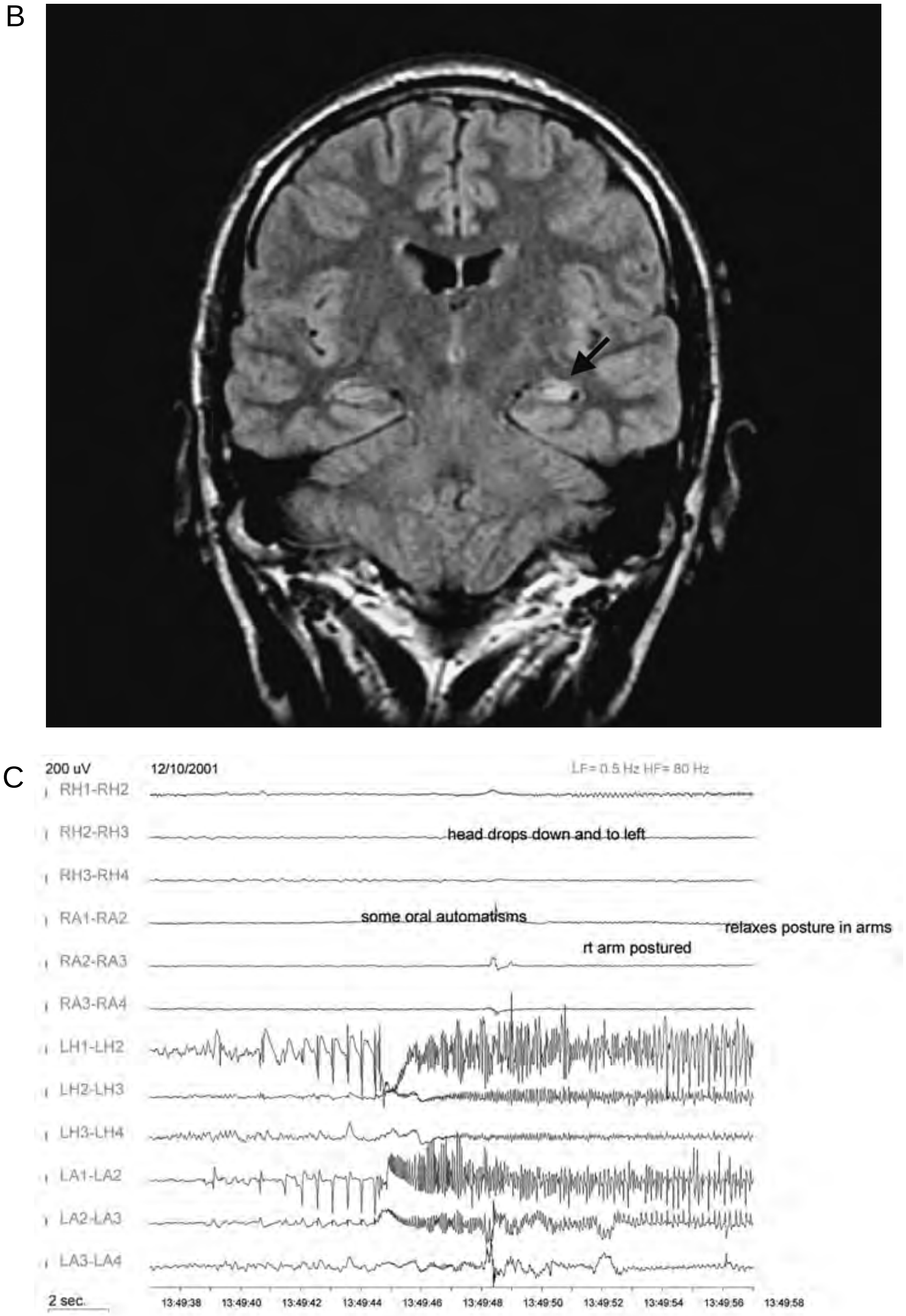

Figure 16–1. Clinical features of mesial temporal lobe epilepsy. *A*. Typical history. *B*. Magnetic resonance imaging (MRI) findings of left hippocampal sclerosis with high T2 signal in shrunken hippocampus (arrow). *C*. Intracranial recordings from left (LH) and right (RH) hippocampus and left (LA) and right (RA) amydala demonstrate well localized 1- to 2-Hz spikes in left hippocampus and amygdala that evolve to low-amplitude fast activity at seizure onset.

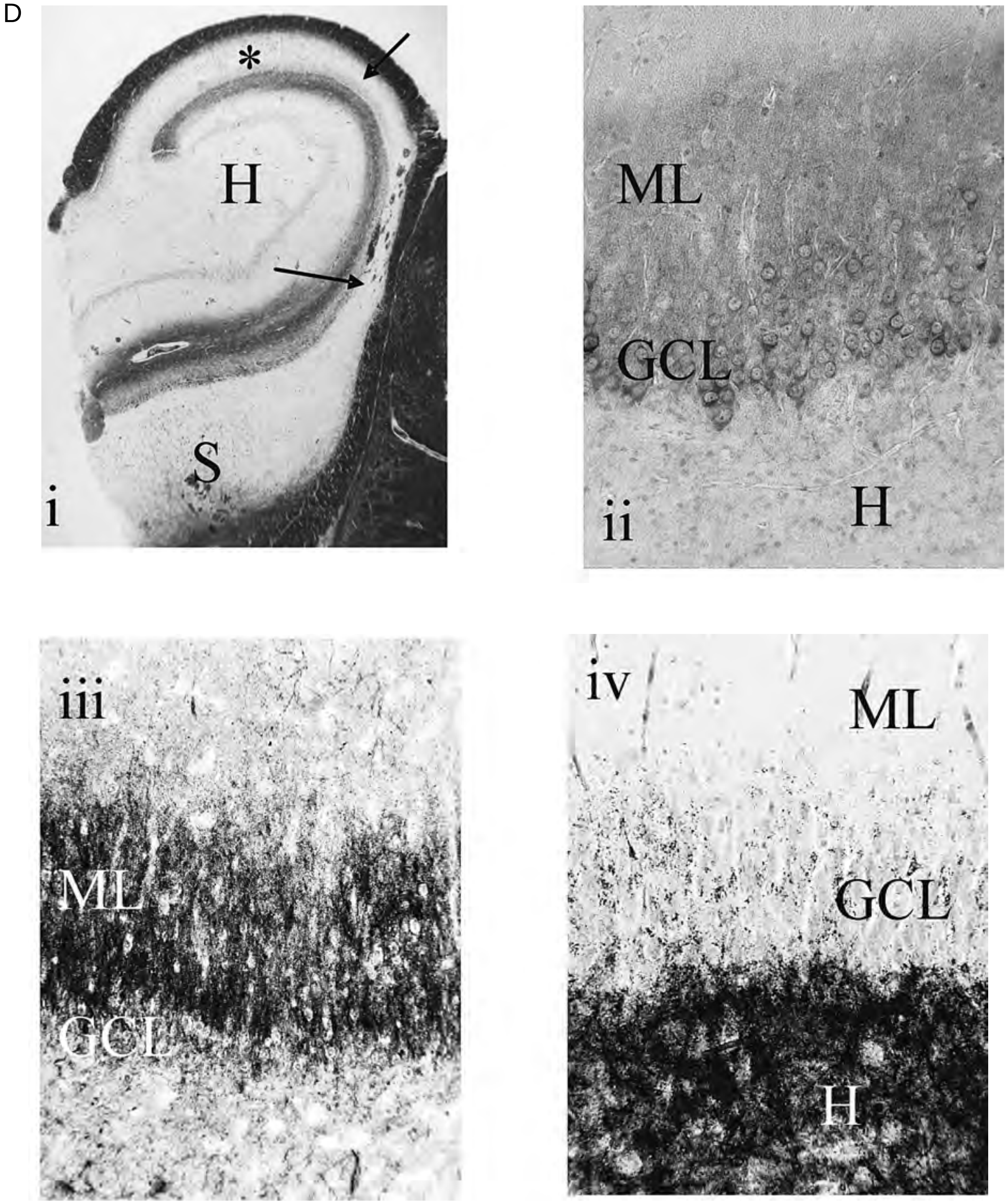

Figure 16–1. *(Continued) D*. Histology of left hippocampal sclerosis. i. Loss of cells in hilus (H) and CA1 (arrows) with preservation of CA2 (star) and subiculum (S); ii. Dynorphin staining demonstrating many fiber sprouting in granule cell layer (GCL) and molecular layer (ML); iii. Mossy fiber sprouting illustrated with Timm's stain; iv. Hippocampus with minimal mossy fiber sprouting for comparison with iii.

abnormalities to occur bilaterally or independently over both temporal regions (reasons why this may be so are discussed below) (Williamson et al., 1993). Ictal scalp recordings usually demonstrate a build-up of 5- to 10-Hz sharp activity localized to the mid/anterior temporal region. This activity may remain localized or commonly spreads to involve a wider field including the contralateral temporal lobe.

Depth electrode studies have further confirmed the electrographic origin of these seizures in the hippocampal formation (King and Spencer, 1995). Although interictal spikes may occur independently from either the hippocampus or extrahippocampal sites (see below), the ictal discharges are usually relatively well localized. Preictal abnormalities can occur with well localized 1- to 2-Hz spikes that recur over seconds or minutes (Fig. 16–1C, see color insert). With clinical seizure onset, there is a 10- to 15-Hz low-amplitude discharge that is initially confined to hippocampal electrodes (Fig.–16 1C, see color insert) but grows in amplitude and then spreads to other regions (King and Spencer, 1995). A second pattern has also been described in which the seizure begins as a low-amplitude, high-frequency discharge without the preictal spiking. These two types of onset can occur in the same patient. The exact location for seizure onset can vary not only from patient to patient but also within the same patient. This suggests that seizure onset and generation is not from a single area in mesial temporal structures but from a distributed network.

Hippocampal Resection

Surgery has provided the most compelling evidence of hippocampal sclerosis as the substrate for the epilepsy. Surgical outcome for intractable TLE is most successful when mesial temporal structures are included in the resection. In patients with drug-resistant epilepsy in whom there is concordance between neuroimaging, electroclinical characteristics of the seizure, and neuropsychological tests, there is a better than 80% chance of "curing" the epilepsy with temporal lobe resection (Arruda et al., 1996). Furthermore, over 80% of patients without tumors rendered seizure-free by temporal lobectomy have hippocampal sclerosis as their main pathology (French et al., 1993). In these patients depth electrode recordings also localized the seizure onset to the sclerosed hippocampus. There is thus a strong correlation between resection of a sclerosed hippocampus and cure of the epilepsy. Temporal lobe surgery, however, also involves removal of or damage to structures outside the hippocampus, including the amygdala and parahippocampal structures and temporal neocortex. Furthermore, many patients, despite successful surgery, remain dependent on antiepileptic drugs. These observations argue that structures beyond the hippocampus are involved in the epileptic network.

16.2.3 Etiology

Pathogenesis of Hippocampal Sclerosis and Developmental Aspects

There are predictable patterns of cell loss and alterations to the intrinsic circuitry of hippocampal sclerosis. However, the factors critical to the initiation of the cell loss and hippocampal reorganization are still debated, and the precise etiology of hippocampal sclerosis remains elusive.

A significant cerebral insult (or initial precipitating injury) occurring early in life, such as a febrile or prolonged seizure, is often reported (30–50% of cases—but up to 80% in one surgical series) in retrospective studies of patients with hippocampal sclerosis (French et al., 1993). The "injury" hypothesis implies that this insult irreversibly damages or alters the hippocampus, resulting in a template for the progression to hippocampal sclerosis following a "latent" interval. There appears to be age-specific sensitivity for this injury, with more severe neuronal loss demonstrated with earlier onset of epilepsy (Davies et al., 1996). The most direct evidence of the association is the observation with serial neuroimaging that hippocampal sclerosis occurs following prolonged febrile convulsions (Van Landingham et al., 1998). Febrile seizures have been modeled in animals by inducing hyperthermic seizures in rats by blasts of hot air or water. The similarities between the animal model and the human condition are that seizures occur in response to high body temperature and that increasing age confers resistance to these seizures (Baram et al., 1997; Walker and Kullmann, 1999). Although fever in humans is associated with other physiological changes, reducing the body temperature is an effective way to reduce the likelihood of seizures; thus, hyperpyrexia is probably the main trigger. There are, however, major differences between hyperthermic seizures in rats and febrile convulsions in humans. Inducing hyperthermia in young Sprague-Dawley rats apparently results in seizures in most of these animals (Baram et al., 1997), but convulsions are relatively rare in children with fever. In experimental models, prolonged hyperthermic seizures in immature rats did not cause spontaneous seizures during adulthood but did increase seizure susceptibility following administration of a convulsant (a "second hit") (Dube et al., 2000). However only 2% to 7% of children with a history of febrile convulsions go on to develop epilepsy (i.e., unprovoked seizures) later in life (Annegers et al., 1987). Because many children with febrile convulsions may have a predisposing susceptibility to seizures, the low incidence of subsequent epilepsy could be explained by a protective effect of febrile seizures. Alternatively, febrile seizures alone are not sufficient to result in development of epilepsy. (Walker and Kullmann, 1999).

Other insults can result in hippocampal sclerosis including neonatal hypoxia and head injuries. In rat models, fluid percussion injury to the dura results in hilar interneuron loss in the hippocampus (Lowenstein et al., 1992). The mechanism by which this occurs is unknown. The neuronal loss is accompanied by enhanced excitability of the hippocampus but again no spontaneous seizures (Lowenstein et al., 1992). These experimental and human studies do not, however, address two fundamental questions: (1) Why is hippocampal sclerosis predominantly a unilateral disease process in humans (see below) following a "global" cerebral insult? (2) What is the nature of the "second hit" that results in the expression of epilepsy?

The second hit does not necessarily have to be environmental but could be the coexistence of various genetic factors or concomitant developmental abnormalities. Temporal lobe epilepsy is generally regarded as an acquired disorder with only a small genetic contribution. There are familial cases of febrile seizures, which are associated with ion channel mutations: sodium channel subunit and γ-aminobutyric acid ionotropic receptor family A ($GABA_A$) subunit mutations) (Table 16–1) (Kullmann, 2002). However, these families usually present with a hetererogeneous group of epilepsies that are distinct from the typical history of hippocampal epilepsy. More recently, the leucine-rich, glioma-inactivated 1 gene has been associated with familial neocortical temporal lobe epilepsies, although the mechanisms by which this mutation results in epilepsy are unknown (Kullmann, 2002). We are at present ignorant of the genetic mutations underlying most of the genetically determined epilepsies, let alone those that contribute to other epilepsies. Genetic predisposition to some forms of temporal lobe epilepsy and febrile convulsions have been described, and there are familial cases of febrile convulsions and TLE but without hippocampal sclerosis (Baulac et al., 2001).

More recent attention has focused on an underlying maldevelopment of the hippocampus as a primary abnormality predisposing to hippocampal sclerosis and to febrile seizures. In an MRI study of families with familial febrile convulsions, a subtle preexisting hippocampal abnormality was detected (Fernandez et al., 1998), and hippocampal sclerosis has also been reported in patients in association with isolated malformations of the hippocampus (Baulac et al., 1998). In addition, an abnormal persistence of calretinin positive Cajal-Retzius cells in the hippocampus has been reported in hippocampal sclerosis specimens (Blumcke et al., 1999b). Cajal Retzius cells, through secretion of the reelin protein, play a critical role in neuronal organization in the developing brain. Higher numbers of Cajal-Retzius cells were particularly prominent in patients with hippocampal sclerosis and a history of febrile seizures. It is plausible that such an injury occurring early in life disrupts normal hippocampal development and maturation (one manifestation of which is an excess of Cajal-Retzius cells), which in turn predisposes to hippocampal sclerosis. As it has been suggested that reelin in the adult cortex has a role in plasticity and axonal remodeling, an increased number of these cells may also be important for the reorganization of circuitry occurring in hippocampal sclerosis (described below).

The final argument supporting a maldevelopmental basis for hippocampal sclerosis comes from the observation that hippocampal sclerosis is often observed in association with subtle cytoarchitactural malformations in the neocortex, also termed microdysgenesis (Hardiman et al., 1988). This may be indicative of a more widespread maldevelopmental process involving both mesial and lateral temporal lobe structures. One cytoarchitectural feature observed in microdysgenesis is also an excess of Cajal Retzius cells in the molecular layer (Garbelli et al., 2001), which interestingly seems to parallel findings in hippocampal sclerosis.

Table 16–1.
Monogenic Epilepsies and Ion Channels Implicated in Human Epilepsy

Hippocampal sclerosis is also well recognized to occur in association with more severe cortical malformations, vascular malformations, and low-grade glioneuronal tumors (Cendes et al., 1995; Li et al., 1999). It possible that in these cases the epileptogenic extrahippocampal lesion "kindles" the hippocampal neuronal loss (i.e., the hippocampal sclerosis in these cases is a secondary event) (see below). It has been shown, however, that in patients with dual pathologies removal of both the lesion and the abnormal hippocampus has the best outcome in terms of seizure control (Li et al., 1999), emphasizing the role of the hippocampus in temporal lobe seizures even when there is a second pathology.

Animal Models of Mesial Temporal Lobe Epilepsy

The interpretation of many of the pathological findings and the electrophysiologic studies in human postsurgical specimens is confounded by: (1) the influence of treatment; (2) the difficulty differentiating cause from effect (i.e., it is possible that the changes are the result, not the cause, of the seizures); and (3) the lack of adequate control tissue for comparison. To overcome these handicaps, animal models of mesial TLE are used. The two most studied are the kindling model and the poststatus epilepticus model. Intrahippocampal injection of tetanus toxin also results in spontaneous seizures even after clearance of the toxin, and this model has also contributed to our understanding of the pathophysiology of mesial TLE (Mellanby et al., 1977). This model does not result in hippocampal sclerosis (Jefferys et al., 1992), and the seizures usually abate, in contrast to the human condition. We discuss the kindling and the poststatus epilepticus models in more detail, as these models possibly have human correlates.

Kindling. Kindling is the repetition of tetanic (trains of) stimuli that initially evoke after-discharges but not seizures (Goddard, 1967; McNamara et al., 1993). Repetition of the same trains of stimuli results in gradual lengthening of the after-discharges, eventually leading to progressively more severe seizures. Once an animal has been kindled, the heightened response to the stimulus seems to be permanent, and spontaneous seizures can occur (McNamara et al., 1993). The hippocampus and amygdala are easily kindled, resulting in a well described progression of limbic seizures. Kindling shares several characteristics with NMDA-dependent long-term potentiation (LTP) of excitatory synaptic transmission. This has led to the suggestion that kindling and LTP have similar underlying mechanisms. In support of this, the rate at which kindling occurs is retarded in rodents treated with NMDA receptor antagonists. There are, however, several differences between kindling and LTP. Although NMDA receptor antagonists can completely block the induction of LTP, they are unable to block kindling completely (Cain et al., 1992). Perhaps a more fundamental difference is that the kindling process requires after-discharges; the repeated induction of LTP without after-discharges does not induce kindling. LTP of glutamatergic synaptic transmission may contribute to kindling by increasing the excitatory synaptic drive and the likelihood of evoking after-discharges but is alone insufficient to explain the cellular mechanisms of kindling (Cain, 1989; Cain et al., 1992).

