of the fluorescing cell populations at 21°C, and 60 percent at 29°C.

The lectin binding properties of both immune reactive and nonreactive hemocytes were studied by shifting half of the *Tum*^{l} larvae parasitized by *L. heterotoma* to 29°C, a condition that ensured a high frequency of encapsulation responses, and maintaining the others at 21°C, a condition which resulted in a low frequency of encapsulated parasites. In immune competent hosts that successfully encapsulated *L. heterotoma*, the percentages of WGA binding hemocytes were significantly higher ($P < 0.001$; *t*-test) than in susceptible hosts that, at either 21° or 29°C, exhibited no encapsulation response (Fig. 5B). Parasite eggs removed from host larvae during the early stages of encapsulation (24 hours after infection) and treated with WGA showed approximately equal numbers of fluorescing and nonfluorescing cells in the outer layer of the developing capsule. In fully formed capsules (48 to 60 hours after infection) no WGA binding hemocytes were observed in the outer layer of the capsule. This suggests either that WGA binding hemocytes are not involved in the terminal stages of capsule formation or that the heavy deposits of melanin obscure their WGA binding activity.

Parasites can develop in an immune competent host if they are not recognized as foreign (for example, molecular mimicry or antigen sharing), or if they produce substances that suppress the host's immune system. Past studies have shown that *L. heterotoma* utilizes the latter mechanism and actively inhibits the blood cell encapsulation responses of *Drosophila* by preventing the differentiation of plasmatocytes to lamellocytes (*3*, *8*). It is believed that the substance which suppresses blood cell differentiation is introduced into the host by the female during oviposition. The low percentages of WGA binding hemocytes found in susceptible *Tum*^{l} hosts may reflect a decrease in the percentage of lamellocytes resulting from parasite suppression of the immune response. However, it is possible that, in addition to blocking the formation of lamellocytes, the parasite also suppresses or modifies those cell surface properties that characterize the hemocytes of immune reactive hosts.

In summary, our studies correlate high percentages of WGA binding hemocytes in circulation with encapsulation reactions that attend parasite encapsulation and melanotic tumor formation in the *Tum*^{l} mutant. The data suggest that the temperature-induced increase in the immune competence of *Tum*^{l} hemocytes at 29°C is due to certain cells acquiring specific surface modifications, identified by WGA binding, which augment their adhesion to one another and to foreign surfaces. These findings support the work of Rizki and Rizki who showed that the percentages of WGA binding hemocytes in other *Drosophila* mutants increased at tumor permissive conditions, and also when heterospecific implants were used to provoke encapsulation responses (*9*). They suggested that aberrant tissues stimulate the differentiation of WGA binding cells, which then adhere to one another and to the tissue surfaces.

Since WGA binds to specific carbohydrate moieties, this lectin does not identify all of the cell surface alterations that may occur on the same or on different immune reactive cells. Moreover, changes in cell surface carbohydrates that are identified by this lectin may be ancillary manifestations in the attainment of immune competence, and not directly responsible for potentiating the adhesion of capsule-forming hemocytes. The fact that many of the hemocytes participating in the development of cellular capsules in *Tum*^{l} do not bind to WGA suggests there are at least two populations of immune reactive cells. The immune competence of one group of hemocytes is manifested by cell surface features which have a high affinity for WGA, while the other, equally competent population lacks this property. It is also possible that cells that do not bind WGA participate in encapsulation reactions only by adhering to WGA binding cells, or to foreign surfaces that become coated with a product believed to be elaborated from the latter cells (*8*).

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Alzheimer's Disease: Cell-Specific Pathology Isolates the Hippocampal Formation

Abstract. *Examination of temporal lobe structures from Alzheimer patients reveals a specific cellular pattern of pathology of the subiculum of the hippocampal formation and layers II and IV of the entorhinal cortex. The affected cells are precisely those that interconnect the hippocampal formation with the association cortices, basal forebrain, thalamus, and hypothalamus, structures crucial to memory. This focal pattern of pathology isolates the hippocampal formation from much of its input and output and probably contributes to the memory disorder in Alzheimer patients.*

The role of the hippocampus and related structures of the ventromedial temporal lobe in learning and memory has been examined in both man and the experimental animal (*1*). Bilateral destruction of these areas is associated with a profound and lasting memory impairment that affects learning in all modalities (*2*).

