bridization was species independent; Scr-1 cDNA from the mouse library was hybridized to sections of brain from an infected hamster (Fig. 4a). Most of the labeled cells appeared to be neurons, as judged by their morphology and anatomic distribution. The Scr-1 probe also hybridized preferentially to tissue sections of brain from three of three patients with neuropathologically confirmed Alzheimer's disease. In Alzheimer's disease, hybridization again was focal—in the cerebral cortex (Fig. 4c); but, in contrast to scrapie, the Scr-1 probe was localized in cellular processes rather than in cell bodies. By staining sections with thioflavine S after in situ hybridization to locate neuritic plaques and tangles, we found that the foci in cerebral cortex correspond to neuritic plaques that occur in the central nervous system of aging humans and with increased frequency in those with Alzheimer's disease [reviewed in (*20*)]. There was no increase in annealing of the Scr-1 probe to the neurofibrillary tangles of Alzheimer's disease or to tissues lacking senile plaques from three control patients (Fig. 4d). The Scr-1 probe also hybridized to relatively rare senile plaques in the brain tissues of three patients with multi-infarct dementia, Pick's dementia, and multiple sclerosis. The annealing of Scr-1 to tissues was specific, since pretreatment with ribonuclease reduced hybridization by 75 to 90 percent; and heterologous probes, as controls for sequence specificity (to measles virus, herpes simplex virus, and cytomegalovirus) hybridized at levels comparable to those of uninfected hamster and schizophrenia controls.

These findings provide evidence that the scrapie agent induces increased expression of at least one mRNA that has a number of intriguing properties as a possible marker for infection. This mRNA is expressed in brain neurons of two rodent species and is increased in some brain areas with the focal pattern of gene expression observed in other slow and persistent infections (*21*). Reduced hybridization after ribonuclease treatment was not complete. This partial resistance to ribonucleases is unusual; it has not been observed in conventional virus infections (*21*) but is a property of interferon mRNA in situ (*22*) and may prove to be of interest in view of the nuclease resistance of scrapie infectivity (*1*). These observations suggest further that increased expression of some cellular RNA's may be common to infection by unconventional agents and to degenerative changes in the aging human brain best exemplified by Alzheimer's disease. The experimental strategy described in this report will be useful in obtaining markers for degenerative human diseases and in selecting probes to investigate the pathogenesis of these conditions.

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Plasticity of Hippocampal Circuitry in Alzheimer's Disease

Abstract. *Two markers of neuronal plasticity were used to compare the response of the human central nervous system to neuronal loss resulting from Alzheimer's disease with the response of rats to a similar neuronal loss induced by lesions. In rats that had received lesions of the entorhinal cortex, axon sprouting of commissural and associational fibers into the denervated molecular layer of the dentate gyrus was paralleled by a spread in the distribution of tritiated kainic acid–binding sites. A similar expansion of kainic acid receptor distribution was observed in hippocampal samples obtained postmortem from patients with Alzheimer's disease. An enhancement of acetylcholinesterase activity in the dentate gyrus molecular layer, indicative of septal afferent sprouting, was also observed in those patients with a minimal loss of cholinergic neurons. These results are evidence that the central nervous system is capable of a plastic response in Alzheimer's disease. Adaptive growth responses occur along with the degenerative events.*

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Several neurological disorders, including Parkinson's, Huntington's, and Alzheimer's diseases, are associated with neuronal loss in specific brain regions (*1*). From animal studies it is well known that the central nervous system (CNS) can modify its neuronal circuitry in response to injury-induced denervation. When one set of axons is lost, the remaining afferents can sprout and replace the lost connections to maintain synaptic density (*2*). It is uncertain, however, whether plastic responses occur in the human brain after neuronal loss induced by aging, injury, or disease.

Recently, severe loss of cells was observed in the entorhinal cortex and subiculum of patients with Alzheimer's disease (AD); this cell loss was suggested to underlie some of the memory deficits in the disease (*3*). The perforant path, arising from the entorhinal cortex, is the major cortical input to the hippocampus, terminating on the granule cell dendrites in the outer two-thirds of the dentate gyrus molecular layer and on the distal portions of pyramidal cell dendrites of the hippocampus and subiculum (*4*). In response to the loss of entorhinal input in rodents, the dentate molecular layer un-

dergoes a major reorganization of its inputs. After clearing of the degenerating nerve terminals, the remaining afferent fibers, an extrinsic afferent from the septum and two intrinsic afferents, the commissural and associational systems, sprout to form new synapses with the denervated target cells. The commissural and associational (CA) fibers, originating in the contralateral and ipsilateral dentate hilus, respectively, normally terminate in the inner third of the molecular layer adjacent to the zone of entorhinal innervation. After an entorhinal lesion, this zone expands until it occupies the inner half of the molecular layer (*5*). Sprouting of the septal input is concentrated in the outer half of the molecular layer (*6*).