Kindling alone is unlikely to explain the occurrence of hippocampal sclerosis in association with other pathology because kindling itself usually results in no or minimal hippocampal damage and sclerosis (Tuunänen and Pitkänen, 2000). Kindling could, however, explain the progression of mesial temporal epilepsy. Eventually spontaneous seizures in the kindling model result in progressive neuronal loss in the hippocampus (Cavazos et al., 1994). Indeed, even following single seizures there is evidence of both apoptotic cell death and neurogenesis in the dentate granule cell layer (Bengzon et al., 1997). This suggests that recurrent seizures may cause further structural and functional changes in the hippocampus. Human evidence for this has mainly been indirect. Epilepsy duration correlates with hippocampal volume loss and progressive neuronal loss and dysfunction (Theodore et al., 1999). There has also been a case reported of hippocampal volumes decreasing with time in hippocampal sclerosis (Van Paesschen et al., 1998) and the appearance of hippocampal sclerosis de novo following secondary generalized brief tonic-clonic seizures (Briellmann et al., 2001).

Poststatus Epilepticus. Seizures are usually self-terminating and brief. Occasionally seizures persist unabated, or repeated seizures can occur without recovery; this situation is termed status epilepticus. Although status epilepticus may occur in individuals with preexisting epilepsy, more than half of patients who present with status epilepticus have no history of seizures (DeLorenzo et al., 1996). In these patients, the status epilepticus is often acutely precipitated by a central nervous system (CNS) infection, cerebral vascular accident, hypoxia, or alcohol. The probability of then developing epilepsy (unprecipitated seizures) is 41% within 2 years compared with 13% for those with acute symptomatic seizures but no status epilepticus (Hesdorffer et al., 1998). This suggests a relation between the prolonged seizures of status epilepticus and subsequent epileptogenesis, although a relation between the length of the seizure and the nature and severity of the precipitant cannot be discounted. In humans, status epilepticus has been shown to result in hippocampal damage and subsequent hippocampal sclerosis. The hippocampus thus has a dichotomous role: as the substrate for epilepsy and as the structure susceptible to damage by prolonged seizures. Animal models of generalized convulsive as well as limbic status epilepticus have supported these findings. Limbic status epilepticus has been induced by the systemic or local administration of kainic acid, systemic administration of pilocarpine (a muscarinic receptor agonist), or protocols using electrical stimulation of limbic areas (Walker et al., 2002). Status epilepticus in these models in adult animals results in hippocampal damage similar to that observed in humans. Following these acute episodes of limbic status epilepticus, many of the animals go on to develop spontaneous limbic seizures after a latent period lasting days to weeks (Walker et al., 2002).

16.2.4 Pathophysiology

One of the major points of confusion in understanding the pathophysiology of epilepsy is the differentiation of a seizure (ictus) from interictal discharges and, indeed, from epilepsy itself. Although obviously linked, they are separate entities. An epileptic seizure is a transient paroxysm of excessive discharges of neurons in the cerebral cortex causing a clinically discernible event. Brief synchronous activity of a group of neurons leads to the interictal spike, and as we discuss, this shares some mechanisms with seizure generation; spikes should, however, be recognized as a distinct phenomenon (de Curtis and Avanzini, 2001). Epilepsy, on the other hand, is the propensity to have seizures; and epileptogenesis is the development of a neuronal network in which spontaneous seizures occur.

Interictal Spike

Epileptiform interictal EEG abnormalities include spikes, which are fast electrographic transients lasting less than 80 ms, and sharp waves, which last 80 to 120 ms (de Curtis and Avanzini, 2001). That these abnormalities are pathological is supported by their rare occurrence ($< 1\%$) in healthy individuals (Gregory et al., 1993) and their strong association with epilepsy (Ajmone-Marsan and Zivin, 1970). Spikes and sharp waves are often followed by a slow wave lasting hundreds of milliseconds. As discussed below, this slow wave probably represents a period of relative refractoriness. It has been established from concomitant field potential and intracellular recordings that the intracellular correlate of the interictal spike is the paroxysmal depolarizing shift (PDS) (Matsumoto and Ajmone-Marsan, 1964), a slow depolarizing potential with a high-frequency (> 200 Hz) burst of action potentials.

In hippocampal slices from healthy animals, PDSs can be observed if $GABA_A$ inhibition is reduced or if “excitability” is increased by increasing potassium, reducing magnesium, reducing calcium, or blocking potassium channels with 4-aminopyridine (de Curtis and Avanzini, 2001). The PDS is characterized by an early phase that is maintained by intrinsic properties of the neuron followed by a later phase that is secondary to recurrent excitation. Thus, the generation of interictal spikes is dependent on two phenomena: the intrinsic burst properties of neurons and the synchronization of neuronal populations. Within the hippocampus, pyramidal cells in area CA3 and some in area CA1 demonstrate burst properties (see Chapter 5). The mechanisms underlying this are different for neurons from these two subfields. The bursting in CA3 pyramidal cells appears to be dependent on regenerative dendritic potentials secondary to activation of calcium and sodium channels (Traub and Jefferys, 1994), whereas the burst properties of some CA1 pyramidal cells is probably due to persistent sodium currents (Su et al., 2001). The effect of a burst of action potentials is to increase synaptic reliability; within the excitatory network of the CA3 pyramidal cells, burst firing in a single CA3 pyramidal cell can generate a synchronized burst throughout the whole network (Miles and Wong, 1983). Because of the propensity for the CA3 pyramidal cells to generate this synchronized burst, this region has often been considered the “pacemaker” for seizure activity. Synchronized bursts can, however, also occur in the CA1 subfield (Karnup and Stelzer, 2001). In some situations the synchronization of CA1 pyramidal cells is secondary to a CA3 generated burst, but synchronization can also occur through a combination of nonsynaptic mechanisms including gap junctions, ephaptic transmission, and changes in the extracellular milieu. The importance of these nonsynaptic mechanisms in neuronal synchronization has been emphasized by the “zero” calcium model of ictal discharges, in which reducing extracellular calcium in a hippocampal slice preparation below that necessary for synaptic transmission results in synchronized epileptiform discharges due to increased axonal excitability and ephaptic transmission (Jefferys, 1995). Furthermore, decreasing extracellular space (indirectly increasing ephaptic transmission) can promote bursting (Roper et al., 1992), whereas intracellular acidification with sodium propionate—indirectly decreasing electrotonic coupling (Perez and Carlen, 2000)—inhibits epileptiform bursts (Xiong et al., 2000). Synchronization of principal cells can occur secondary to oscillations in the inhibitory interneuron network; indeed, single basket cells have been shown to synchronize the discharges of pyramidal cells through synchronized somatic inhibition (Cobb et al., 1995). Although the precise mechanisms of neuronal synchronization in the hippocampus are still unclear, the observation of high-frequency oscillations superimposed on spike discharges has led to the hypothesis that the same physiological mechanisms that subtend fast oscillations in the hippocampus are also responsible for pathological synchronization (Perez and Carlen, 2000;Traub et al., 2001).

The interictal spike is terminated by activation of hyperpolarizing $GABA_A$ and $GABA_B$ receptor-mediated currents and calcium-dependent potassium currents (Alger and Nicoll, 1980; Domann et al., 1994; Scanziani et al., 1994). There is also some evidence of a contribution by other potassium currents, such as the sodium-dependent potassium current (Schwindt et al., 1989). Blocking the after-hyperpolarization, however, only results in moderate prolongation of the burst in CA3; and exhaustion of the immediately releasable pool of glutamate has also been proposed to be a critical process in burst termination (Staley et al., 1998). Furthermore, large depolarizations (rather than hyperpolarizations) herald the termination of brief epileptic after-discharges (Bragin et al., 1997). This depolarization can be replicated by focal microinjection of potassium, and it has been hypothesized that potassium ions released by discharging neurons result in propagating waves of depolarization, which block spike generation in neurons akin to spreading depression (Bragin et al., 1997).

Nevertheless, interictal spikes activate hyperpolarizing currents resulting in a postspike refractory period, during which neuronal activity is inhibited (de Curtis and Avanzini, 2001). The effective activation of these currents by the interictal spike thus raises the possibility that spikes can be anti-ictogenic. There is evidence that this may be the case or at least that spikes are intrinsically different from a seizure. Depth EEG recordings in humans suggest that the interictal spike can originate from a much wider field than the ictal zone (see above). Therefore, it is not uncommon to find spikes originating in either hippocampus, whereas seizure activity is confined to one hippocampus.

A seizure is not the evolution of spike discharges but can begin as a distinct high-frequency rhythm (see above). Spike discharges can precede the seizure with progressively less effective after-hyperpolarizations, but ictal activity remains a distinct phenomenon. Furthermore, activation of interictal spikes occurs after the seizure, raising the possibility that this is a compensatory antiepileptic response (de Curtis and Avanzini, 2001). Critical experiments in entorhinal cortex-hippocampal slice preparations, in which there is partial preservation of the trisynaptic loop, have confirmed the antiepileptic potential of spikes. Spike discharges generated in CA3 inhibited epileptic activity in the entorhinal cortex, so sectioning of the Schaffer collaterals led to potentiation of entorhinal cortex seizure activity (Fig. 16–2) (Barbarosie and Avoli, 1997).

Most of the ictal activity described thus far in the hippocampal slice preparation has been brief and difficult to relate to seizures in vivo that last tens of seconds. Can such prolonged activity be mimicked in the slice, and does it differ from briefer discharges? Prolonged ictal activity (seizure-like activity) has been induced in the slice with high extracellular potassium (Traynelis and Dingledine, 1988). In this preparation, the CA3 subfield generates regular interictal spikes, which "drive" the generation of prolonged rhythmic "seizure" activity in the CA1 region; interestingly, the CA3 region in this preparation is resistant to generating this ictal activity (Traynelis and Dingledine, 1988). Inducing seizure-like activity in brain slices by other means (e.g., the lowering magne-

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Figure 16–2. Interictal spikes inhibit seizure activity. Spontaneous epileptiform activity induced by Mg^{+2}-free artificial cerebrospinal fluid (ACSF) before and after a Schaffer collateral cut. Before the lesion (*top*), synchronized interictal discharges are recorded in the CA3, entorhinal cortex (EC), and dentate gyrus (DG). Sectioning the Schaffer collaterals (*bottom*) abolishes interictal discharges in the EC and discloses ictal epileptiform activity that is recorded in the three areas. Expanded traces of the experiment shown in the *top* and *bottom* are illustrated in the *middle*. Note that before the Schaffer collateral cut Mg^{+2}-free-induced interictal discharges consist of multiple components, whereas after the cut (Interictal) they are markedly reduced in duration and number of events. (*Source*: Adapted from Barbarosie and Avoli, 1997.)

sium level or GABA$_A$ receptor blockade) can result in the generation of such activity in other regions in the hippocampal/parahippocampal formation such as the subiculum (Behr and Heinemann, 1996), area CA3 (Borck and Jefferys, 1999), and the entorhinal cortex (Jones, 1989). That small areas can generate seizure-like activity in the slice supports the hypothesis that a network of only a few thousand neurons is necessary to sustain seizure activity (Borck and Jefferys, 1999). That the maintenance of seizure-like activity is different from the mechanisms underlying briefer epileptiform discharges is suggested by their differing pharmacology; NMDA receptor antagonists can terminate brief epileptiform discharge but are ineffective during the "maintenance phase" of seizure-like activity (Borck and Jefferys, 1999).

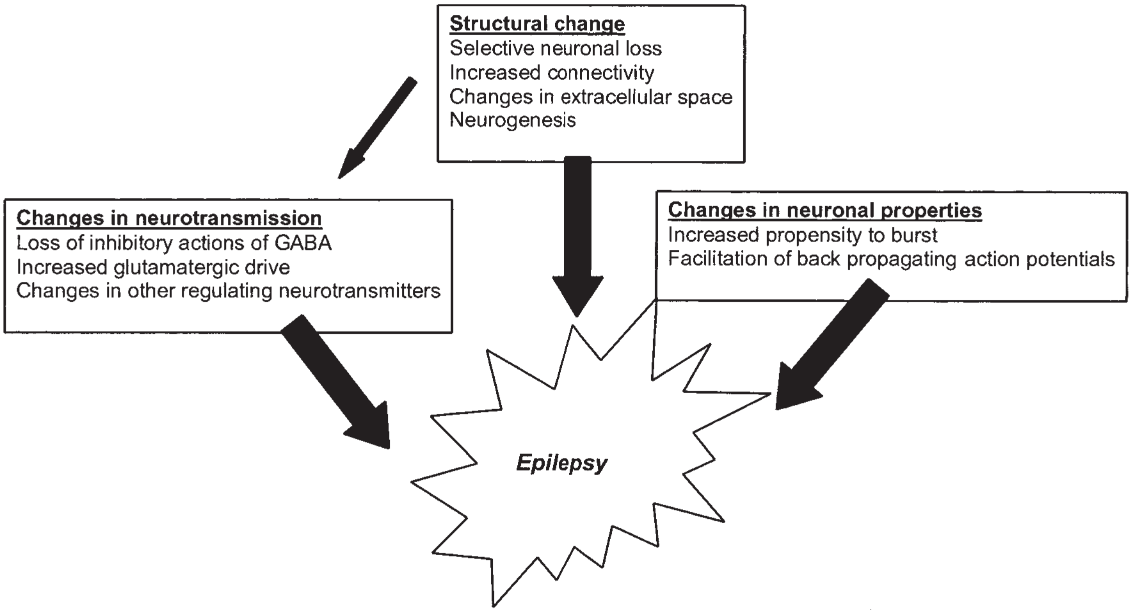
So how do spontaneous seizures (epilepsy) occur in vivo? The brain slice studies can give us an insight into specific questions concerning the generation of epileptiform discharges. The interpretation and the in vivo extrapolation of such studies are, however, complicated by certain observations: Seizure-like activity can be generated in vitro by quite disparate means, and the mechanisms and structures involved in the generation of such activity can differ from study to study. Seizure activity relies on oscillatory synchronization; thus, mechanisms similar to those described in Chapter 8 undoubtedly contribute to the emergence of in vivo seizure activity. Furthermore, it is likely that mechanisms similar (but not necessarily identical) to those that underlie spike discharges and longer "epileptic after-discharges" promote in vivo seizure activity. The transition from normal, physiological oscillatory behavior to epileptiform behavior is likely to be due to greater spread and neuronal recruitment secondary to enhanced connectivity, enhanced excitatory transmission, or a failure of inhibitory mechanisms. Indeed, in human studies, the EEG becomes less chaotic in large areas of cortex at the start of an ictus, suggesting that widespread synchronization is occurring (Martinerie et al., 1998). To understand how a network that usually maintains oscillatory behavior becomes "epileptic," it is paramount to consider the changes that occur in the hippocampus during epileptogenesis.

One problem with much that has been described is that it is associational (i.e., changes are observed and are assumed to contribute to the epileptogenic process). This may not hold true for many of the changes, which could be compensatory (i.e., protecting against the epileptogenic process). Unfortunately, it has been difficult to distinguish between these possibilities, and we are far from a comprehensive model of epileptogenesis. The changes that occur can be divided into structural change (neuronal loss, reorganization, synaptic reorganization, changes in glia and extracellular space), changes in neurotransmission, and lastly changes in neuronal properties. We discuss how each of these changes may contribute to the epileptogenic process (Fig. 16–3).

Structural Change

Neuronal Loss. Hippocampal sclerosis is typically a unilateral process, affecting either hemisphere equally, with involvement of the whole length of the hippocampus. In some cases more focal damage may be observed (Babb et al., 1984). and in others there is bilateral sclerosis (Van-Paesschen et al., 1997a). In so-called classic hippocampal sclerosis, selective loss of pyramidal cells is seen in the CA1 subfield and in the hilar region with accompanying astrocytic gliosis. In some patients neuronal loss is restricted to the CA1 subfield (de Lanerolle et al., 2003). Pyramidal cells of CA2 and dentate granule cells appear more resistant (Bruton, 1988). In severe hippocampal

Figure 16–3. Structural changes, changes in neurotransmission, and changes in the intrinsic properties of neurons all contribute to the development of epilepsy.



sclerosis almost total neuronal loss is seen in all hippocampal subfields and may be accompanied by marked deposition of corpora amylacea. In the pattern of hippocampal sclerosis termed "end folium gliosis," encountered in 3% to 4% of surgical cases (Bruton, 1988), the neuronal cell loss appears confined to the hilus and includes loss of both principal cells and interneurons. This pattern of hippocampal atrophy is less easily detected on preoperative MRI and is associated with a later onset of epilepsy than classic hippocampal sclerosis and a worse postoperative seizure outcome (Van Paesschen et al., 1997b).

Quantitative histological studies have been carried out in hippocampal sclerosis series, and pathological grading systems have been proposed to categorize the severity of neuronal loss in hippocampal sclerosis; for example, in one system, grade I hippocampal sclerosis correlates with less than 10% of neuronal dropout in CA1 up to grade IV hippocampal sclerosis, which shows more than 50% neuronal loss in all subfields (Wyler et al., 1992). This is based on a semiquantitative assessment of neuronal loss in histological sections. Such analyses have proved useful as they allow pathological correlation with clinical parameters (e.g., the age of the patient at epilepsy onset and the duration of seizures) (Davies et al., 1996) and with neuroimaging features. These grades may also reflect a progressive evolution of hippocampal sclerosis from grades I to IV, mirroring ongoing hippocampal atrophy that has been occasionally reported in sequential neuroimaging studies.

Marked cytological alterations have been observed in surviving neurons in hippocampal sclerosis using immunohistochemistry, electron microscopy, and confocal imaging techniques (Blumcke et al., 1999a). These changes include enlargement and accumulation of neurofilaments in end folial neurons, abnormal dendritic nodular swellings, and ramifications of these cells. These features more likely represent secondary or adaptive cellular changes due to the altered connectivity in the reorganized hippocampus rather than a primary cellular abnormality.

Neuronal loss and gliosis may also be present in adjacent limbic structures, including the amygdala (Yilmazer-Hanke et al., 2000) and parahippocampal gyrus, which along with hippocampal sclerosis are collectively referred to as mesial temporal sclerosis. Neuronal loss may also involve the entorhinal cortex, and volume loss has been demonstrated in the entorhinal cortex with quantitative MRI studies (Salmenpera et al., 2000). Neuropathological studies of this cortical region in patients with hippocampal sclerosis have suggested significant loss of layer III cells (Du et al., 1993), although other studies have suggested a more variable pattern of cell loss involving all layers, including loss of pre-alpha cells (Yilmazer-Hanke et al., 2000). The observed loss of entorhinal neurons could indicate either primary or secondary involvement of this region in the pathophysiology of temporal lobe seizures. An important observation, however, has been the demonstration of entorhinal cortex neuronal loss in the absence of hippocampal sclerosis (Yilmazer-Hanke et al., 2000; Bernasconi et al., 2001). This and the observation that the entorhinal cortex can alone maintain seizure-like activity (Jones, 1989) perhaps implicate a more specific role for this region in seizure generation.