Memory impairment is an early, prominent manifestation of Alzheimer's disease, and the hippocampal formation and parahippocampal gyrus are among the brain areas most consistently and heavily implicated in its pathology (*3*). Pathologic alterations (*4*) and neurochemical deficits (*5*) have also been observed in association cortices, as well as in subcortical structures such as the amygdala (*6*), locus coeruleus (*7*), and the cholinergic

neurons of the nucleus basalis of Meynert (*8*).

The degree to which these changes contribute to the memory impairment in Alzheimer's disease is unknown. Although several studies have focused on changes of the medial temporal lobe (*3*) (for instance, quantifying neurofibrillary tangles or senile plaques), they have not provided information on the distribution of the pathologic changes. In the higher primate much of the input and output pathways of the hippocampal formation is formed by axons that arise from specific and discrete cell populations, namely, the neurons of layers II, III, and IV of the entorhinal cortex and of the subiculum (Fig. 1). We have reexamined the distribution of hippocampal and parahippocampal pathology in the brains of Alzheimer patients and now report a specific pattern of cellular changes in the major projection neurons of the hippocampal formation, thus isolating it from the association cortices, basal forebrain, thalamus, and hypothalamus.

Serial sections (50 μm) from the left temporal lobe of five patients with senile dementia (Alzheimer's disease) (*9*) were studied (71, 76, 84, 85, and 98 years of age). Control materials were serial sections from the brains of five nondemented elderly patients (58, 70, 76, 76, and 83 years of age). Sections were stained with Nissl, Congo red, or hematoxylin-eosin (*10*).

The Nissl sections from the Alzheimer brains showed conspicuous alterations in the cellular architecture of both the hippocampal formation and the parahippocampal gyrus in all cases. In the subiculum and adjacent CA1 pyramids there was a marked cell loss creating a patchy cell-poor appearance. No alterations were observed in the adjacent presubicular and parasubicular cortices or in other pyramidal cell fields of the hippocampus. In the entorhinal cortex, there was a nearly total loss of the large clusters of layer II stellate cells that characterize this cortex, and a decrease in density in the superficial parts of layer III and the large multipolar neurons of layer IV. The deeper parts of layer III and layers V and VI were unaffected. These alterations were not observed in the brains of nondemented controls. Even in the brain of the 83-year-old control, the characteristic normal cellular architecture of the subiculum and entorhinal cortex was well preserved.

There was a close correlation between areas showing a disruption of cellular architecture with the Nissl stain and areas showing selective staining of neurofibrillary tangles with Congo red (*10*) in all Alzheimer brains. Large numbers of neurofibrillary tangles were seen in the subiculum and hippocampal CA1 field (Fig. 2A), whereas the adjoining parts of the hippocampal formation such as the presubiculum and CA3 field contained only an occasional tangle. A striking laminar predilection was obvious in the entorhinal cortex (Fig. 2B). In this area, neurons of layer II and layer IV were involved. In all Alzheimer brains, the cells of layer II were affected heavily, while the involvement of cells of layer IV varied. Some neurons in the superficial parts of layer III also contained tangles, but its deeper parts and layers V and VI were always largely unaffected. The control brains showed birefringence only in blood vessels and in occasional cells in scattered locations, and no systematic laminar or structural pattern was ever observed.