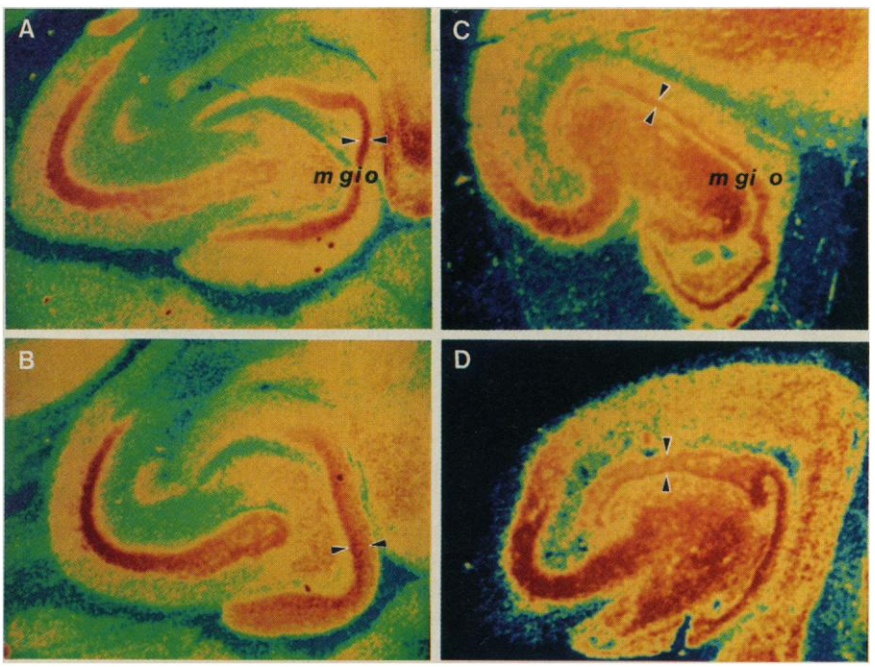

Fig. 1. Color-enhanced autoradiograms of [^{3}H]KA binding to human and rodent hippocampal sections. Increasingly higher binding-site densities are indicated by blue, green, yellow, and red. (A) Control rat hippocampus. (B) Rat hippocampus after an entorhinal cortex lesion. (C) Hippocampus from a 73-year-old male control patient. (D) Hippocampus from a 69-year-old male patient with AD. Abbreviations: g, granule cell layer of the dentate gyrus; m, mossy fibers; i, inner portion of the dentate gyrus molecular layer corresponding to the CA system terminal zone; o, outer portion of the molecular layer corresponding to the perforant path terminal zone. Arrows indicate the region of high-density KA-binding sites corresponding to the CA terminal zone. Expansion of this region is visible in (B) and (D).

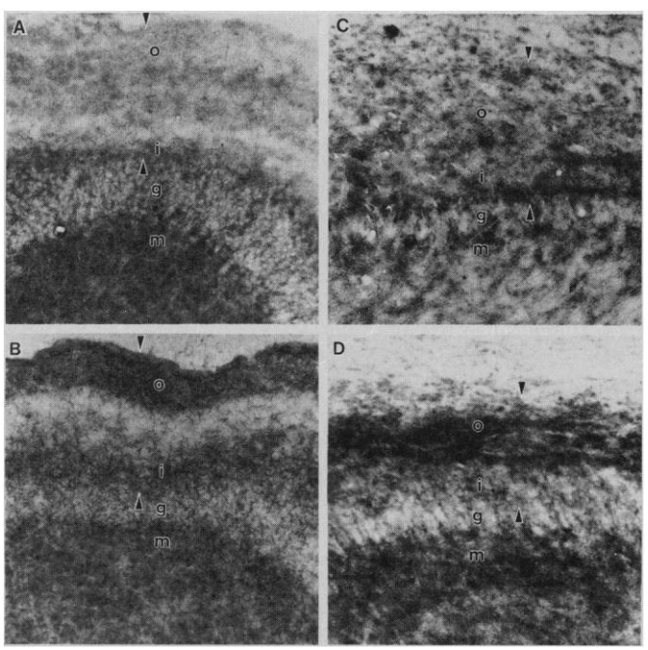

Fig. 2. Acetylcholinesterase staining of the dentate gyrus molecular layer in rodent and human hippocampal sections. (A) Control rat hippocampus. (B) Rat hippocampus after an entorhinal cortex lesion. (C) Hippocampus from a 73-year-old male control patient. (D) Hippocampus from a 71-year-old AD patient. Abbreviations: g, granule cell layer of the dentate gyrus; m, mossy fibers; i, inner portion of the dentate gyrus molecular layer; and o, outer portion of the dentate gyrus molecular layer. Arrows indicate the total width of the molecular layer. Intensification of AChE activity in the outer molecular layer, indicative of sprouting of the cholinergic septal input, is visible in (B) and (D).