The extent of any temporal neocortex neuronal loss does seem to correlate with the severity of hippocampal damage (Bruton, 1988). Neocortical neuronal loss also appears to be layer-specific, with cortical layers II and III more severely affected.

Mechanisms of Neuronal Death in Status Epilepticus. The pattern of neuronal death in hippocampal sclerosis is mirrored by neuronal death seen in postmortem specimens following status epilepticus (DeGiorgio et al., 1992). Imaging studies have demonstrated the occurrence of hippocampal sclerosis following status epilepticus and prolonged seizures, further emphasizing the vulnerability of the hippocampus to neuronal damage. Insights into the mechanisms underlying this damage have largely been derived from animal models of status epilepticus (Meldrum, 1991). They have shown that although a certain amount of neuronal damage is secondary to physiological compromise that occurs during status epilepticus (e.g., hypoxia, hypoglycemia, hypotension) a large proportion of the damage is independent of these factors. This neuronal damage is due to excitotoxicity in which the presence of epileptic activity mediates neuronal death through the activation of glutamate receptors. Excessive influx of calcium (and zinc at mossy fiber synapses) through primarily NMDA receptors, but also through α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors lacking the GluR2 subunit, results in a cascade of reactions leading to cell death (Weiss et al., 2000;Lipton and Rosenberg, 1994;Tanaka et al., 2000).

Specific Neuronal Vulnerability in Hippocampal Sclerosis. Loss of the principal pyramidal cells in hippocampal sclerosis is established, but it is difficult conceptually to conceive how removal of principal excitatory neurons can contribute to a state of hyperexcitability. Undoubtedly, neuronal loss may contribute to synaptic rearrangements and perhaps increased connectivity, but more important perhaps is the vulnerability of specific subsets of interneurons in the hippocampal formation, which may influence the intrinsic circuitry of the hippocampus and seizure propagation. Most interneurons contain the neurotransmitter GABA but can be further subdivided according to their connectivity, calcium-binding protein content, and neurotransmitter receptor status (Freund and Buzsaki, 1996).

Neuropeptide Y (NPY)- and somatostatin-containing inhibitory interneurons are normally numerous in the hilus and form a dense plexus of fibers in the outer molecular layer of the dentate gyrus which co-localizes with glutamic acid decarboxylase (GAD) (Amaral and Campbell, 1986). Loss of these interneuronal subtypes in the hilus was noted in hippocampal sclerosis (deLanerolle et al., 1989;Mathern et al., 1995a). NPY-containing axons also appeared to be reorganized in the dentate molecular layer in hippocampal sclerosis, and ectopic expression of NPY in granule cells has been observed following seizures (Vezzani et al., 1999). This is likely to represent plasticity in NPY inhibitory mechanisms in the epileptogenic hippocampus. A more recent quantitative study using in situ hybridization however, has suggested that NPY and somatostatin neurons in the fascia dentata are lost in proportion to the overall cell loss and are not specifically "targeted" in the disease process (Sundstrom et al., 2001).

The calcium-binding proteins calbindin D-28-K, parvalbumin, and calretinin label different, nonoverlapping subsets of inhibitory hippocampal interneurons, and the resistance or susceptibility of these cell populations in hippocampal sclerosis may directly affect hippocampal epileptogenesis. The normal distribution of calbindin is not restricted to interneurons but is also present in the dentate gyrus granule cells, mossy fibers, and CA2 pyramidal cells; parvalbumin and calretinin are present only in interneurons. Calbindin-positive interneurons are mainly involved in the inhibition of principal cells in the dendritic region, whereas calretinin-positive interneurons probably selectively innervate other interneurons (Magloczky et al., 2000). An early study had suggested preferential survival of calbindin and parvalbumin immunoreactive neurons in hippocampal sclerosis (Sloviter, 1991). Furthermore, increased complexity of the terminal processes of powerful inhibitory interneurons, the chandelier cell (which may be parvalbumin- or calbindin-positive) has also been demonstrated in hippocampal sclerosis (Arellano et al 2004). More recent quantitative studies, however, have shown selective loss of parvalbumin-immunoreactive neurons in the hilus disproportionate to the overall cell loss (Zhu et al., 1997). The distribution of calbindin-positive interneurons in the dentate gyrus in hippocampal sclerosis was shown not to differ from controls in one study but striking enlargement of their cell bodies with enhanced expression of calbindin and modification of the dendritic trees and synapses of these cells was noted (Magloczky et al., 2000). Marked plasticity and reorganization of calbindin-positive interneurons in the CA1 subfield in the hippocampus has also been shown, which may predate the pyramidal cell loss (Wittner et al., 2002). The findings support a complex set of changes in interneural anatomy with changes in interneuron targets. Calretinin cells do not appear to show abnormal distribution in hippocampal sclerosis (Blumcke et al., 1996); but increased numbers of a subset of calretinin-positive neurons, the Cajal-Retzius cells, occurs in hippocampal sclerosis in some patients (Blumcke et al., 1999b). Studies have demonstrated an expansion of calretinin-positive axonal networks in the molecular layer of the dentate gyrus in hippocampal sclerosis (Blumcke et al., 1996, 1999b; Magloczky et al., 2000). These fibers are likely to represent those of the excitatory supramamillary pathway terminating on granule cells rather than local axons. This observation may indicate enhanced excitation of granule cells by this pathway (Magloczky et al., 2000).

The observed relative resistance of certain calbindin-containing neurons to neuronal damage has led to the suggestion that calbindin itself may be neuroprotective (Leranth and Ribak, 1991). However, in calcium-binding protein knockout mice, the absence of these proteins does not appear to affect the numbers of interneurons or excitotoxic-mediated cell loss in epilepsy (Bouilleret et al., 2000). Furthermore, in hippocampal sclerosis there is loss of calbindin expression by granule cells (Magloczky et al., 2000). Granule cells are typically more resistant to damage in hippocampal sclerosis than other principal neurons and it has controversially been proposed that the calbindin loss actually protects these cells from Ca^{2+}-mediated neuronal damage (Nagerl et al., 2000). The proposed mechanism underlying this proposal is that lack of calbindin results in larger intracellular free calcium transients that inactivate calcium channels, thus limiting intracellular calcium accumulation; on the other hand, buffering of free calcium transients with calcium-binding proteins permits greater accumulation of intracellular calcium. Thus, although these calcium-binding proteins may identify neurons that are resistant to damage, the calcium-binding protein itself is probably not neuroprotective.

Selective loss of hilar mossy cells, an excitatory interneuron with distinctive dendritic arborizations, has been described in hippocampal sclerosis cases compared to patients with generalized seizures (Blumcke et al., 2000b). These excitatory interneurons project to inhibitory basket cells, and their loss may result in reduced feedforward granule cell inhibition supporting the experimental "dormant basket cell hypothesis" (Sloviter, 1991). However it is recognized in animal models that basket cells also receive direct excitatory input from the granule cells and perforant path fibers, thus bypassing the mossy cells (Kneisler and Dingledine, 1995).

Pathophysiological Role of Neuronal Damage. Are epileptogenesis and neuronal damage directly related? Following status epilepticus, those animals that develop spontaneous seizures have greater hilar interneuronal loss, perhaps resulting in decreased inhibitory drive (Gorter et al., 2001). It has also been suggested that damage to CA3 and the Schaffer collaterals may prevent spikes generated in CA3 from inhibiting epileptic activity in the limbic system (see above). Selective neuronal death can also result in a change in the nature of inhibition. Dendritically expressed inhibitory postsynaptic potentials (IPSPs) modify the transmission of excitatory postsynaptic potentials (EPSPs) to the soma, whereas somatic and perisomatic IPSPs depress the excitability of the neuron (Cossart et al., 2001). Basket cells, which make multiple perisomatic and somatic synapses, have extensive axonal arborizsations leading to connection of one basket cell with many pyramidal cells. By synchronously modulating the excitability of a group of pyramidal neurons, one basket cell can synchronize pyramidal cell activity (Cobb et al., 1995). The loss of oriens/lacunosum-moleculare interneurons in CA1 results in loss of distal dendritic inhibition, whereas the preservation and increased connectivity of basket cells may result in greater pyramidal cell synchronization (Fig. 16–4) (Cossart et al., 2001). The selective loss of certain interneuronal populations in hippocampal sclerosis is thus probably pro-epileptogenic; but is neuronal loss necessary for epileptogenesis? That there is a distinction

Figure 16–4. Inhibition is decreased in dendrites but increased at the soma. *A*, *B*. Recordings from the apical dendrites of CA1 pyramidal cells. *A*. The cumulative probability plot of the amplitudes of miniature events was shifted to the left, indicating decreased frequency of amplitudes larger than 15 pA in epileptic animals. The kinetics of miniature events (normalized averages) did not seem modified (*inset*). *B*. Whole-cell recordings of spontaneous (control) and miniature (TTX, 1 μM) inhibitory postsynaptic currents obtained at the reversal potential of glutamatergic events in a control and a kainate-treated animal. Continuous 4-s recordings are shown. The frequency of spontaneous events is lower in the epileptic tissue. *C*, *D*. Somatic recordings from CA1 pyramidal cells. *C*. The cumulative probability plot of the amplitudes of spontaneous IPSCs was shifted to the right, indicating an increased frequency of large-amplitude events in experimental animals (n = 6 cells) as compared to controls (*n* = 6 cells). *D*. Whole-cell recordings of spontaneous (control) and miniature (TTX, 1 μM) inhibitory postsynaptic currents obtained at the reversal potential of glutamatergic events. The frequency of spontaneous events was similar in control and epileptic neurons, but the number of large-amplitude events seemed to be increased in the epileptic neuron. (*Source*: Adapted from Cossart et al., 2001, with permission.)

between neuronal damage and epileptogenesis is indicated by kindling, in which epileptogenesis occurs in the setting of no or minimal neuronal damage, and similarly in the intrahippocampal tetanus toxin model (see above). Indeed, kindling may protect against neuronal damage induced by kainic acid (Kelly and McIntyre, 1994), raising the intriguing possibility that epilepsy itself can be neuroprotective.

Granule Cell Dispersion. The observation of disorganization or dispersion of granule cells into the molecular layer of the dentate gyrus in hippocampal sclerosis was first described in detail by Houser (Houser, 1990; Houser et al., 1992). Dispersed granule cells appear separated from the normally compact cell layer, which gives the impression of an undulated irregular border with the molecular layer. In some cases the deep (hilar border) of the granule cell layer is also ill-defined. As a result, in hippocampal sclerosis the cell layer appears broadened with a mean width of 180 μm in TLE patients compared to 100 μm in control subjects (Houser, 1990). The dispersed cells often appear elongated or fusiform in shape, reminiscent of migrating neurons. Less often a bilaminar arrangement of granule cells is observed (Houser et al., 1992; Thom et al., 2001) or nests of GCs are present in the hilus (Houser, 1990; Thom et al., 2001). The incidence of granule cell dispersion in hippocampal sclerosis surgical series is of the order of 40% (Houser, 1990; Thom et al., 2001).

It has been suggested that granule cell dispersion represents a primary abnormality of neuronal migration or an underlying hippocampal malformation (Houser et al., 1992). There are occasional reports of granule cell dispersion in association with cortical malformations in the absence of a history of seizures and with bilateral hippocampal involvement (Harding and Thom, 2001). Disorganization of the granule cell layer and ectopic localization of neurons have also been noted in several animal models with cortical malformations such as the reeler, and *p35* mutant mice. The presence of granule cell dispersion in human hippocampal sclerosis has also been correlated with epileptic events occurring early in life, including febrile seizures (Houser, 1990), suggesting a vulnerability of these neurons during this time period. It has further been shown that the presence of granule cell dispersion correlates well with the severity of hippocampal neuronal loss (Thom et al., 2001). This suggests that granule cell dispersion represents an epiphenomenon of hippocampal sclerosis rather than a primary abnormality, the migration of granule cells perhaps being influenced by neurotrophin secretion during seizures or other cellular signals. Interestingly, an inverse correlation between the levels of reelin protein and the extent of granule cell dispersion in the hippocampus has been shown, suggesting a possible functional role for Cajal Retzius cells in this process (Frotscher et al., 2003).

In animal models of epilepsy, such as the pilocarpine model, there is evidence to suggest that abnormally migrated granule cells are newly generated cells, neurogenesis being stimulated by the seizures (Parent et al., 1997) (see Chapter 9). Rapid dispersion of granule cells has been demonstrated following injury (Omar et al., 2000) and it has been shown that newly generated cells can migrate as far as CA3 and integrate into the CA3 neuronal network (Scharfman et al., 2000). Abnormal connections formed by new cells may contribute to seizure development (Parent et al., 1997), although experimental inhibition of neurogenesis does not prevent granule cell axon reorganization (see below) in epilepsy models (Parent et al., 1999). Studies have confirmed that neurogenesis also occurs in adult human dentate gyrus (Eriksson et al., 1998), and neuronal progenitor cells have been isolated from the dentate gyrus (Roy et al., 2000). This pool of precursor cells may have important physiological roles, but it is conceivable that in human epilepsy, stimulated by seizures, an increased rate of granule cell neurogenesis occurs, leading to the abnormal cell localization and reorganization observed in hippocampal sclerosis. There is also evidence emerging that radial glial cells in this region could act as neuronal precursors, and neurogenesis may be stimulated by NPY (Howell et al., 2003).

Another study has demonstrated the stem cell intermediate filament protein nestin in granule cell neuronal precursors in young patients with mesial TLE and surgery before the age of 2 years (Blumcke et al., 2001). Similar cells were not found in adults with hippocampal sclerosis; whether the nestin-positive cells represent newly generated cells or a delay in hippocampal development in these younger patients is not clear. Studies of cell cycle proteins, including Ki67, showed low expression in the dentate gyrus subgranular layer in adult hippocampi from patients with epilepsy (Del Bigio, 1999). Although this is an insensitive technique for measuring cells with a low turnover rate, it does imply that neurogensis in hippocampal sclerosis is a rare event and likely to be dependent on age.

Even if migrated granule cells do represent newly generated cells, it is unknown whether there are any differences in the physiological properties of these less mature cells. Electrophysiological studies in human hippocampal sclerosis have already demonstrated the existence of distinct populations of granule cells, with one group showing abnormal excitability (Dietrich et al., 1999). We also know from animal studies that there is considerable potential for adaptability and plasticity of granule cells, such as increased basal expression of GAD (Sloviter et al., 1996), NPY induction (Vezzani et al., 1999), loss of calbindin expression (Magloczky et al., 1997), and altered ionotropic and metabotropic glutamate and GABA neurotransmitter receptor profiles on granule cells (Loup et al., 2000). It is plausible that such plasticity could be enhanced in newly generated granule cells and that it could contribute to seizure propensity.

Mossy Fiber Sprouting. In 1974, using Golgi techniques, Scheibel and colleagues identified aberrant axons from granule cell neurons ascending into the molecular layer of the dentate gyrus in hippocampal specimens from patients with epilepsy (Scheibel et al., 1974). It has long been considered that reorganization of the excitatory glutamatergic mossy fiber pathway is a key event in the development of chronic seizures (Sutula et al., 1989). Mossy fiber sprouting in human hippocampal sclerosis specimens results in aberrant innervations of other granule cells and also of CA3 pyramidal neurons, resulting in both feedback and feedforward excitation (Babb et al., 1991; Mathern et al., 1995b). In addition, aberrant mossy fibers in animal models innervate interneurons, suggesting that new inhibitory circuits are established (Kotti et al., 1997).

Mossy fiber sprouting in the supragranular layer of the dentate gyrus can be demonstrated using the Timms histochemical method, which highlights the zinc-rich mossy fiber synaptic terminals (Babb et al., 1991), or with dynorphin immunohistochemistry (Houser et al., 1990). Increased expression of growth-associated protein GAP-43 in the supragranular layer is thought to be indicative of active mossy fiber sprouting in hippocampal sclerosis specimens (Proper et al., 2000). Similarly increased syanaptogenesis in this region has been demonstrated by studying the distribution of 5′nucleotidase activity, which localizes in regions with more active synaptic turnover (Lie et al., 1999). Overall reorganization of synaptic terminals in hippocampal sclerosis has also been demonstrated in human specimens using immunohistochemistry for synaptic antigens such as synaptophysin, which shows a loss in the hilus and increased labeling in the dentate gyrus molecular layer (Honer et al., 1994; Davies et al., 1998; Proper et al., 2000). Similarly, prominent immunolabeling for chromogranins (neuropeptide precursors that can be co-released with catecholamines and peptides) in the inner molecular layer of the dentate gyrus has been shown to correspond with reorganized mossy fibers in patients with epilepsy (Kandlhofer et al., 2000). In parallel with increased synaptogenesis, elaboration and increased complexity of granule cells dendrites in the inner molecular layer has been demonstrated in hippocampal sclerosis patients (von Campe et al., 1997). The hypothesis that mossy fiber collaterals form granule cell–granule cell synapses has been confirmed by visualizing dentate granule cells and their mossy fibers after terminal uptake and retrograde transport of biocytin in epileptic rats secondary to status epilepticus (Okazaki et al., 1995).

Sprouting of mossy fibers is thought to result from epilepsy-induced loss of target cells (e.g., hilar mossy cells). However, in animal models it may be an early event, occurring within 4 weeks following the start of kindling (Elmer et al., 1996) and independent of hippocampal cell loss, possibly regulated by neurotrophic factors (Adams et al., 1997). Preliminary studies also indicate that mossy fiber sprouting is likely to be independent of any granule cell neurogenesis (Parent et al., 1997).

Pathological Role of Mossy Fiber Sprouting. It has long been considered that reorganization of the excitatory glutamatergic mossy fiber pathway is a key event in the development of chronic seizures. The dentate granule cells of the hippocampus probably act as a brake against seizure propagation through limbic circuitry (Perlin et al., 1992; Lothman and Bertram, 1993). This is mediated by the relative inexcitability of dentate granule cells through strong tonic GABAergic inhibition and relatively hyperpolarized membrane potentials. Dentate granule cells do not show the burst properties characteristic of hippocampal pyramidal cells in response to reduced GABAergic inhibition. Furthermore, dentate granule cell synchronization is difficult to achieve because of the low rate of connectivity between the granule cells.

Epileptogenesis may change the properties of dentate granule cell receptors (see below); but, importantly, mossy fiber sprouting greatly increases their connectivity. Even with sprouting, it is difficult to recruit dentate granule cells into epileptiform activity perhaps because of the extensive and increased synaptic input from GABAergic interneurons. Epileptiform activity can be induced in the dentate granule cells when mossy fiber sprouting is present by increasing extracellular potassium or by reducing $GABA_A$ receptor-mediated inhibition (Cronin et al., 1992; Wuarin and Dudek, 1996). This has led to the compelling hypothesis that epileptogenesis results in a potentially hyperexcitable granule cell layer that can be recruited into epileptic activity either by a rise in extracellular potassium, which could occur secondary to a sustained discharge from the entorhinal cortex, or through breakdown of inhibition (see below).