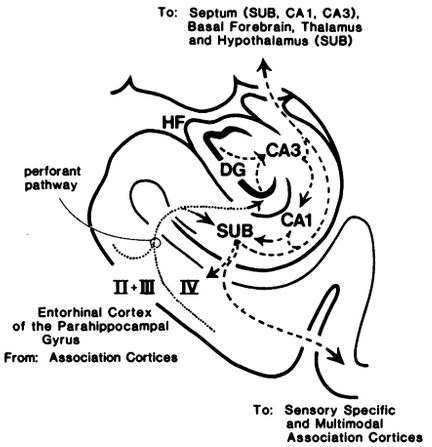

Fig. 1. Connections of the hippocampal formation in the higher primate. Cortical input to the hippocampus is from the projection neurons of layers II and III of the entorhinal cortex, while hippocampal output to the cortex is from projection neurons of the subiculum. CA, cornu ammonis; DG, dentate gyrus; HF, hippocampal fissure; SUB, subiculum.

The foregoing changes are significant when considered in relation to higher primate hippocampal connectivity. Layers II and III of the entorhinal cortex receive cortical input from several sensory association and limbic cortices, and, in turn, give rise to the perforant pathway (*11*), the major cortical afferent source for the hippocampus and dentate gyrus (*12*). Cellular damage in these specific cortical layers would effectively disconnect the cerebral cortex from the hippocampus with regard to its cortical input. In contrast, the subiculum and CA1 zones of the hippocampal formation receive the output of intrinsic hippocampal circuits and are the major sources of hippocampal output to the thalamus, hypothalamus, basal forebrain, amygdala, and cerebral cortex (*13*). Pathology in Alzheimer's disease thereby deprives these structures of hippocampal output. The subiculum also projects strongly to

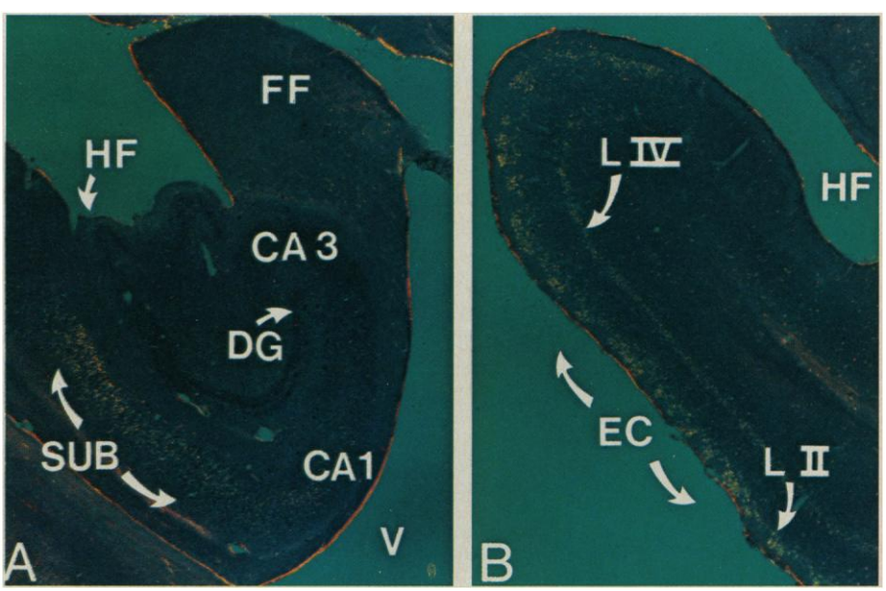

Fig. 2. Distribution of neurofibrillary tangles in the hippocampal formation in Alzheimer's disease. Congo red–stained sections from the brain of a 71-year-old patient with a 6-year history of Alzheimer's disease, viewed under cross-polarized light. Neurofibrillary tangles assume a brilliant yellow-green appearance against a dark background. (A) The subicular CA1 fields contain numerous neurofibrillary tangles that are, by comparison, sparse in adjacent CA3 and presubicular fields. (B) Neurofibrillary tangles are present in a laminar distribution in the entorhinal cortex involving the characteristic clusters of neurons in layer II and the large multipolar neurons of layer IV. CA, cornu ammonis; DG, dentate gyrus; EC, entorhinal cortex; FF, fimbria-fornix; HF, hippocampal fissure; L II and L IV, layer II and layer IV; SUB, subiculum; V, ventricle.

entorhinal cortex layer IV, which projects to the basal forebrain and cortex (*14*).