A high density of the kainic acid (KA) subtype of excitatory amino acid receptors is restricted to the inner third of the dentate molecular layer, corresponding to the terminal zone of the CA system (*7*). We used [vinylidene-^{3}H]KA (60 Ci/mmol; New England Nuclear) and in vitro autoradiography to determine whether KA receptors redistribute to parallel the expansion of the CA system after an entorhinal cortex lesion in rats and in response to the neuronal loss in the entorhinal cortex in patients with AD. Acetylcholinesterase (AChE) activity was used as a marker of the cholinergic septal input. Our results indicate that the lesion-induced expansion of the CA system was accompanied by an expanded distribution of KA receptors in rats and that a similar expansion occurred in the hippocampus in patients with AD. Furthermore, in AD patients who did not have a severe loss of cholinergic input to the hippocampus, intensified AChE activity was observed in the outer molecular layer.

Male Sprague-Dawley rats (150 to 200 g) received unilateral knife-cut lesions of the angular bundle (*8*). Thirty days after the operation the rats were decapitated and the brains were removed rapidly and frozen in powdered dry ice. Human brain samples were obtained postmortem from patients with clinically and neuropathologically defined AD and from nondemented, age-matched control patients (*9*). The autoradiographic procedure was similar to that described previously (*10*). Adjacent sections were stained for AChE activity and for Nissl substance (*8*).

In Sprague-Dawley rats, the pattern of KA binding in the dentate gyrus molecular layer on the control side (contralateral to the lesion) was similar to that previously observed in untreated animals (*7*). A distinct band of high-density KA-binding sites, measuring 70 ± 6 μm (mean ± standard error; n = 3) in coronal sections, occupied the inner third of the molecular layer (the CA system terminal zone) (Fig. 1A). On the deafferented side (ipsilateral to the lesion), the region of high-density KA-binding sites had expanded to 110 ± 6 μm (a 57 percent increase) (Fig. 1B), such that is now occupied approximately 40 percent of the molecular layer. This correlates with the magnitude of the CA afferent fiber

sprouting (*5*). These results provide anatomical evidence that receptor induction is correlated with afferent sprouting.

The overall KA-binding pattern in hippocampal samples, from the control patients (*11*) was very similar to that observed in the normal rat brain. A region with a high density of KA-binding sites, measuring 150 ± 20 μm (n = 4), occupied the inner third of the molecular layer (Fig. 1C). In AD patients, this region had expanded to 260 ± 30 μm (n = 4) (a 73 percent increase), so that it now occupied over half of the molecular layer (Fig. 1D). These results suggest that the human brain is capable of neuronal plasticity in response to pathologically induced neuronal loss.

Sprouting of the cholinergic septal input to the outer dentate molecular layer in rats with entorhinal lesions can be measured by intensification of AChE activity in that zone (*6*). The use of this response as a marker for sprouting in AD is often precluded by the loss of cholinergic input to the hippocampus (*12*). However, we observed numerous AChE-positive plaques in the denervated dentate molecular layer, which is consistent with an aberrant sprouting response in this region. Furthermore, in those AD patients in which significant cholinergic input to the hippocampus was present, intensification of AChE activity was observed in the outer half of the dentate molecular layer (Fig. 2). These results indicate that cholinergic neurons in AD patients are also capable of a sprouting response.

It appears that the neuronal loss in the entorhinal cortex of AD patients acts as a stimulus in a manner similar to that of the lesion in the rat brain. The loss removes the perforant path input to the hippocampus and dentate gyrus, inducing a compensatory response from adjacent CA system afferents and from septal afferents, if present. The observed expansion of a receptor field and the increase in the activity of a transmitter-metabolizing enzyme are in marked contrast to the numerous reports of reductions in transmitter-related parameters in this disease (*13*).

Compensatory growth in the course of a degenerative disease indicates that the resultant circuitry cannot simply be considered as a loss of neural elements. The sprouting of septal and CA inputs may enhance the efficacy of remaining entorhinal inputs to compensate for cell loss, but also may paradoxically increase the vulnerability of hippocampal cells to excitotoxic activity and facilitate cell death. In rodent models the rearrangement of circuitry after entorhinal lesions has been shown to be behaviorally significant (*14*). The influence of neuronal plasticity on the behavior of AD patients is unknown.

Loss of neurons, whether occurring slowly in the course of a disease or rapidly through injury, produces a similar end result. The striking similarity between the human CNS response to AD-induced denervation and the rat CNS response to lesion-induced denervation suggests that rats with lesions of the entorhinal cortex may be a model for some aspects of AD. The model may provide fundamental anatomical and behavioral principles for examining the neural degeneration and rearrangement in this disease. Comparison of the characteristics of sprouting in the diseased and injured CNS may yield clues to the etiology of AD and suggest strategies for ameliorating lost function. Potential therapeutic approaches include manipulation of functional plasticity (*15*) and excitatory amino acid transmission (*16*).

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