Is mossy fiber sprouting necessary for epileptogenesis? Blocking mossy fiber sprouting with the protein synthesis inhibitor cycloheximide does not prevent epileptogenesis (Longo and Mello, 1998). The interpretation of these experiments is confounded, however, by the likelihood that inhibiting protein synthesis inhibits other antiepileptogenic processes. In a recent study, the presence (or not) of dynorphin-positive mossy fiber sprouting in hippocampal sclerosis correlated with the postsurgical outcome; those with sprouting more often having a seizure-free outcome (de Lanenerolle et al., 2003). It is also becoming increasingly apparent that there is sprouting of axons from other neuronal populations, including sprouting of CA1 pyramidal cell axons, resulting in an increase in interconnectivity that results in increased excitability of the CA1 subfield (Esclapez et al., 1999). Sprouting of excitatory axons leading to increased interconnectivity may be a powerful means of generating hyperexcitable circuits that can maintain and propagate epileptic activity.

Dormant Basket Cell Hypothesis. Immediately following 24 hours of perforant path stimulation, there is loss of paired-pulse inhibition to perforant path stimulation in the dentate granule cell layer (Sloviter, 1987). The mechanism underlying this change was proposed to be the loss of excitatory input onto basket cells due to excitotoxic loss of mossy cells (Sloviter, 1987). However, application of NBQX, an AMPA receptor antagonist, was shown to inhibit the loss of mossy cells but to have no effect on the loss of paired-pulse inhibition (Penix and Wasterlain, 1994). Similarly, in the tetanus toxin model, disinhibition occurs in the absence of hilar cell loss (Whittington and Jefferys, 1994). Other factors are therefore likely to be responsible for the loss of paired-pulse inhibition, such as loss of affinity and activity of $GABA_A$ receptors in the hippocampus or a shift in the chloride reversal potential to more positive values (Kapur et al., 1994). These changes may be due in part to altered phosphorylation of $GABA_A$ receptors (Kapur et al., 1994) but could also be due to altered $GABA_A$ receptor subunit composition and expression (Sperk et al., 1998). Perhaps one of the main mechanisms is decreased recruitment of basket cells by excitatory inputs from dentate granule cells, the perforant path, and CA3 pyramidal cells through upregulation of presynaptic metabotropic glutamate receptor activity (Doherty and Dingledine, 2001).

Loss of paired-pulse inhibition was proposed to be an epileptogenic phenomenon, but subsequent studies have shown that paired-pulse inhibition becomes increased during the latent period (i.e., with epileptogenesis) and that epileptogenesis is associated with increased recruitment of basket cells (Milgram et al., 1991; Sloviter, 1992).

Synaptic rearrangement also occurs in CA1; and the hyperexcitability in CA1 with epileptogenesis has been proposed to be secondary to reduced recruitment of inhibitory interneurons (Bekenstein and Lothman, 1993). The main evidence for this is the loss of the IPSP from the EPSP–IPSP sequence on distant stimulation of the Schaffer collaterals. This evidence is perhaps flawed because this study does not adequately differentiate loss of the IPSP from prolongation of the EPSP. Subsequent data have shown increased excitability of CA1 interneurons following epileptogenesis (Sanabria et al., 2001). There are, however, other mechanisms underlying possible disinhibition in the CA1 region including changes in the pattern of inhibition (see above) and postsynaptic changes (see below).

Glial Cells and Extracellular Space. There are alterations in astrocytic function in the gliotic hippocampus. Astrocytes show physiological changes characteristic of immature astrocytes, including prolonged depolarization, that may contribute to seizure generation (Hinterkeuser et al., 2000; Schroder et al., 2000). Indeed, there is altered expression of ionotropic glutamate receptors on astrocytes in hippocampal sclerosis which may facilitate seizure spread (Seifert et al., 2004). In another study of rat and human hippocampi in TLE, the proliferation of glial cells in areas of neuronal loss were associated with alterations in extracellular potassium, which also may affect conduction of seizure activity (Heinemann et al., 2000). Altered levels of glial glutamate transporters (e.g., EAAT2), which has been shown in hippocampal sclerosis (Proper et al., 2002), may also influence the extracellular pool of glutamate. Furthermore, calcium oscillations in astrocytes can result in glutamate release, which may contribute to epileptic activity (Tian et al., 2005).

During seizures there is considerable shrinkage of the extracellular space due to intracellular accumulation of sodium chloride; indeed, a single seizure can result in a 10% to 30% decrease in extracellular space (Lux et al., 1986). This may result in increased nonsynaptic transmission through ephaptic and ionic mechanisms. Indeed, decreased extracellular space during seizures has been indirectly shown to have a role in seizure maintenance and spread; hypotonic extracellular solutions that decrease extracellular space are proconvulsant, whereas hypertonic solutions (increasing the extracellular space) can terminate seizure discharges (Roper et al., 1992). Although overall there is an expansion of the extracellular space in hippocampal sclerosis (Hugg et al., 1999; Wieshmann et al., 1999), how this relates to local changes in extracellular space and neurotransmission are unknown.

Neurotransmitter Systems

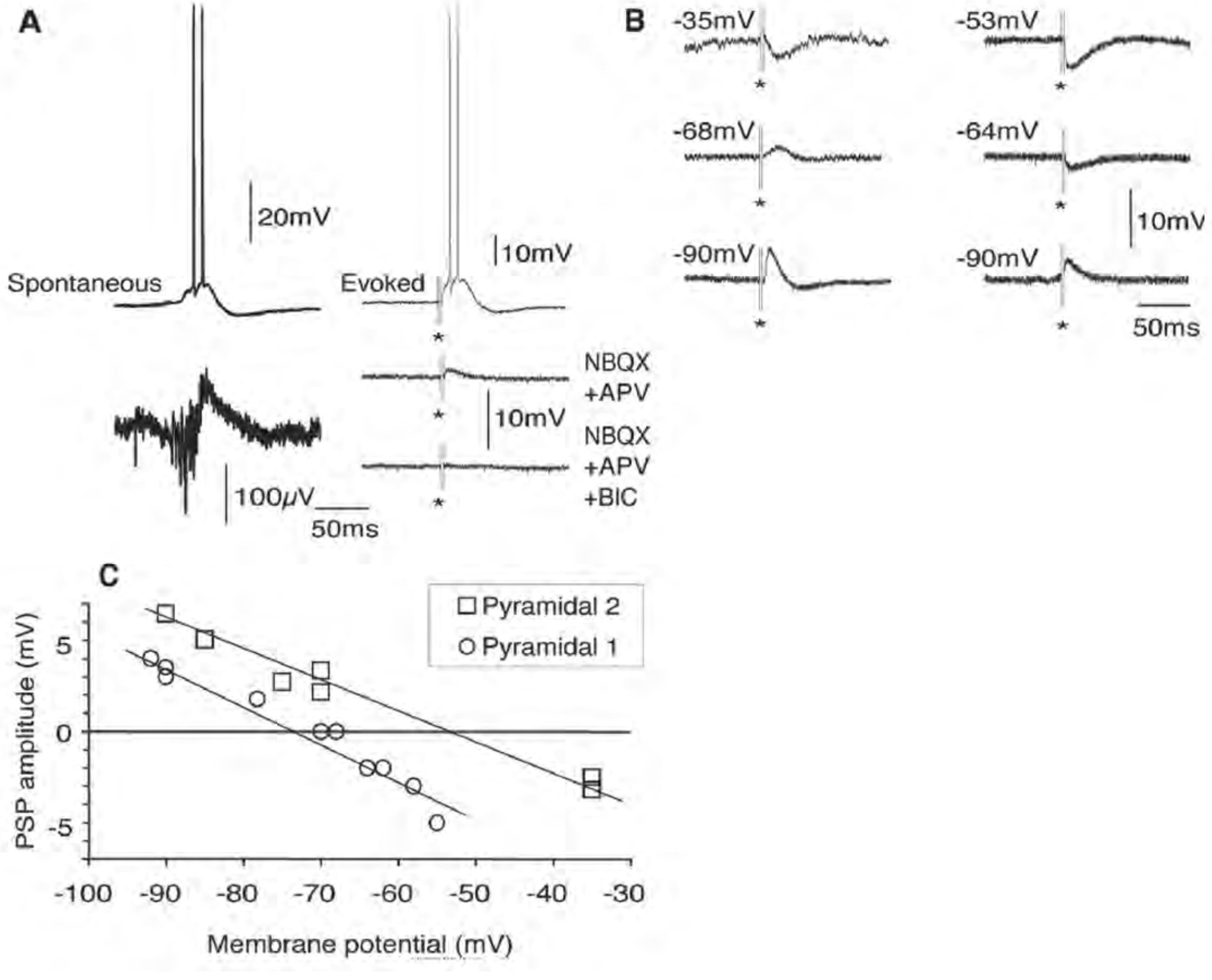
GABAergic Mechanisms. Alteration in the distribution of neurotransmitter receptors has been extensively investigated as a pathogenic mechanism in the hyperexitability of the hippocampus in TLE. The "GABA" hypothesis proposes that a deficit in inhibitory GABAergic transmission is implicated in seizures. $GABA_A$ and to a lesser extent $GABA_B$ receptor subtype expression (Barnard et al., 1998) and reuptake mechanisms have been studied in human hippocampal sclerosis tissues. Many alterations in GABA transmission may represent an adaptive mechanism in the brain in response to repetitive seizures, and increased expression of $GABA_A$ receptors has been documented in animal models of epilepsy as a compensatory mechanism (Fritschy et al., 1999). There is also upregulation of GAD, the main GABA-synthesizing enzyme, in interneurons following acute seizures. GABA and GAD are also upregulated in the mossy fibers and dentate granule cells (Sloviter et al., 1996). The finding of a mossy fiber-like GABAergic signal has raised the possibility that mossy fibers co-release glutamate and GABA (Walker et al., 2001); seizures may upregulate the GABAergic component (Gutierrez and Heinemann, 2001). In human hippocampal sclerosis, selective upregulation of $GABA_A\alpha 2$ subunit in granule cells has been observed, highlighting the plasticity of this neurotransmitter system in hippocampal sclerosis (Loup et al., 2000). The changes that are observed differ with time and between regions. Thus, specific $GABA_A$ receptor changes occur during acute seizures. As acute seizures continue they can become less responsive to benzodiazepines, which is mirrored by decreased potency of benzodiazepines on GABA-mediated synaptic currents in dentate granule cells (Kapur and Macdonald, 1997). In contrast, the potency of GABA itself and pentobarbitone remained unaltered, suggesting that rapid changes in GABA receptor properties occur during seizures. Although the pathophysiological consequences of these changes are difficult to predict, they have implications for the treatment of acute, prolonged seizures. During epileptogenesis the $GABA_A$ receptor changes are more complex and are region-specific. In the dentate granule cells, there is an increase in the number of $GABA_A$ receptors per synapse, leading to increased quantal size (Nusser et al., 1998). An especially interesting finding is that the increased GABA receptor-mediated signaling to dentate granule cells becomes more sensitive to zinc (Buhl et al., 1996; Brooks et al., 1998). To understand the potential involvement of zinc in epilepsy, it is necessary to consider the role of the sprouted mossy fibers. Mossy fiber terminals contain zinc and release it during synaptic activity (Assaf and Chung, 1984; Howell et al., 1984). Thus, it is conceivable that in the epileptic hippocampus zinc released from mossy fibers results in disinhibition, unmasking the potentiated excitatory dentate granule cell circuits (Buhl et al., 1996). This hypothesis is confounded by the observation that the zinc released from sprouted mossy fibers failed to affect $GABA_A$ receptor-mediated currents induced by local release of caged GABA (Molnar and Nadler, 2001). Furthermore, mice that lack the zinc transporter (ZnT3) and so lack synaptically available zinc have an exaggerated response to a convulsant; this observation does not, however, preclude a role for zinc in epileptogenesis (Cole et al., 2000).

Decreased sensitivity of $GABA_A$ receptor-mediated signals to zolpidem (a selective benzodiazepine agonist) has also been noted (Brooks et al., 1998). Using a combination of patch-clamp recording and single-cell mRNA amplification, it was found that the increased zinc sensitivity and decreased benzodiazepine sensitivity of the $GABA_A$ receptor was associated with (and possibly explained by) decreased expression of the $\alpha 1$ subunit and increased expression of the $\alpha 4$ subunit (Brooks et al., 1998). In addition, there were changes in β subunit expression that may affect benzodiazepine efficacy and the efficacy of barbiturates, steroids, zinc, and loreclezole, a new antiepileptic drug (Brooks et al., 1998). These changes were seen during the latent period that predated the onset of epilepsy, suggesting a role in the epileptogenic process. $GABA_A$ receptor-mediated transmission in CA1 undergoes different changes. In contrast to dentate granule cells, $GABA_A$ receptors on CA1 pyramidal cells are less responsive to applied GABA following epileptogenesis (Gibbs et al., 1997). There are also changes that suggest there may be a decrease in the presynaptic GABA reserve, although the synaptic consequences of this are unknown (Hirsch et al., 1999).

The effects of $GABA_A$ receptor activation on membrane potential depend on the chloride reversal potential. High internal chloride such as occurs developmentally can result in depolarizing $GABA_A$ receptor-mediated potentials. Could such depolarizing $GABA_A$ receptor-mediated potentials occur in epileptic tissue and contribute to epileptogenesis? Evidence from a study of hippocampal slices from patients who underwent temporal lobectomy suggest that this is so (Cohen et al., 2002). Synchronous rhythmic activity was found to be generated in the subiculum of slices of temporal lobe from patients with temporal lobe epilepsy, and this synchronous rhythmic activity was abolished by the $GABA_A$ receptor antagonist bicuculline and by glutamate receptor antagonists, suggesting that both excitatory and inhibitory signaling contributed to the spontaneous interictal-like events. Further studies in these cells demonstrated that the GABAergic synaptic events reversed at depolarized potentials (Fig. 16–5). Thus, depolarizing GABAergic responses potentially contributed to human ictal activity in the subiculum. The mechanism by which such depolarizing $GABA_A$ receptor-mediated potentials occur is unknown, but might be downregulation of the K^+/Cl^- co-transporter KCC2 that maintains the low intracellular chloride.

$GABA_B$ receptor changes have also been demonstrated in human hippocampal sclerosis tissue. $GABA_B$ receptors inhibit neurotransmitter release from presynaptic terminals and

Figure 16–5. Depolarizing γ-aminobutyric acid-A ($GABA_A$) receptor-mediated potentials may contribute to seizure generation. Pyramidal cells active during interictal-like events showed excitatory GABAergic transmission. *A.* Discharges in pacemaker cells were triggered by orthodromic stimulation (right, upper trace). Depolarizing synaptic potentials persisted when glutamatergic transmission was suppressed (middle trace). They were suppressed by blocking $GABA_A$ receptors (lower trace). BIC, bicuculline. *B*, *C*. Reversal potential for GABAergic transmission in cells that fired during interictal events was more depolarized than at rest (*B*, left; *C*, squares). Reversal in inhibited cells was hyperpolarized from rest (*B*, right; *C*, circles). Records were obtained in NBQX and APV. Linear fits are indicated. PSP, postsynaptic potential. (*Source*: Adapted from Cohen et al., 2002, with permission.)



cause late inhibitory synaptic potentials (Barnard et al., 1998). Increased expression of $GABA_B1$ receptor has been shown in the subiculum of hippocampal sclerosis cases and in surviving CA1 neurons and granule cells with augmented receptor binding in CA3 (Billinton et al., 2001), although functional interpretation of these findings is difficult. The upregulation could represent a greater number of inhibitory synapses, increased postsynaptic $GABA_B$ receptors, or increased presynaptic $GABA_B$ receptor number, leading to decreased neurotransmitter release mainly from inhibitory axonal terminals. More recently, downregulation of mossy fiber presynaptic $GABA_B$ receptors has been found in tissue from epileptic animals. It resulted in decreased mossy fiber heterosynaptic depression, and may contribute to increased signal flow through the hippocampus (Chandler et al., 2003). Decreased presynaptic $GABA_B$ receptor activity on interneurons has also been proposed to underlie the enhanced inhibition that occurs in the dentate gyrus during epileptogenesis (Haas et al., 1996).

Changes in GABA uptake have also been described in the dentate gyrus (During et al., 1995; Patrylo et al., 2001). Both animal and human data support a decrease in clearance of synaptically released extracellular GABA, perhaps owing to decreased expression or impairment of GABA transporters (During et al., 1995; Mathern et al., 1999; Patrylo et al., 2001). This has been speculated to lead to increased interictal inhibitory "efficacy." Because the rises in extracellular potassium that occur during seizures may result in reversal of GABA uptake, decreased GABA transporter function results in impairment of extracellular GABA rises during seizure activity, possibly resulting in impaired inhibition during seizure activity. The change in $GABA_A$ receptor subunits resulting in a greater response to endogenously applied GABA (Brooks et al., 1998) could compensate for decreased extracellular GABA rises. Nevertheless, these data raise the possibility that decreased GABA transporter function could be proepileptogenic during times of seizure activity but promote inhibitory transmission during the interictal period.

Glutamatergic Mechanisms. Upregulation of excitatory metabotropic glutamate receptors (mGluR1 subunit) has been observed in the dentate gyrus in both human and animal models of hippocampal sclerosis, and it could contribute to the development of chronic seizures through increased excitatory transmission (Blumcke et al., 2000a). In addition, upregulation of the presynaptic inhibitory metabotropic receptor subunit mGluR4 in the dentate gyrus and granule cells in hippocampal specimens was also observed, which may contribute to the dampening of seizure activity (Lie et al., 2000). In studies employing in situ hybridization techniques, increases in pyramidal and granule cell AMPA receptor mRNA (Mathern et al., 1997) and in granule cell NMDAR1 and NMDAR2 subunit mRNA have been shown, results that are supported by autoradiographic studies (Brines et al., 1997). Studies in kindled models have supported the hypothesis that during epileptogenesis there is enhanced transmission from the entorhinal cortex to dentate granule cells (Behr et al., 2001). One of the major mechanisms underlying this is undoubtedly an increase in NMDA receptor-mediated neurotransmission (Mody and Heinemann, 1987). Kindling results in fast, long-lasting posttranslational modifications in the function of dentate granule cell NMDA receptor channels, leading to increases in the mean open time and burst and cluster duration and to decreases in the channel-blocking effect by magnesium (Kohr et al., 1993). Similar changes in NMDA channels have been reported in human epileptic tissue (Lieberman and Mody, 1999). Modification of the NMDA receptor channels probably results from a decrease in the activity of intracellular phosphatases, leading to increased phosphorylation of the receptors (Kohr et al., 1993; Lieberman and Mody, 1994).

There is more uncertainty concerning changes in AMPA receptor neurotransmission. Certainly, changes in AMPA receptor subunit composition are seen in animal models prior to neurodegeneration; there is a decrease in the expression of the GluR2 subunit in vulnerable cells (Grooms et al., 2000). This evidence supports a role for calcium flux through GluR2 lacking AMPA receptors in mediating neuronal death (Grooms et al., 2000). Conversely, there appears to be upregulation of GluR2 in less vulnerable neurons such as the dentate granule cells (de Lanerolle et al., 1998).