In conclusion, our findings reveal a remarkably specific cellular pattern of pathology in Alzheimer patients. By giving rise to the major cortical input to the hippocampal formation, the cells affected in the entorhinal cortex constitute the major gateway of information from the association and limbic cortices. The cells damaged in the subiculum and CA1 field are equally critical since they are the major recipients of hippocampal output, and this constitutes the gateway of hippocampal influence on various parts of the neuraxis, such as the association cortices, the amygdala, the basal forebrain, and the diencephalon. Cellular damage to these key projection neurons of hippocampal circuitry isolates the hippocampal formation by disconnecting major input and output pathways, and it is difficult to conceive that the hippocampal formation in the brains of Alzheimer patients is functionally useful. This isolation of the hippocampal formation may be no less devastating (*15*) with regard to memory than removal or destruction of the entire structure, and contribute to the contextual memory defect that is a major component of the amnesia in Alzheimer's disease.

Our results are of further interest when viewed in relation to the cholinergic deficiency (*5*) in the cortex of Alzheimer patients and to reports of diminished cell density in the cholinergic neurons of the nucleus basalis of Meynert, both of which have been linked with memory impairments (*16, 17*). The basal forebrain has strong connections with medial temporal lobe structures, directly with neurons of the subiculum and layer IV of the entorhinal cortex (*14*) or indirectly via the amygdala (*18*).

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11. The perforant pathway arises from layers II and III of the entorhinal cortex, and courses through the subiculum. It terminates along the outer dendritic branches of the subicular and hippocampal pyramids, and on the outer two-thirds of the dendritic field of dentate gyrus granule cells. It initiates a multisynaptic excitatory sequence of intrinsic hippocampal circuits that terminate in the CA1 field and the subiculum.
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Insulin-Mediated Regulation of Neuronal Maturation

Abstract. *Exposure to insulin increased stimulus-evoked transmission at synapses formed in culture by cholinergic retinal neurons derived from fetal rats. This effect occurred at physiological concentrations and was long lasting. The findings support the hypothesis that insulin may serve as a developmental signal to regulate the emergence of effective neurotransmission across nascent synapses.*

Insulin and its receptors are found within the central nervous system (*1*). Although the significance of this discovery is unclear, one possibility is that insulin may play a role in brain development. Consistent with this possibility is the detection of insulin-like immunoreactivity, insulin receptors, and insulin-mediated effects on macromolecular synthesis in the fetal nervous system (*2*). However, a functional role for insulin in neuronal maturation has not been identified. Using a cell culture system, we examined the effect of insulin on the developmental step in which a presynaptic neuron becomes capable of information transfer. We found that insulin could precociously induce in cholinergic neurons derived from fetal rat retina the capability of releasing acetylcholine at synapses in response to an excitatory stimulus.

The cell culture system we used consisted of retinal and muscle cells (*3–4*). Cholinergic neurons dissociated from perinatal rat retinas rapidly form functional synapses in culture with rat striated muscle cells (*3*). Muscle cells are useful in studies of postsynaptic responses of cholinergic neurons because the membranes of muscle cells have areas with a high density of cholinergic receptors and because their physiological response to acetylcholine has been extensively studied in vivo and in culture (*5*). In addition, their relatively large size permits prolonged intracellular monitoring of postsynaptic responses.

The formation of functional retina-muscle synapses undergoes a sequence of developmental steps (*3, 4, 6*). Early in the maturational process, there is a period in which the neuronal release of acetylcholine occurs spontaneously but cannot be evoked by stimuli such as potassium or glutamate, a putative excitatory neurotransmitter. This "nontransmitting" phase is followed by the emer-