Changes in glutamate uptake during epileptogenesis could also have an important role. There is burgeoning evidence that glutamate may escape from the synaptic cleft to activate extrasynaptic receptors, or even receptors at neighboring synapses (Kullmann and Asztely, 1998). Glutamate "spillover" can activate presynaptic glutamate receptors on GABAergic terminals, resulting in decreased inhibitory drive, and can increase NMDA receptor-mediated signaling (Min et al., 1999; Semyanov and Kullmann, 2000). Extrasynaptic accumulation of glutamate may play a role in epilepsy: Rodents lacking the gene coding for the glial glutamate transporter GLT-1 (EAAT2) show lethal spontaneous seizures (Tanaka et al., 1997). Rather surprisingly, chronic administration of antisense oligonucleotide to knock down the same transporter produces a different phenotype, characterized by neurodegeneration rather than seizures (Rothstein et al., 1996). Reduction of expression of the neuronal transporter EAAC1 (EAAT3), however, also causes seizures in rats. Subtle alterations in transporter levels have been reported in hippocampal tissue taken from patients with TLE (Mathern et al., 1999), although it is difficult to determine to what extent this reflects selective neurodegeneration. In an animal model of hippocampal sclerosis there was downregulation of the glial glutamate transporter that could contribute to glutamate spillover but upregulation of the neuronal glutamate transporter, which has been hypothesized to play a dominant role in reversed glutamate uptake (Ueda et al., 2001). Furthermore, marked extracellular glutamate rises have been recorded in humans using in vivo microdialysis prior to seizure onset, leading to the suggestion that these glutamate rises are an initiating factor in spontaneous seizures (During and Spencer, 1993).

Other Neurotransmitters. Alterations of many other transmitter systems have been described in association with acute limbic seizures and with hippocampal sclerosis. Perhaps one of the most intriguing roles for many of these transmitters is in seizure termination, and acute alterations have been implicated in the progression of seizures to status epilepticus. Adenosine, opioids, NPY, and galanin have all been proposed to play an important role in seizure termination (Young and Dragunow, 1994; Mazarati et al., 1998, 1999; Vezzani et al., 1999), whereas accumulation of substance P has a pro-epileptogenic effect (Mazarati et al., 1999).

Adenosine is a potent inhibitor of neurotransmitter release and has been shown to be effective for terminating brief seizures. Indeed, accumulation of adenosine seems to be a credible contender for a prominent role in seizure termination, as seizures promote adenosine release (Berman et al., 2000). Adenosine antagonists shorten the stimulation protocol or lessen the chemoconvulsant dose necessary to induce status epilepticus (Young and Dragunow, 1994). Also, adenosine agonists are effective at stopping both the induction and maintenance of status epilepticus (Handforth and Treiman, 1994). To what degree changes in adenosine anticonvulsant activity contribute to epileptogenesis or to the failure of seizure termination (status epilepticus) is unknown. Although regional changes in adenosine receptor density have been described during epileptogenesis (Ekonomou et al., 2000), it may reflect cell loss and synaptic rearrrangement.

Opioid release has also been suggested as a major mechanism underlying the termination of seizures. The observed loss of dynorphin-like immunoreactivity in the hippocampus during sustained seizure activity is consistent with loss of a potent endogenous antiepileptic (Mazarati et al., 1999). Opioid antagonists facilitate the establishment of status epilepticus, and agonists inhibit both the induction and maintenance of status epilepticus.

In addition to opioids, a variety of other modulatory neuropeptides exist. NPY is such a peptide that has potent effects on neurotransmission. Cloning has revealed five NPY receptors, Y1–Y5 (Vezzani et al., 1999). In human hippocampal sclerosis, there is increased NPY, upregulated presynaptic Y2 receptors that inhibit neurotransmitter release, and downregulated Y1 receptors that are expressed postsynaptically and are excitatory (Furtinger et al., 2001). Furthermore, Y5 receptor knockout mice and NPY knockout mice have an exaggerated response to kainic acid with prolonged seizures (Baraban et al., 1997; Marsh et al., 1999).

Galanin is another bioactive peptide that is widely distributed throughout the CNS. Galanin in the hippocampus is predominantly inhibitory, decreasing the release of excitatory amino acids. In the hippocampus, galanin immunoreactivity is confined to axons, the bulk of which are the axons of medial septal neurons (Mazarati et al., 1998). Status epilepticus in two models—perforant path stimulation and lithium pilocarpine—resulted in the disappearance of galanin immunoreactive fibers in the hippocampus; this may have resulted from loss of medial septal neurons or through exhaustion of galanin stores (Mazarati et al., 1998). This would have a disinhibitory effect. Soon after the status epilepticus, however, galanin immunoreactive-positive neurons appeared in the hilus; they increased in number after the first day but gradually declined a few days later. This increase in galanin-immunoreactive neurons in the hippocampus is possibly a compensatory response to prolonged ictal activity and depletion of galanin from septal afferents. Galanin injected into the hilus prevented the induction of status epilepticus and also stopped established status epilepticus. Conversely, antagonists of galanin receptors facilitated the development of status epilepticus (Mazarati et al., 1998). Further confirmatory evidence of the importance of galanin comes from studies of transgenic mice in which overexpression of galanin had an antiepileptic effect, and galanin knockouts were more susceptible to the induction of status epilepticus (Mazarati et al., 2000).

Changes in Neuronal Properties

Although most recent research into epileptogenesis and seizure generation has concentrated on changes in the neuronal network, alterations in intrinsic neuronal properties could also contribute to this process. Importantly, ion channel mutations in which there may be only subtle changes to the kinetics of ion channels can result in epilepsy.

As discussed in the section on the interictal spike, CA3 pyramidal cells can generate burst firing, whereas few pyramidal cells in CA1 demonstrate such firing properties. Alterations in intrinsic membrane properties can dramatically affect the firing properties of such neurons and could promote burst firing. Such burst firing in a dense excitatory network has the potential to generate synchronized bursts and may thus promote epileptic activity. In the pilocarpine model of epileptogenesis, the proportion of bursting CA1 pyramidal cells increases dramatically, such that more than half demonstrate bursting properties (Fig. 16–6) (Su et al., 2002). This may be due to upregulation of a T-type calcium channel that can produce a significant calcium tail current following an action potential, resulting in significant afterdepolarization (Su et al., 2002). Persistent sodium currents may also contribute to this propensity for bursting.

More recently, downregulation of dendritic A-type potassium channels has been found in the pilocarpine epilepsy model (Bernard et al., 2004). This downregulation is partly due to increased channel phosphorylation by extracellular signal-regulated kinase but also to decreased transcription. These potassium channels limit the back-propagation of action potentials from the soma into the distal dendrites. The functional consequence of back-propagating action potentials is likely to be an amplificatioin of EPSPs and thus increased excitation. The effect of downregulation of A-type potassium channels on dendritic calcium spikes and burst firing is unknown.

Epileptogenesis can thus lead to an acquired channelopathy in neurons that may promote burst firing and hyperexcitability.

\Figure 16–6. Upregulation of intrinsic burst-firing in CA1 pyramidal cells from SE-experienced versus control animals. *A*. Representative responses of CA1 pyramidal cells from sham-control (a) and SE-experienced animals (b) 30 days after pilocarpine treatment. In each panel, the responses of CA1 pyramidal cells to long (leftmost traces) and brief (rightmost traces) depolarizing current pulses (injected through the recording microelectrode) are shown. Top and bottom traces depict the neuronal response and the current stimulus, respectively. *B*. Most of the CA1 pyramidal cells in the sham-control group ($n = 42$) and in the naive-control group ($n = 15$) were regular firing cells, with only a small fraction displaying burst discharges to threshold stimulation (white bars). In contrast, CA1 pyramidal cells in the SE-experienced group showed a high incidence of intrinsic bursting (54%, $n = 97$), with bursting neurons displaying all-or-none bursts of three to four clustered spikes in response to either brief or long current injection (Ab). (*Source*: Adapted from Su et al., 2002. with permission.)

16.2.5 Conclusion

The hippocampus through its physiological role can initiate and maintain oscillatory behavior. It is this property along with the plasticity of the hippocampus and its vulnerability to neuronal damage that contribute to the pathological role of the hippocampus in epileptic activity. In association with neuronal damage associated with hippocampal sclerosis, there is a vast array of changes in organization, connectivity, receptors, intrinsic neuronal properties, and astrocyte function that can contribute to epileptogenicity. The great challenges are to differentiate pro-epileptogenic changes from antiepileptogenic, compensatory changes, and to determine which are the critical processes. It is likely that epileptogenesis is not a single process but that many diverse processes can result in the expression of epilepsy.

16.3 Alzheimer’s Disease

16.3.1 Introduction

Alzheimer’s disease (AD) is the most common cause of dementia worldwide. AD is a neurodegenerative disorder in which the accumulation of amyloid plaques and neurofibrillary tangles represents the pathological hallmark of the disease. Five million people in the United States and 400,000 in the United Kingdom are estimated to have AD, with a disease onset typically occurring early in the eighth decade of life. Disease prevalence increases with age: 1 in 2000 people aged below 60 years are affected and 1 in 200 aged over 60 years. In the elderly population the prevalence rises dramatically, with AD occurring in 20% of those aged ≥ 80 years and in 50% of those ≥ 90 years of age.

Memory loss is the predominant feature of AD. Impairment of episodic memory occurs early during the course of the disease and is usually the most prominent symptom throughout the disease course. Progressive dysfunction of other cognitive domains is also observed in AD, and the final stages of the disease are characterized by severe global impairment of cognitive function.

The neuropathological changes in AD are thought to be manifest initially in the entorhinal cortex (EC), progressing from there to the hippocampus, with increasing involvement of the neocortex as the disease progresses (Braak and Braak, 1991). However, significant neocortical pathology is already present by the time dementia is clinically diagnosed, which has prompted efforts to identify the clinical manifestations of AD in its earliest stages, when the pathological changes are primarily restricted to the medial temporal lobe structures. This has resulted in the introduction of the concept of “mild cognitive impairment” (MCI), representing the predementia stage of AD. Within the broad overview of AD provided in this chapter, particular attention is devoted to the involvement of the hippocampal formation in AD and MCI and on the implications that improved identification of this involvement has for earlier diagnosis and treatment of AD.

16.3.2 Clinical Features

Memory impairment of insidious onset typifies the initial stages of AD. Patients exhibit poor memory for autobiographical events, current affairs, and the names and faces of acquaintances. Frequently they exhibit difficulty remembering familiar routes, and "getting lost" is a commonly experienced symptom early in the disease. These early symptoms may be attributed initially to cognitive decline as a consequence of normal aging; but as the disease progresses the memory impairment becomes severe enough to disrupt activities of daily living, and patients begin to lose their functional independence. The central nature of the memory problem in AD is reflected in the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984) for the diagnosis of probable AD, which state that the presence of progressive memory impairment and impairment of at least one other cognitive function are required for a clinical diagnosis of AD. However, atypical forms of AD are well recognized, in which the prevailing symptomatology reflects dysfunction of nonmemory cognitive domains, such as speech or visuoperceptual function, but these are much less common than the classic amnestic presentation.

Impairment of other cognitive functions becomes increasingly prominent during the course of the disease. Word-finding difficulty is a relatively frequent early symptom, and speech output is often diminished. Executive functions, such as problem solving and abstract reasoning, decline progressively. Later in the disease there is impairment of word comprehension, reflecting a breakdown in semantic memory function. Other characteristic early symptoms include limb apraxias (higher order motor disorders affecting the execution of skilled or learned limb movements), impaired calculation skills, and disorders of visuospatial and visuoperceptual function. The progressive involvement of multiple cognitive domains in AD reflects the accumulation of pathological changes predominantly in the frontal, temporal, and parietal lobes; the occipital lobes are usually relatively spared in the early stages of AD.

The disorders of cognitive function are accompanied by a variety of neuropsychiatric symptoms, which include depression, changes in personality, delusions, and hallucinations, with depression often preceding diagnosis. Behavioral disturbances such as aggression, agitation, and nocturnal wandering increase with disease severity. Loss of insight is common. In the late stages of the disease, parkinsonian signs (e.g., limb rigidity, motor slowing) and various involuntary limb movements are observed, and seizures may also be observed in severe AD. Finally, patients become bedbound and entirely dependent on caretakers, and in this state they are vulnerable to intercurrent medical complications such as sepsis.

From the time that AD is diagnosed initially, the disease runs approximately 5 to 10 years until the time of death, with a mean survival of 8 years.

Mild Cognitive Impairment

There is increasing awareness that AD may be associated with a prolonged prodromal or "preclinical" phase, during which the cognitive dysfunction is relatively subtle and circumscribed and insufficiently severe to warrant a diagnosis of dementia. The introduction of drug treatments for AD, in the form of the acetylcholinesterase inhibitors, has helped stimulate efforts to identify this prodromal phase in AD, culminating in the introduction of the concept of mild cognitive impairment (MCI) (Smith et al., 1996). Although there exists a degree of debate about the defining criteria for MCI, the most widely accepted criteria for the diagnosis of MCI are based on the presence of significant objective memory decline in the context of normal activities of daily living and intact function in other cognitive domains.

Figures for the prevalence of MCI in the population vary considerably, ranging from 3.0% to 16.8% depending on definitions (DeCarli 2003). Epidemiological data support the notion that MCI represents a precursor state of AD: Patients with MCI "convert" to AD at a rate of approximately 12% per annum, compared with an annual rate of 1% to 2% in the normal elderly population.

The current definition of MCI is predicated on impaired memory. However, the heterogeneity in the clinical presentation of AD suggests "amnestic MCI" that may represent a particular precursor state of AD. Future efforts to categorize the initial clinical phases of AD will include the identification of equivalent MCI states affecting other cognitive domains and will aim to differentiate MCI (due to AD) from cognitive impairment as a consequence of other cognitive disorders, as well as from the memory decline that accompanies the normal aging process. In this context, the fact that the pathological damage in the earliest phases of AD is largely restricted to the entorhinal cortex and hippocampus may be used to direct diagnostic investigations, including structural and functional neuroimaging techniques and clinical neuropsychological assessments of memory.

Pattern of Cognitive Deficits in AD

The deficit in memory that characterizes early AD is primarily impaired episodic memory. Patients are unable to learn new material and have difficulty recalling recent events. A number of factors contribute to this disruption of episodic memory, including deficiencies in encoding and storing new information and heightened sensitivity to the disruptive effects of proactive interference. AD patients also exhibit impaired priming performance (facilitated performance by prior exposure to stimuli); patients with AD, Huntington's disease, and Korsakoff syndrome are equally impaired on tests of verbal recognition and recall, but only AD patients show additional impairment of verbal priming, indicating that AD is also associated with a deficit of implicit memory.

Studies on the rate of long-term forgetting, or the rate of loss of memory after successful learning, have yielded conflicting results. Some studies have shown that AD patients have faster forgetting rates than either controls or patients with depression or Korsakoff syndrome (Hart et al., 1987), but others have failed to demonstrate accelerated forgetting in AD (Becker et al., 1987). Studies of retrograde amnesia in AD have revealed that recent memories are more affected than remote memories.

The diagnostic utility of memory tests in AD varies according to the severity of cognitive impairment. In the presymptomatic phase of the disease, the tests currently considered to predict with greatest accuracy the progression to AD are tests of verbal learning and immediate visual recall. By contrast, for established AD, tests of delayed recall are most sensitive at differentiating between early AD and normal controls but are of limited value in tracking disease severity in AD because performance on these tests often declines rapidly to a plateau. Recognition memory tests, involving verbal and visual subtests, are less sensitive than recall tasks for detecting early AD but are more useful for staging disease severity.

Disorders of speech and language also occur early in the course of AD. Word-finding difficulty is often the first problem to become manifest, and it is associated with compensatory circumlocution. Naming is initially preserved but becomes progressively more impaired during the course of the disease. Neologisms, verbal and literal paraphasias, also become more prominent and are accompanied by loss of word comprehension, reflecting a breakdown of verbal semantic knowledge. Severe AD may be associated with palilalia (the repetition of words and phrases) and logoclonia (repetition of the final syllable of a word), and speech may deteriorate into unintelligibility. Ultimately, some patients may become entirely mute.

Topographical disorientation is another characteristic early feature of AD. Patients complain of "getting lost," initially only in unfamiliar environments, but subsequently they experience difficulty finding their way around familiar places, including their own homes. The presence of topographical disorientation may help differentiate early AD from other dementias; for instance, patients with frontotemporal lobe degeneration, typified by focal atrophy of the frontal and anterior temporal lobes, typically do not get lost. This topographical disorientation in AD has been variously ascribed to impaired visuospatial function as a result of damage to the parieto-occipital region; to topographical agnosia, representing an impairment of the ability to recognize those cues or landmarks that are required to permit successful navigation through an environment; and to an impaired memory for places as a consequence of damage to the hippocampus or parahippocampal regions. The last possibility is consistent with the observation that the medial temporal lobe structures are preferentially affected in the earliest stages of the AD disease process.

Apraxia (impairment of sensorimotor integration due to disorders of higher cerebral function) is observed in most patients with mild to moderate AD. Ideational apraxia, in which there is an inability to construct the idea of a purposeful movement, such that patients are unable to perform these movements (e.g., using a manual tool), may occur as a result of damage to the parietal and frontal associations areas, particularly in the left hemisphere. Ideomotor apraxia, in which the construct of a purposeful movement is intact but the execution of the movement is faulty, is associated more with damage to the parietal association areas as well as damage to the premotor cortex and the supplementary motor areas.

Visual agnosia (inability to recognize objects) is a common feature of AD in its more advanced stages and results from damage to the visual association areas. Subtypes of visual agnosia include apperceptive agnosia, in which the disorder of object perception is exemplified by difficulty recognizing unusual views of common objects; it is typically associated with damage to the right parietal lobe. In associative agnosia, there is no perceptual deficit but, instead, inability to assign the correct semantic meaning to the perceived objects, resulting again in misidentification of objects. In this instance, the cortical damage most frequently involves the left occipitotemporal region. More specific forms of agnosia include prosopagnosia, in which there is impairment of familiar face recognition, typically associated with damage to the right temporal lobe.

Together, amnesia, aphasia, apraxia, and agnosia form the core disorders of cognitive function in AD. However, the global nature of the cortical involvement in established AD is reflected in a multitude of additional cognitive deficits, prominent among which are disorders of attention and calculation. The involvement of the frontal lobes in the pathological process results in "neurological" symptoms such as impaired executive function and reduced problem-solving ability, as well as a variety of "neuropsychiatric" symptoms including changes in personality and disturbances of social conduct.

Structural Imaging

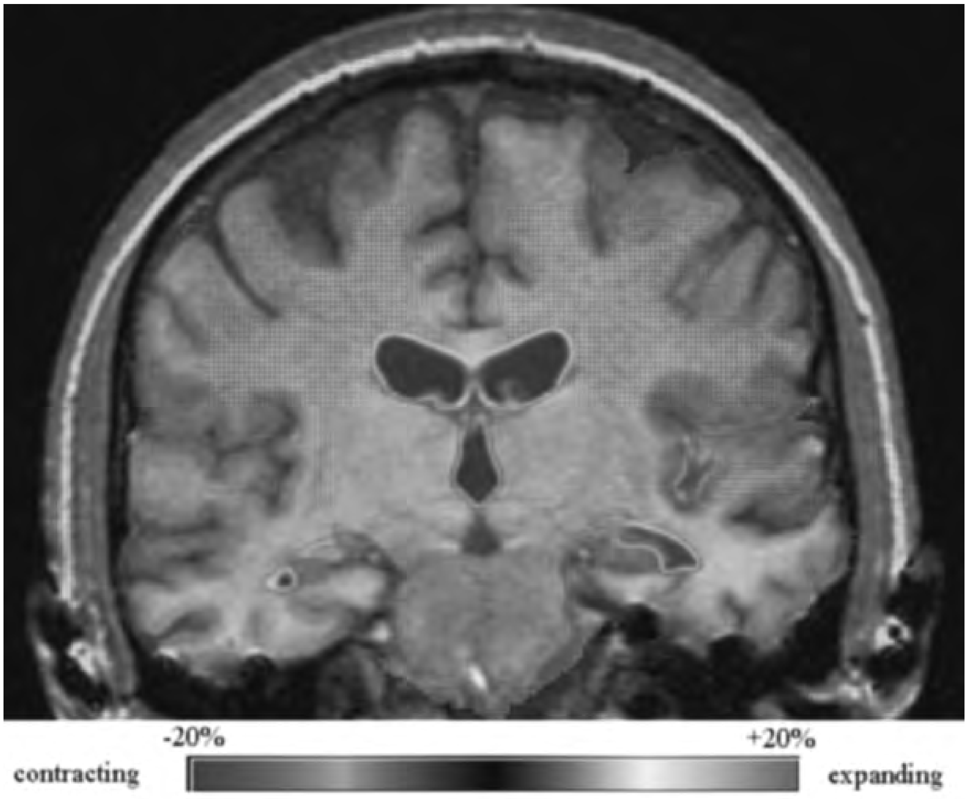
Generalized cerebral atrophy is a characteristic gross pathological feature of AD. The utility of cerebral atrophy as a biomarker of disease is reflected in the NINCDS-ADRDA diagnostic criteria (McKhann et al., 1984), which state that the diagnosis of probable AD is supported by "evidence of cerebral atrophy on CT or MRI and progression documented by serial observation." The presence of cerebral atrophy in AD may be determined using a variety of techniques. Qualitative assessment of brain atrophy (visual inspection of brain scans) has the benefit of general applicability but the disadvantage of wide interobserver variability. Of the various quantitative techniques currently in use, volumetric analyses have been shown to have greater diagnostic specificity and sensitivity than linear measurements of atrophy. Analyses may be cross-sectional (i.e., based on a single scan) or longitudinal (repeated measurements performed over serial scans). The diagnostic utility of data obtained from cross-sectional imaging studies is limited by the variability in brain size among individuals, reflecting differences in head size in the normal population, and by the reduction in total brain volume that occurs as a consequence of normal aging. Longitudinal studies have greater diagnostic specificity and sensitivity than cross-sectional studies but are necessarily disadvantaged by the need for at least two scans and as a consequence cannot provide corroborative information at the time of the initial diagnostic inquiry.

Most volumetric MRI studies have relied on manual segmentation of brain regions of interest. Information on the structural brain changes in AD can also been obtained using semiautomated, increasingly sophisticated techniques such as voxel-based morphometry, in which the distribution of atrophy in the AD brain is determined by comparison with a nonatrophied "template" brain (Good et al., 2002), and fluid registration MRI, in which longitudinal patterns of atrophy throughout the brain can be observed by tracking the brain changes over time on a voxel-by-voxel basis (Fox et al., 2001) (Fig. 16–7, see color insert).

Prior understanding of the pattern of pathological involvement of the cerebral cortex has prompted the assessment of regional brain volume measurements in AD as an alternative to measurements of whole-brain volume. Most studies have concentrated on the medial temporal lobe, but other researchers have investigated structures such as the frontal lobes, the cingulate gyrus, the superior temporal gyrus, and the corpus callosum. As with the measurement of whole-brain atrophy, there are several methods for assessing atrophy of the medial temporal lobe structures. They include the use of a visual rating scale for medial temporal lobe atrophy, in which the degree of atrophy is classified according to a five-point grading scale following visual inspection of MRI scans (Wahlund et al., 2000). Linear measures of atrophy include measuring the height of the hippocampus and parahippocampal gyrus, the interuncal distance, and the width of the temporal horn of the lateral ventricles. Area measurements include changes in the cross-sectional area through the hippocampus and in the surface area of the entorhinal cortex. Of these various measurement techniques, perhaps the most useful in the clinical domain is that based on visual rating of medial temporal lobe atrophy, which has benefits in terms of ease of use and widespread applicability and compares favorably with quantitative volumtric analysis in the diagnostic differentation of patients with AD.

Hippocampus. In recent years increasing emphasis has been devoted to volumetric analyses of structural change derived using quantitative MRI techniques, with the hippocampus representing the primary region of interest in most instances. The use of volumetric MRI measures of hippocampal atrophy as surrogate markers of disease in AD is validated by the demonstration of a strong correlation between MRI-determined hippocampal volumes and neuronal numbers in the hippocampus in AD (Bobinski et al., 1999). A number of

Figure 16–7. Coronal MRI at the mid-hippocampal level using voxel-compression mapping overlay to show the change in brain volume over 12 months in a patient with Alzheimer's disease (AD). Particular features to note are the marked involvement of the temporal lobes including the hippocampi, the relative symmetry of the structural changes, and the diffuse involvement of both gray and white matter.



studies have demonstrated that AD is associated with significant hippocampal volume loss. Data from cross-sectional studies are complemented by longitudinal data indicating that AD is associated with an increased rate of hippocampal atrophy (Jack et al., 1998). Although most of these studies have concentrated on the structural changes in patients with established dementia, hippocampal atrophy has also been found to be present in the presymptomatic stage of AD, as determined by scan data acquired on familial AD (FAD) patients prior to symptom onset (Convit et al., 1997).

Determination of hippocampal atrophy can distinguish AD from normal aging with a high degree of specificity and sensitivity. At a fixed specificity of 80%, the sensitivity of hippocampal volumetric measurements for differentiating patients with mild-to-moderate AD is approximately 88% (Jack et al., 1997).

Several imaging techniques have been applied to the hippocampus in recent years. MRI-based high-dimensional brain mapping (Csernansky et al., 2000) is a technique in which a control MRI template is transformed onto individual scans in such a way that changes in the shape of the hippocampus are detectable. Using this technique, AD was found to be associated with a symmetrical deformity of hippocampal shape that differentiated it from the morphological changes seen with normal aging. The particular deformities affecting the head of the hippocampus and along the lateral aspect of the body of the hippocampus are consistent with pathological changes affecting the CA1 field in AD. A similar technique was subsequently used by the same authors to demonstrate hippocampal deformations in patients with mesial TLE (Hogan et al., 2004).

A technique has also been developed for visualizing neuritic plaques in autopsy-acquired human brain tissue using magnetic resonance (MR) microscopy, which provides greater spatial resolution than MRI (Benveniste et al., 1999). Technical limitations currently militate against in vivo application, but advances in scan technology may permit the future use of a similar technique in the antemortem diagnosis of AD.

Entorhinal Cortex. In view of the early, severe involvement of the EC in the AD pathological process, it has been argued that EC atrophy may be a more sensitive marker of AD than hippocampal atrophy. However, the theoretical benefits of assessing EC volume changes in AD are partially offset by the difficulty of determining with confidence the boundaries of the EC on MRI scans, which have led some to undertake measurements of the parahippocampal gyrus (which contains the EC in its entirety) instead, with the presence of atrophy in this structure taken as a surrogate marker of EC atrophy. Despite these perceived difficulties, a technique for delineating the EC from volumetric MRI scans was developed (Insausti et al., 1998) using the cytoarchitectonic boundaries of the EC as the guidelines for segmentation of the EC volume. In various modified forms (modifications in segmentation protocol resulting primarily from the difficulty of establishing the medial border of the EC at the junction with the perirhinal cortex along the medial lip of the collateral sulcus), this technique has been used to demonstrate significant bilateral EC atrophy in established AD (Fig. 16–7, see color insert). Longitudinal studies on AD patients, with volume measurements of the EC and the hippocampus performed on scans with an average scan interval of 21 months, reveal a significantly greater rate of atrophy affecting the EC (mean annual volume loss 7.1% per annum) than the hippocampus (mean annual volume loss 5.9%), which would support the idea that the pathological changes in AD are more severe in the EC than in the hippocampus.

Structural Imaging in MCI. Neuroimaging studies reveal that MCI is also associated with atrophy of the EC and the hippocampus. Volumetric MRI analysis indicates that there is a progression of atrophy of these structures from normal aging through MCI to AD; the degree of atrophy in these structures is sufficiently great to differentiate effectively between MCI patients and age-matched controls and between MCI and AD patients, with significantly greater atrophy observed in the latter group. The etiological relation between MCI and AD is underlined further by the observation that the presence of hippocampal atrophy in MCI patients is predictive of future conversion to AD (Jack et al., 1999).

A comparison of hippocampal and EC volumes in normal control subjects, patients with MCI, and patients with early AD revealed that assessment of EC volumes is most effective for discriminating between control subjects and MCI patients, whereas measurement of hippocampal volumes provided better discrimination between patients with MCI and those with AD, which suggests that atrophy of the EC precedes that of the hippocampus and is more pronounced in the initial stages of AD (Pennanen et al., 2004).

Medial Temporal Lobe Atrophy in Non-Alzheimer Dementias. Atrophy of medial temporal lobe structures discriminates effectively between AD and age-matched controls but is less effective at differentiating between AD and other diseases that cause dementia. Hippocampal atrophy has been found in other neurodegenerative disorders such as dementia with Lewy bodies and frontotemporal lobar degeneration (FTLD), as well as in vascular dementia. With regard to the latter, the determination of hippocampal atrophy to differentiate between different dementias is complicated further by the frequent coexistence of AD and vascular pathology. With regard to other temporal lobe structures, atrophy of the EC has also been observed in FTLD and, in particular the clinical subtype of FTLD described as semantic dementia (SD). In this instance, the severity of EC atrophy exceeds that noted in AD patients of comparable disease severity, although the atrophy is predominantly left-sided, in keeping with the language dominance of the left hemisphere (Chan et al., 2001).

The fact that the presence of hippocampal or EC atrophy alone is insufficiently specific to discriminate effectively between these disorders suggests that greater diagnostic differentiation may require evaluation of the particular distribution of atrophy in these regions. One example of this is provided by the left/right asymmetry of medial temporal lobe atrophy in SD, which contrasts with the symmetrical atrophy that is typical of AD. An alternative approach is to examine the distribution of atrophy in regions of interest. For instance, in AD there is an even distribution of atrophy along the rostrocaudal length of the hippocampus, whereas in SD there is asymmetrical atrophy affecting primarily the rostral portion of the left hippocampus.

Memory Impairment and Medial Temporal Lobe Atrophy in Alzheimer's Disease. Deficits of episodic memory correlate with atrophy of the hippocampus but not with atrophy of structures outside the medial temporal lobe, such as the caudate nucleus and the lateral temporal cortex. Although differing results have been observed across a number of studies, partly as a consequence of differences in methodology and in data interpretation, most studies have revealed that performance on tests of memory correlate with hippocampal volume but not with the volumes of the amygdala or of the whole temporal lobe. Hemispheric differences are also found; the volume of the left hippocampus has been found to correlate with verbal recall, whereas the volume of the right hippocampus correlates with performance on tests of visual or spatial recall. Studies of the relation between hippocampal volume and immediate and delayed recall have yielded differing results: In one study, hippocampal volumes correlated with both recall tasks (de Toledo-Morrell et al., 2000), whereas in another a positive correlation was observed between the volumes of the hippocampus and parahippocampal gyrus with delayed, but not immediate, recall (Kohler et al., 1998). The volume of the right parahippocampal gyrus was positively associated with delayed visual recall. Hippocampal and parahippocampal gyrus volumes have also been found to correlate with a different verbal learning task (Libon et al., 1995).

In summary, most studies attempting to correlate the memory deficits in AD with atrophy of specific brain regions have implicated the hippocampus as the main region of interest. The difficulty of establishing the nature of the memory task that provides the best correlation with hippocampal volume in AD are reminiscent of the problems experienced in the clinical setting with identifying the memory tests that are most able to detect early AD.

Functional Imaging

The neuronal death and the dysfunction of surviving neurons in affected brain regions in AD results in a reduction in neuronal activity, which in turn produces an alteration in metabolic demands, with a lowering of glucose metabolism and oxygen uptake. Cerebral blood flow (CBF) to affected brain regions is likewise reduced. Cerebral hypometabolism and hypoperfusion are detectable using various functional imaging techniques, and the information derived from functional imaging complements the structural data obtained from computed tomography (CT) and MRI. To date, most of the functional imaging studies in AD have compared patients with AD with age-matched normal control subjects rather than with patients with other neurodegenerative disorders.

Positron Emission Tomography. Cerebral glucose metabolism can be measured by positron emission tomography (PET) imaging of the radioactive tracer ^{18}F-fluorodeoxyglucose (^{18}F-FDG). PET can also be used to measure oxygen metabolism or CBF using $^{15}O_2$- or $^{15}O_2$-labeled water. Statistical parametric mapping (SPM) (Friston et al., 1995) is commonly used to analyze scan data on a voxel-by-voxel basis.

Positron emission tomography (PET) scans in AD demonstrate bilateral temporoparietal hypometabolism and hypoperfusion. The reductions in CBF and oxygen uptake have been found to correlate with the severity of the dementia. A number of studies have demonstrated correlations between memory scores and metabolism or blood flow in AD, and an association between hippocampal atrophy and regional glucose metabolism has also been demonstrated. In advanced AD, hypoperfusion changes are more widespread and additional reductions in CBF are seen in the frontal lobes.

The efforts to identify the earliest structural abnormality in AD using MRI are mirrored by studies performed using functional imaging paradigms. These have shown that hypoperfusion of the EC in cognitive normal elderly subjects is predictive of progression to MCI (de Leon et al., 2001), indicating that abnormalities of brain function may be detected at the very earliest stages of the disease.

The relation between the functional imaging data obtained from PET studies and data from structural imaging studies remains unclear. Although both imaging modalities have identified abnormalities involving the EC in the earliest stages of AD, PET studies have also shown hypofunctioning of the temporoparietal regions and the posterior cingulate cortex in early AD, whereas structural scans have revealed atrophy predominantly affecting the medial temporal lobe regions at this stage of the disease process. Given that these regions receive significant inputs from the hippocampal formation, it is possible that the PET data reflect a disconnection syndrome, in which deficits are observed in regions with disturbed activity due to reduced afferent input from damaged regions upstream in the projection. An alternative explanation for this apparent discrepancy might rest with methodological considerations. With regard to the structural imaging data, reproducible and easily validated protocols for the measurement of brain volumes are primarily restricted to the temporal lobe structures, within which atrophy is also readily detected on visual inspection. By contrast, the anatomical landmarks of regions such as the posterior cingulate gyrus are less easily identified on MRI, as a consequence of which volume loss in these regions is more difficult to detect and may therefore be underreported. In terms of the PET data, the absence of observed hypometabolism in the hippocampus and parahippocampal regions in early AD may reflect in part the low spatial resolution of the current generation of PET scans. Finally, the nature of the relation between regional brain atrophy and detection of regional hypofunction has yet to be established fully for progressive degenerative disorders such as AD.

In one study, Klunk et al. (2004) employed PET imaging using a novel tracer, named Pittsburgh compound-B (PIB), designed as a marker for brain amyloid. In patients with early AD, PET scans demonstrated increased retention of PIB (compared with control subjects) in regions of association cortex that are known to contain significant numbers of amyloid deposits. By contrast, there was no significant difference in PIB retention between AD patients and controls in those brain regions largely devoid of amyloid deposition, such as the cerebellum. The evidence from this study that PET, in conjunction with targeted tracer compounds, may be used to provide quantification of the pathological changes in AD raises the possibility that PET may be used in the future to detect, and possibly to track over time, the key pathological changes in AD.

Single Photon Emission Computed Tomography. Single photon emission computed tomography (SPECT*)* has an advantage over PET in that it is less expensive and more widely available, but it is less informative in that it provides only semiquantitative perfusion images and has poorer spatial resolution. CBF is measured by detection of radioactive tracers such as the lipophilic technetium ^{99m}Tc-hexamethyl propyleneamine oxamine (^{99m}Tc-HMPAO). There is a reduction in CBF in the temporoparietal regions of patients with mild-to-moderate AD (Battistin et al., 1990), with a correlation between the reduction in blood flow and hippocampal atrophy. However, the low diagnostic sensitivity and relatively poor spatial resolution of SPECT has limited its use as a diagnostic tool.

Functional MRI. The different magnetic properties of oxygenated and deoxygenated blood can be measured using the technique of blood oxygen level-dependent (BOLD) functional MRI (fMRI), with increased levels of blood oxygenation resulting in greater signal intensity. As with PET, fMRI scan data can be analyzed using SPM.

During a verbal episodic memory task patients with mild AD showed reduced activation of anterior prefrontal cortex when compared with control subjects and, instead, exhibited increased activation in a number of other brain regions, with the latter believed to represent compensatory reallocation of brain resources in response to the frontal lobe dysfunction. In a cued recall task, AD patients failed to demonstrate any increased activity in the left hippocampus—a phenomenon observed in control subjects—but, instead, were found to have increased activity in other cortical regions. These observations of increased functional activation during cognitive tasks as compensation for dysfunction of brain regions normally associated with those tasks raises the possibility that fMRI may be used in the diagnosis of AD. Functional brain changes have also been detected prior to the clinical onset of AD; subjects at risk for AD exhibit different patterns of brain activation in the absence of any clear cognitive impairment.

Particular attention has been devoted to the regional activation patterns with the hippocampal formation in view of the early pathological involvement of the hippocampus in AD. A comparison of the fMRI activation patterns in patients with AD and patients with isolated memory decline reveals that AD patients exhibit reduced activation in all hippocampal regions. By contrast, patients with selective memory impairment either exhibited diminished activation in all hippocampal regions (similar to the pattern observed in AD) or reduced activation affecting only the subiculum. In the latter cases, the preferential involvement of the subiculum in these cases may reflect neuronal loss in the subiculum or loss of input to the subiculum in patients at risk of developing AD.

The observation that regional BOLD fMRI activation correlates with excitatory input, as manifested by the EPSP, rather than the regional output (spiking activity), suggests that reductions in regional fMRI activation patterns in AD may reflect damage in upstream neuronal populations providing excitatory inputs to the region in question (Logothetis et al., 2001). However, several outstanding issues remain with regard to the association of abnormal fMRI activation and structural pathology in AD. Specifically, the relation between fMRI activation and cerebral atrophy, as demonstrated on structural MRI, remains unclear, particularly in terms of the potential confounding effect of atrophy on fMRI activation patterns. In addition, the presence of vascular pathology in AD may represent another confounding factor in fMRI analysis in that changes in activation may be attributable to alterations in hemodynamic response as well as to changes in neural activity.

Magnetic Resonance Spectroscopy. Detection of changes in the concentration of brain metabolites using magnetic resonance spectroscopy (MRS) represents an alternative imaging modality that can be applied to disease states affecting the brain. Two metabolites that are of greatest interest are *N*-acetyl aspartate (NAA) and myoinositol (MI), which are markers of neuronal and glial cell metabolism, respectively. MRS has demonstrated levels of NAA that are reduced by 10% to 15% in AD, with the magnitude of metabolite reduction correlating with disease severity. Other studies (Jessen et al., 2000) have shown a 15% to 20% increase in MI, and a combination of NAA and MI measurements increases the ability of MRS to differentiate AD from normal aging. At present, MRS is less useful for distinguishing AD from other neurodegenerative diseases, and the utility of this imaging technique beyond the research domain has yet to be established.

16.3.3 Genetics

Most AD cases occur in sporadic form, with familial AD (FAD) accounting for less than 5% of all cases. Apart from the earlier age at onset (typically before the age of 65 years) no consistent differences in the clinical features of sporadic and familial AD have been identified. This similarity in clinical presentation has underpinned the belief that greater understanding of the defects occurring as a result of the genetic mutations associated with FAD will, in turn, yield key insights into the mechanisms of disease in AD.

Amyloid Precursor Protein

Most cases in which the patients have early-onset AD (aged ≤ 50 years) are attributable to familial forms of AD. Causative genetic mutations have been identified in these cases. The first reported FAD-associated mutations were those in the amyloid precursor protein (APP) on chromosome 21 (Chartier Harlin et al., 1991). The exact function of APP remains undetermined, with a role in growth promotion, signaling mechanisms, and cell adhesion having been variously suggested (Breen et al., 1991; Milward et al, 1992; De Strooper and Annaert, 2000). APP is cleaved at its N- and C-termini by β- and γ-secretases, respectively, to produce the peptide Aβ, comprising 40 to 42 amino acids, which is the main constituent of the amyloid plaques in AD. Despite the uncertainty over the role of APP, its role in the pathogenesis of AD appears unequivocal; all currently identified mutations of the APP gene result in an increased amount of Aβ or an increased proportion of Aβ containing 41 or 42 amino acids, which are more amyloidogenic and therefore predispose to the formation of amyloid plaques.

Presenilins

The APP mutations account only for a small proportion of early-onset FAD. Most of these cases are caused by mutations of the *PS1* gene on chromosome 14, coding for the protein presenilin 1 (PS1). In all, *PS1* mutations are responsible for 30% to 50% of all cases of familial AD. Shortly after the discovery of the *PS1* gene, a second presenilin gene, *PS2*, on chromosome 1 was identified (Li et al., 1995). Known *PS2* mutations account for less than 10% of all FAD cases. More than 50 different mutations of *PS1* have been described, but to date only two causative *PS2* mutations have been identified.

The presenilins are transmembrane domain proteins. As with APP, the function of the presenilins remains unclear, although some clues can be derived from understanding the function of the homologous proteins SEL-12 and SPE-4 in *Caenorhabditis elegans*. SEL-12 is involved in receptor trafficking and localization, mediated via the *lin-12/Notch* pathway, and SPE-4 plays a role in intracellular protein sorting during spermatogenesis. The demonstration of a relation between PS1 and γ-secretase function (De Strooper et al., 1999) has fueled speculation that PS1 represents γ-secretase or that PS1 and γ-secretase form part of a macromolecular complex that mediates the cleavage of APP. Accordingly, presenilin mutations may alter the membrane conformation of APP, resulting in a different position of cleavage by γ-secretase; and thus generation of the more amyloidogenic forms of Aβ. The observation that mice underexpressing PS1 exhibit a reduction in LTP following repeated tetanic stimulation of the CA1 region (Morton et al., 2002) suggests that PS1 is also implicated in the maintenance of LTP.

Apolipoprotein E

Linkage to chromosome 19 was observed in families with late-onset FAD (Pericak-Vance et al., 1991). The responsible susceptibility gene was found to encode for apolipoprotein E (ApoE), a 299-amino-acid lipid transport protein that mediates the intracellular uptake of lipids through binding to the low density lipoprotein (LDL) receptor. Three alleles for the ApoE gene exist: *APOE-ϵ2*, *APOE-ϵ3* (the common form), and *APOE-ϵ4*. The likelihood of developing AD has been found to correlate with the number of *APOE-ϵ4* genes (Saunders et al., 1993); ϵ4 heterozygotes had a greater risk and earlier disease onset than non-ϵ4 individuals, and ϵ4 homozygotes had the greatest risk of all. Homozygous ϵ4 patients were also found to have a greater number of amyloid plaques than patients homozygous for the ϵ3 allele. When compared with age-matched normal controls (in whom the ϵ4 allele is found in 16% of cases), the presence of the ϵ4 allele is more frequent in both late-onset AD with a positive family history (52% of all cases) and sporadic AD (40% of all cases).

One theory concerning the role of ApoE4 in the pathogenesis of AD proposes that ApoE4 binds more easily to Aβ than ApoE3, resulting in increased deposition of amyloid in plaques. Another theory is based on the observation that ApoE4 binds less well to the microtubule-associated protein tau than ApoE3 or ApoE2. This has the effect of destabilizing microtubules, which in turn results in the formation of neurofibrillary tangles.

16.3.4 Pathophysiology

Neuropathology

On macroscopic examination, the AD brain can vary in appearance from normal to severely atrophic, with cases of early-onset AD often exhibiting the most marked atrophy. Typically, the AD brain features widening of the sulci and ventricular enlargement, which is most prominent in the lateral ventricles. There is generalized atrophy of the cerebral cortex, but closer inspection of the temporal lobes may reveal a greater degree of atrophy affecting the amygdala, hippocampus, and parahippocampal gyrus.

The definitive diagnosis of AD relies on the demonstration of histological features that were first described around the turn of the twentieth century. Most prominent among them are the amyloid plaques and neurofibrillary tangles, and their significance is reflected in the various published criteria for the pathological diagnosis of AD, which are based on an evaluation of the frequency of neuritic plaques, on quantitative assessment of both plaques and tangles, and most recently on the number of tangles and neuropil threads in the cerebral cortex. Other pathological changes in AD include granulovacuolar degeneration and Hirano bodies, both of which primarily affect the hippocampus, as well as amyloid angiopathy and in severe AD mild spongiosis. Little is known about the pathogenesis of granulovacuolar degeneration or Hirano bodies or their relation to the natural history of AD, but the predominance of hippocampal involvement in both instances may provide another explanation for the prominence in AD of symptoms of hippocampal dysfunction.

Amyloid Plaques. Extracellular amyloid plaques (APs) are visualized best using silver stains or immunohistochemical techniques using an antibody to Aβ. APs are divided into two main types: diffuse plaques and neuritic plaques. Diffuse plaques are composed of homogeneous deposits of fibrillary material but contain only scant numbers of amyloid fibrils and do not stain with the Congo red stain for amyloid. Neuritic plaques are more heterogeneous in composition, with a central dense core of amyloid fibrils surrounded by glial and abnormally swollen neuritic processes, occasionally containing paired helical filaments. Neuritic plaques stain with Congo red. The two types of plaque differ crucially in that the Aβ in neuritic plaques occurs in the form of insoluble, possibly neurotoxic β-pleated sheets. APs are observed mainly in the neocortex, with only small numbers seen in the hippocampal formation during the early stages of AD; plaque density in the cortex increases with disease severity, although the progression of AP deposition does not follow a clear hierarchical pattern (Arriagada et al., 1992).

Neurofibrillary Tangles. Neurofibrillary tangles (NFTs) are found in the perikarya of neurons. They are stained with the Bielschowsky silver stain (a stain for thioflavine S) and by immunohistochemistry using an antibody to the tau protein. They are commonly flame-shaped in appearance and occupy the cell body and proximal portion of the apical dendrite of the affected neuron. NFTs are usually intraneuronal but occasionally extracellular and represent the insoluble remains of a dead neuron (the "ghost tangle"). Ultrastructural examination reveals that NFTs are primarily comprised of paired helical filaments, which are composed of a number of proteins including tau, β-amyloid, ubiquitin, and neurofilament proteins such as actin.

A hierarchical staging system for the neuropathological changes in AD has been elaborated based on the distribution of NFTs in the cerebral cortex (Braak and Braak, 1991). The first neurons to exhibit NFTs and neuropil threads (NTs)—straight and paired helical filaments composed of abnormally phosphorylated tau protein—are the pre-alpha projection neurons in the transentorhinal cortex, a transition zone between the entorhinal cortex and the adjacent isocortex. Other areas with early development of NFTs are the entorhinal cortex (EC) and field CA1 of the hippocampus. In stages I and II (the "transentorhinal stages") these minor pathological changes are restricted to the EC and hippocampus. Stages III and IV (the "limbic stages") are characterized by moderate numbers of NFTs and NTs in the transentorhinal cortex, EC, and CA1, with additional scant numbers of NFTs in CA4, the subiculum, and the parasubiculum. Small numbers of NFTs and NTs are also found in association cortices. In stages V and VI (the "isocortical stages") all hippocampal subfields and isocortical association areas are severely affected. The progression of these pathological changes is depicted in Figure 16–8, see color insert.

Granulovacuolar Degeneration. In marked contrast to the widespread distribution of APs and NFTs, granulovacuolar degeneration is restricted primarily to one neuronal population: the pyramidal cells of the hippocampus. This pathological change is observed in up to 50% of AD cases. Vacuoles (3–5 μm diameter) are found in the cytoplasm of the pyramidal neurons, either singly or in combination. Within each vacuole is an electron-dense granular core. These features can be seen on light microscopy using either silver staining or hematoxylin and eosin (H&E) preparations.

Granulovacuolar degeneration is not specific to AD. It is also a pathological feature of other neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) and the Parkinson-dementia–ALS complex of Guam and has been found in young adults with Down's syndrome.

Hirano Bodies. Hirano bodies are ovoid eosinophilic inclusions about 10 to 30 μm in length. They can be visualized using the H&E stain. They are most commonly observed adjacent to the hippocampal pyramidal cells, when they indent the neuronal perikaryon, although they can also be found in isolation in the stratum lacunosum. Electron microscopy reveals that Hirano bodies are comprised of parallel filaments 60 to 100 nm in length.

Hirano bodies have been observed in many disorders as well as in the aged normal brain. Although they are most commonly seen in the hippocampus, Hirano bodies have been observed in most other structures of the CNS.

Medial Temporal Lobe Pathology in Alzheimer's Disease. *Entorhinal cortex:* Within the EC, the stellate cells of layer II are the first to exhibit NFTs and NTs. These cells are consistently involved in AD. In one study of patients with pathological diagnoses of definite AD, severe infiltration of stellate cells by NFTs was observed in 100% of cases (Hyman et al., 1990). These degenerative changes are accompanied by neuronal loss, which is prominent even in the early stages of AD. By late AD, severe loss of layer II cells in observed. Examination of the perforant path reveals a number of associated changes. Myelin cuffing and argyrophilic degenerative changes are seen throughout the course of the perforant path as well as in its termination zone in the outer two-thirds of the molecular layer of the dentate gyrus. Increased acetylcholinesterase staining in the outer two-thirds of the dentate molecular layer suggests that there is partial cholinergic reinnervation of the hippocampus in response to the deafferentation of the dentate gyrus.

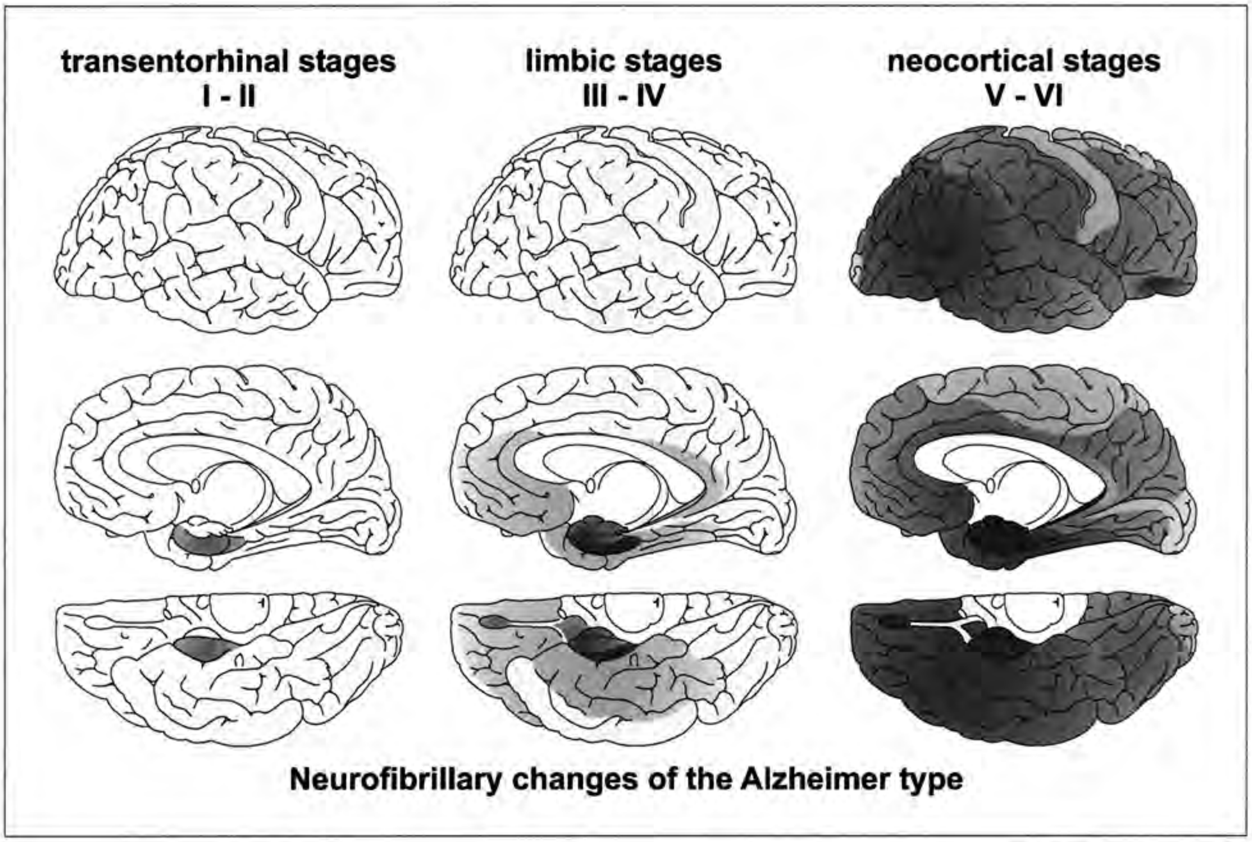


Figure 16–8. Distribution patterns of neurofibrillary changes in AD, including both neurofibrillary tangles and neuropil threads. In stages I and II the pathological changes are largely restricted to the transentorhinal region (tangles may also be found in CA1). Stages III and IV are characterized by severe involvement of the transentorhinal and entorhinal regions, with additional changes in CA1 and the subiculum. The isocortex is minimally affected. In stages V and VI the entire hippocampal formation is involved, with severe neuronal loss in CA1. The temporal lobes and cortical association areas are severely affected, but typically there is relative sparing of the primary sensory cortices. (*Source*: Courtesy of Dr. Heiko Braak.)

Of the other layers of the EC, NFT formation is observed in most of the pyramidal cells of layer IV. By contrast, significantly fewer NFTs are observed in layers III, V, and VI, although in layer III the superficial layer of neurons is more severely affected, compounding the disruption of perforant path input to the hippocampus. Assessment of neuritic plaque density reveals a different pattern of laminar involvement, with most plaques observed in layer III. Layers IV, V, and VI are associated with similar plaque density, but relatively few plaques are seen in layer II. Neither NFTs nor neuritic plaques have been demonstrated in layer I.

Hippocampus: Of the hippocampal subfields, NFT and AP density is greatest in CA1 and the subiculum. The CA1/subiculum interface zone is particularly affected, with large number of plaques and tangles observed in all cases. In CA3 and CA4, only small numbers of plaques and tangles are observed. The outer two-thirds of the molecular layer of the dentate gyrus is heavily infiltrated by NFTs and neuritic plaques, and NFTs are also seen in the dentate granule cells. Pathological changes in the mossy fiber zone are negligible. In stark contrast to the subiculum, NFTs and plaques are largely absent from the presubiculum. In terms of the hierarchical staging of AD pathology, the CA1 pyramidal cells are the first hippocampal neurons to exhibit changes and, in fact, represent the second neuronal population to be affected in AD, after the stellate cells of the EC. The nonpyramidal cells of CA4 and the subicular neurons are next to be affected; the granule cell layer of the dentate gyrus, CA3, and the presubiculum are involved only in the late stages of AD. Interestingly, NFTs are observed in the inner third of the dentate molecular layer in the most severely affected AD cases, which suggests that in the late disease stages there is additional deafferentation of the input to the dentate gyrus from the hilar cells. Although the hippocampus is affected throughout its extent, morphometric studies have found that there is a proportional increase in the number of neurons exhibiting NFTs and granulovacuolar degeneration in the posterior hippocampus when compared with the distribution of changes observed in the hippocampi of age-matched control subjects (Ball, 1987).

The degree of overall hippocampal pyramidal cell loss in AD has been estimated to be around 43% to 47%, with an increase in neuronal loss correlating with disease severity. Some disagreement exists with regard to the distribution of neuronal loss affecting the hippocampus proper; some observers have reported that the greatest proportion of neuronal loss occurs in CA1, with additional neuronal loss observed in CA4, the subiculum, and the prosubiculum and relative sparing of the dentate granule cells and neurons in CA3 and CA2 (Doebler et al., 1987; West et al., 1994). In a later study, no significant difference was noted in the amount of cell loss in CA1 in AD and normal aging; instead, the greatest differences in neuronal numbers were observed in the granule cell layer and the subiculum (Simic et al., 1997). An inverse correlation exists between hippocampal neuronal density and the number of neurons with NFT infiltration or granulovacuolar degeneration. In terms of the clinical significance of the severity of hippocampal involvement, the degree of neuronal loss in CA1, CA4, and the subiculum is found to correlate with the duration and severity of AD.

Amygdala: In the amygdala, NFT density is highest in the accessory basal and cortical nuclei and lowest in the medial, lateral, and central nuclei. APs were most prominent in the accessory basal and medial basal nuclei and least numerous in the medial, lateral, lateral basal, and central nuclei. Neuronal loss is greatest in the medial group of nuclei. The projections between the amygdala and the hippocampal formation are severely disrupted in AD: There is a prominent afferent projection from the accessory basal nucleus both to the hippocampus and to layer III of the EC. The return projection to the amygdala arises primarily from CA1, the subiculum, and layer IV of the EC, all of which exhibit marked pathological damage.

Pathological Changes in MCI. Postmortem analysis reveals that the pathological changes associated with MCI primarily affect the EC and the hippocampus. Patients with very mild dementia at the time of death exhibit severe neuronal loss primarily affecting layer II, with neuronal numbers being reduced by almost 60% (Gomez-Isla et al., 1996). In layer IV, there is a 40% reduction in neuronal numbers, whereas layers I, III, and V are less affected. The degree of neuronal loss is greater in cases of severe dementia, with the drop in neuronal counts in layers II and IV rising to about 90% and 70%, respectively. These reductions in neuronal numbers are accompanied by increased deposition of NFTs and neuritic plaques. A comparison of the severity of EC pathology in MCI and AD reveals that AD is associated with greater volume loss affecting layer II, but the absence of any corresponding decrease in the number of layer II neurons indicates that the development of frank dementia may be associated with other structural changes, such as alterations in the extent of dendritic arborization.

The distribution of pathological damage in the EC in MCI patients bears comparison with the initial stages of pathology described in AD (Braak stages I and II). Given that the onset of frank dementia in AD is associated with more advanced pathological involvement, equivalent to Braak stages III and IV, it might reasonably be assumed that the syndrome of MCI represents the clinical correlate of the earliest pathological (“transentorhinal”) stages of the AD disease process.

Comparison with the Pathological Changes of Normal Aging. The APs and NFTs are also observed in the brains of nondemented aged individuals. Diffuse plaques are found throughout the cerebral cortex, with additional plaques in the amygdala, EC, and CA1. Smaller numbers of neuritic plaques are also found in these regions but with proportionally greater quantities in the medial temporal lobe structures. NFTs are commonly present in the nondemented elderly brain and are most prominent in the hippocampus and parahippocampal regions (including the EC), with NFT numbers in CA1 correlating with age.

These AD-like changes have led to the suggestion that some “normal” elderly subjects may in fact have covert, or preclinical, AD. However, the observation that normal aging and AD are associated with different patterns of neuronal loss in the hippocampus suggest, instead, that the two do not share a common pathological substrate. The demonstration of Alzheimer-type pathology in “normal” elderly individuals and the concomitant difficulty distinguishing these changes from those observed in the earliest stages of AD mirrors the problem in clinical practice with respect to the differentiation between individuals exhibiting minor cognitive decline in keeping with increasing age, and patients manifesting the earliest symptoms of AD.

Cholinergic Deficit in AD

The cholinergic innervation to the hippocampal formation arises from various components of the basal forebrain. The medial septal nucleus and the nucleus of the diagonal band provide most of the inputs, with a smaller afferent projection originating from the basalis of Meynert. By comparison, the cerebral cortex receives its major cholinergic input from the basalis of Meynert, with additional lesser projections from the pedunculopontine and lateral dorsal nuclei. Cholinergic afferents are distributed to all cortical regions, but the limbic and paralimbic cortices (including the parahippocampal areas) are the recipients of a particularly strong projection. As a consequence, lesions of the basal forebrain in monkeys result in widespread behavioral abnormalities, within which disruption of memory functions are particularly prominent (Berger-Sweeney et al., 1994).

In AD, NPs and NFTs are observed in the basalis of Meynert, the nucleus of the diagonal band, and the medial septal nuclei. There is marked neuronal loss in the nucleus basalis and the nucleus of the diagonal band. The severity of the neuropathological changes in the basalis of Meynert have been found to correlate with clinical disease severity. The concomitant depletion of cortical cholinergic axons results in a reduction in the activity of choline acetyltransferase (ChAT) in the cortex and diminished choline uptake in AD. ChAT activity is reduced by 60% in cortical biopsies obtained from patients with AD.

The observation that in AD the basal forebrain nuclei are affected by plaques and tangles, in conjunction with the reduction in cortical cholinergic innervation and the demonstration that disruption of the cholinergic system causes impaired learning and memory underpin the "cholinergic hypothesis of AD." The hypothesis proposes that the cognitive dysfunction associated with AD is at least partly attributable to impairment of cholinergic neurotransmission. Support for the hypothesis comes from studies demonstrating that the reductions in ChAT activity and acetylcholine (ACh) synthesis correlate with dementia severity in AD (Wilcock et al., 1982). However, the primary role of the cholinergic system in the pathogenesis of AD is cast into question by the observation that there is relative preservation of the cholinergic neurons of the nucleus basalis in MCI and early AD (Gilmor et al., 1999). Furthermore, the cholinergic system is not selectively affected in AD; pathological changes are also observed in a number of brain stem nuclei, including the locus coeruleus, the ventral tegmental area, and the rostral raphe nuclei. These nuclei, in turn, provide major components of the noradrenergic, dopaminergic, and serotoninergic innervation of the cerebral cortex, and it is likely that their involvement contributes to the cognitive dysfunction observed in AD.

Impairment of Synaptic Function in AD

Synaptic density microdensitometry performed on pathological specimens obtained from the frontal and temporal lobes has revealed significant reductions in the density of presynaptic boutons in AD (approximately 60% of that observed in control brains). The antemortem Mini Mental State Examination score, employed as a global measure of dementia severity, was more closely correlated with synaptic density than with amyloid plaque density or ChAT activity. Consistent with the known involvement of the entorhinal cortex in AD, there is a reduction in the markers for synaptic vesicle proteins in the termination zone of the perforant path in the outer molecular layer of the dentate gyrus. This is accompanied by a reduction in synaptic density in the inner molecular layer, although this is associated with an expansion in the size of the remaining synapses, resulting in the maintenance of the total synaptic contact area in this region (Scheff et al, 1996).

Abnormalities of synaptic transmission in early AD have been demonstrated using in vitro and in vivo preparations. In vitro studies using hippocampal slices prepared from PDAPP transgenic mice overexpressing human mutant amyloid precursor protein have demonstrated alterations in synaptic transmission and LTP (Larson et al., 1999). Enhanced paired-pulse facilitation of synaptic transmission was observed in slice preparations taken from young PDAPP mice. In addition, there was a small (about 10%) reduction in the size of the CA1 dendritic EPSPs following stimulation of the Schaffer collaterals and commissural fibers. By contrast, slice experiments performed on slices taken from aged mice revealed diminution, rather than enhancement, of paired-pulse facilitation and a marked (around 55%) reduction in CA1 field EPSPs. LTP could be induced in both young and aged preparations, but this was associated with an abnormally rapid decay function. In vivo studies using PDAPP mice have demonstrated impaired induction and maintenance of LTP in CA1 following high-frequency stimulation (Giacchino et al., 2000). PDAPP mice also exhibited attenuation of paired-pulse facilitation, indicating impaired presynaptic function in these animals. Although these changes were most prominent in aged transgenic mice, the demonstration of abnormalities in young transgenic mice prior to the development of AD-related pathological changes indicates that defects of hippocampal synaptic transmission may precede the onset of gross neurodegenerative changes.

Mouse Models of AD

The discovery of the pathogenic APP mutation in 1991 stimulated efforts to create a mouse model in which the characteristic cognitive and pathological features were expressed. Gene-targeted knockout models provide information on the possible actions of the proteins coded by the mutant genes, whereas studies using transgenic mice explore the consequences of the overexpression of mutant AD genes.

Knockout Models. APP knockout mice with functionally inactivated alleles of APP are observed to have mild impairment of forelimb grip strength and decreased locomotor activity associated with reactive gliosis. PS1 knockout mice were found to have disrupted development of the axial skeleton as a result of impaired somitogenesis. Examination of the brains of these mice reveals thinning of the ventricular zone and severe regional neuronal loss. Cerebral hemorrhages were seen in all mouse embryos. PS2 knockout mice were found to have mild pulmonary fibrosis and hemorrhage but no pathological brain changes. Absence of PS2 did not affect APP processing. However, the double homozygous PS1/PS2 knockout mice are more severely affected than the PS1 mice, suggesting that PS1 and PS2 have partially overlapping functions.

Transgenic Models. Several lines of transgenic mice are currently in existence. The PDAPP mice overexpressing V717F β-APP (an FAD-associated mutation) exhibit amyloid plaques with dystrophic neurites surrounding β-amyloid cores. Transgenic mice with overexpression of human APP_{695} (a 695-amino-acid length APP isoform representing one of the more abundant APP isoforms in the AD brain and a potential source of the Aβ peptide) have abnormally high levels of Aβ and β-amyloid deposits in the amygdala, hippocampus, and cortex between 6 and 12 months of age. Neither of these two lines of mice was found to have significant neuronal loss. None of the APP transgenic mice developed NFTs, although abnormally phosphorylated tau immunoreactivity has been observed. Deficits of spatial memory are noted by 9 to 10 months of age, and APP transgenic mice also exhibit impaired LTP (Chapman et al., 1999). PDAPP mice have been found to exhibit an age-related deficit in learning a succession of spatial locations in the watermaze (Chen et al., 2000). By contrast, object recognition was normal. In these mice, impaired performance on the watermaze task was found to correlate with amyloid plaque density.

The PS1 transgenic mice have elevated levels of Aβ_{1-42}. In view of the fact that patients with familial AD due to PS1 mutations have amyloid plaques comprised primarily of this longer Aβ peptide, these observations provide further evidence that PS1 is involved in this aspect of plaque deposition.

Earlier studies involving APP and PS1 transgenic mice were able to provide reliable models of amyloid deposition, but the absence of any significant tau pathology meant that these studies were of limited value as realistic models of the human AD disease process. However, subsequent research has now provided evidence of a causal association between amyloid and tau pathology. The observation that bigenic mice expressing both mutant APP and mutant tau are found to have significantly greater quantities of intracytoplasmic tau tangles in the limbic system and olfactory cortex than similarly aged mice expressing the mutant tau gene alone can be taken as evidence that that the formation of NFTs is influenced by amyloid protein (Lewis et al., 2001). Furthermore, injection of Aβ_{1-42} fibrils into the hippocampi of transgenic mice expressing the mutant P301L tau gene results in a five-fold increase in the number of NFTs in the amygdala (Gotz et al., 2001), which suggests that NFT deposition may be driven by Aβ_{1-42}. These data represent a significant advance in our understanding of the underlying nature of the neurodegeneration of AD, particularly in terms of the link between the two key aspects of AD pathology: β-amyloid deposition and tangle formation.

16.3.5 Treatment Options

The introduction of pharmacological treatments for AD during the last decade has resulted in a fundamental change in the approach to the clinical management of a condition previously considered to be associated with an inexorable and unalterable decline. At present, cholinesterase inhibitors are licensed for use in Europe and the United States, and the NMDA antagonist memantine is currently licensed for use in certain European countries. Both treatment options represent symptomatic therapy aimed at attenuating the rate of cognitive and functional decline by enhancing synaptic function. However, neither of these licensed treatments has been demonstrated to exert any effect on the underlying pathological process and so neither is likely to affect the natural history of the disease. As a consequence, efforts have been directed toward the development of treatments that may interrupt the disease process, in the anticipation that any disease-modifying drug may prove successful in altering the natural history of AD.

Enhancement of Cholinergic Function

Therapeutic strategies aimed at redressing the cholinergic deficit have led to the development of AChE inhibitors, which block the breakdown of ACh, thereby increasing ACh concentrations in the synaptic cleft. Clinical trials have demonstrated that usage of these drugs results in improved cognitive function. AChE inhibitors are currently licensed for the treatment of mild to moderate AD. There is accumulating evidence that they also attenuate the progression of noncognitive symptoms in AD, such as agitation and aggression, and may serve to maintain activities of daily living. However, meta-analyses of randomized clinical trials relating to the use of AChE inhibitors indicate that these drugs provide only a modest benefit in AD (Doody et al, 2001; Kaduszkiewicz et al., 2005), and to date there is little evidence that these drugs have the capacity to alter the natural history of the disease.

AChE inhibitors are at present the only drugs licensed for the treatment of mild to moderate AD. However, other drugs are being developed that are designed to enhance the cholinergic system by alternative means. They include ACh agonists, m1 muscarinic receptor agonists, and stimulators of ACh release. There have been some reports that cigarette smoking may have a protective effect in AD, which may relate to the effect on nicotinic receptors, but these findings have yet to be fully substantiated. In addition, any potentially beneficial effect on cholinergic transmission is at least partly offset by the increased risk of cerebrovascular disease associated with smoking, which increases not only the risk of AD per se but also the risk of developing concomitant vascular dementia.

NMDA Receptor Antagonists

Enhancement of glutamate-mediated synaptic function has been implicated in the pathophysiology of several neurodegenerative disorders including AD, Huntington's disease, and Parkinson's disease. The aminoadamantane compounds amantadine and memantine have been found to confer neuroprotection by noncompetitive inhibition of glutamate activity at NMDA receptor. The low affinity of both compounds for the NMDA receptor and their fast voltage-dependent channel unblocking kinetics have been cited as the explanation for their low toxicity and good clinical tolerance. This contrasts with the neurotoxicity and psychotogenicity associated with compounds that are more potent NMDA receptor antagonists, such as MK-801 and phencyclidine. A clinical trial involving the use of memantine in patients with severe AD has demonstrated a good safety profile and clinical improvement as measured using cognitive and functional assessment scores (Winblad and Poritis, 1999). As a consequence, memantine has been licensed for use in patients with moderately severe to severe AD.

Antiamyloid Immunotherapy

PDAPP mice immunized with Aβ_{1-42} at 6 weeks of age, prior to the development of pathological damage, developed anti-Aβ_{1-42} antibodies and were observed to have significant reductions in amyloid deposition and plaque formation (Fig. 16–9) (Schenk et al., 1999). Injections of Aβ_{1-42} into older mice, in whom AD pathological changes were established, resulted in

Figure 16–9. Hippocampal $A\beta$ deposition, neuritic plaque formation, and astrocytosis in 13-month-old mice injected with phosphate-buffered saline (PBS) and $A\beta_{42}$. There is marked $A\beta$ deposition in the outer molecular layer of the dentate gyrus of the PBS-injected mice (*a*), which contrasts with the absence of $A\beta$ observed in the $A\beta_{42}$-injected mice (*b*). Dystrophic neurites labeled with the APP-specific monoclonal antibody 8ES were found in the hippocampal sections of the PBS-injected (*c*) but not the $A\beta_{42}$-injected mice (*d*). Plaque-associated astrocytosis is abundant in the retrosplenial cortex of the PBS-injected (*e*) but not the $A\beta_{42}$-injected (*f*) mice. (*Source*: Schenk et al., 1999, with the permission of Dr. Dale Schenk and Nature Publishing Group.)

reduced neuritic plaque burden and associated neuritic dystrophy and gliosis compared with nonimmunized mice. The pattern of amyloid pathology in the hippocampus was strikingly different in the treated mice, with an absence of diffuse deposits and an altered pattern of $A\beta$ immunoreactivity.

Transgenic mice expressing the mutant human APP_{695} transgene that were vaccinated with the $A\beta_{1-42}$ peptide exhibited a reduction of impairment when tested ozn a reference memory version of the watermaze task over a number of weeks subsequent to immunization (Janus et al., 2000). However, immunized transgenic mice remained impaired compared with nontransgenic control mice. As with the PDAPP mice, $A\beta_{1-42}$ immunization was associated with reduced amyloid plaque density when compared with nonimmunized mice.

Although initial safety trials of antiamyloid immunization indicated that the treatment could be used in human subjects, a subsequent Phase II safety trial was terminated following the development of subacute meningoencephalitis in 5% of the study population. Interestingly, a pathological study of the brain of the first immunized patient to come to postmortem (Nicoll et al., 2003) revealed large areas of cerebral cortex containing very few amyloid plaques and plaque-associated dystrophic neurites, although quantities of NFTs and neuropil threads were comparable to those observed in nonimmunized cases. Microglial cells were also found to be associated with immunoreactivity to Aβ, indicating that the immunization had been successful in generating an appropriate immune response, which in turn resulted in clearance of amyloid plaques.

Secretase Inhibitors

Whereas the vaccination approach is designed to clear amyloid from the brain, an alternative approach is to prevent formation of amyloid plaques by interfering with the production of $A\beta_{1-42}$ from its precursor protein. With this aim in mind, attention has been focused on the discovery of potential inhibitors of the β- and γ-secretase enzymes that cleave APP to form $A\beta_{1-42}$. Despite extensive animal studies, at present no secretase inhibitor has been put forward openly for clinical trials.

Other Therapeutic Options

Information derived from epidemiological observations and from research into factors influencing the development of AD pathological changes have stimulated trials with a number of treatments with potentially disease-modifying effects. Following the initial report in 1990 of a reduced incidence of AD in patients with arthritis taking nonsteroidal antiinflammatory drugs (NSAIDs), a number of other reports have drawn attention to the apparent protective effect of long-term NSAID use (in t'Veld et al, 2001). The underlying mechanism for this neuroprotection has not been established, but the observation that amyloid plaques are surrounded by immune cells (e.g., microglia) suggests that NSAIDs may serve to reduce the degree of immune-mediated neuronal destruction. An alternative explanation relates to the suppression by NSAIDs of oxygen free radical-mediated cellular damage. Finally, there may also be a direct effect on the key underlying pathological processes in AD; some NSAIDs have been demonstrated to suppress $A\beta_{1-42}$ formation. Although the epidemiological data are convincing, conclusions arising from clinical trials assessing the value of these drugs in AD must be tempered by the known profile of adverse effects associated with these drugs, particularly the risk of peptic ulceration and gastrointestinal bleeding.

Other long-term studies have documented the beneficial effects of compounds with antioxidant properties; most prominent among them are gingko biloba and vitamins C and E. Preliminary studies have indicated that all three preparations may delay the progression of AD. Similar benefits have been shown for estrogen preparations, but progress in clinical trials has been delayed following the demonstration of an increased risk of breast cancer, stroke, and myocardial infarction in a large study using hormone replacement therapy (Rapp et al, 2003).

The observation that individuals with high serum cholesterol levels are more likely to develop AD, coupled with the results from some studies suggesting that cholesterol promotes the formation of β amyloid, has led to clinical trials involving the use of statins (cholesterol-lowering agents) in patients with mild AD. The first published reports have indicated that patients on statin therapy experience a small, but not significant, reduction in the rate of cognitive decline over a 6-month period.

Certain metals have been implicated in the AD disease process. Zinc and copper cations play a critical role in the aggregation of β amyloid; in addition, the combination of these cations with β amyloid results in oxidative damage as a consequence of the generation of hydrogen peroxide. Clinical trials involving the use of metal chelators (agents that bind to metal ions) are currently under consideration (Scarpini et al, 2003).

Ultimately, any drug with true disease-modifying potential must fulfill a number of core criteria. First, a reduction in the rate of clinical decline must be demonstrated over several years, in view of the duration of the disease. Second, treatment benefits would have to be observed beyond the period of dosing to exclude the possibility of a symptomatic effect only. Ideally, benefits would be noted not only in memory and other cognitive functions but also in terms of more global measures such as activities of daily living. Finally, it would be desirable to supplement these clinical improvements with some evidence of attenuation of disease progression. In the absence of the ability to monitor directly the effect of treatments on the pathological brain changes, various surrogate markers of disease progression have been proposed, including structural (regional and global cerebral atrophy) and functional (cerebral hypometabolism) markers. Significant, consistent alterations in the longitudinal measurement of these surrogate markers of disease, observed over meaningful time spans, would provide convincing evidence of disease modification.

The discovery of multiple potential treatment avenues has markedly altered the clinical approach to AD. The anticipated development of genetic models of disease that more accurately reflect the core aspects of the AD disease process in conjunction with advances in our understanding of the pathophysiology of the disease are likely to give rise in the future to treatments that have the potential not only for effecting symptomatic alleviation but also for slowing down—perhaps even arresting—the pathological progression of Alzheimer's disease.

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