could alter the excitability of layer 4 neurons either by direct synaptic inputs or by affecting NMDA receptors in layer 4. Examining the effect of subplate neurons on synaptic responses in the visual cortex should lend insight into how these neurons influence geniculocortical interactions during the critical period.


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19. The projection patterns of LGN axons in animals injected with kainic acid into the white matter were examined at P47 (two animals), P48 (one animal), P55 (three animals), P60 (one animal), P70 (one animal), and P100 (one animal). The transneuronal pattern of labeling in visual cortex after an ipsilateral eye injection (2 mCi of [$^3$H]proline in 45 μl of 0.9% saline) was revealed by autoradiography (*1*). In one animal (injected with kainic acid on P5) the pattern of LGN axon terminals in primary visual cortex was revealed by transneuronal transport of wheat germ agglutinin–horseradish peroxidase (WGA-HRP) (*20*).
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21. The lack of segregation of geniculocortical afferents within layer 4 in the subplate-lesioned animals is probably not a direct effect of the kainic acid on LGN axons because injections of kainic acid directly into layer 4 (two animals) or layer 1 (one animal) did not prevent segregation. Nor is the failure of column formation due to a reorganization of the retinogeniculate projection, as retinal ganglion cell axons are restricted to the appropriate eye-specific layers throughout the LGN in kainic acid–injected animals.
22. Kainic acid injections into the white matter in neonates produce long-term histological changes in layer 4. By several months after injection, many of the small stellate cells are missing from layer 4. Because injection of kainic acid into the cortical plate during the first postnatal week has no effect on cortical organization, the subsequent changes in cortical histology are likely to be secondary to changes (such as altered geniculocortical interactions) caused by the absence of subplate neurons.
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# Technical Comments

## Age-Associated Inclusions in Normal and Transgenic Mouse Brain

Two research groups have used transgenic techniques in attempts to induce cerebral amyloidogenesis in mice (*1, 2*). In (*1*), the transgene construct included the human amyloid precursor protein (APP) promoter and sequence encoding the amyloid β protein (βAP). After 6 months of age, these transgenic mice developed clusters of βAP-positive granules primarily in the hippocampus. Ultrastructurally, these lesions consisted of fibrillar material, perhaps within dendrites (*1*). In recent immunohistochemical studies of a laminin-binding protein (LBP) in brain (*3*), we noted that inbred C57BL/6J mice exhibited abnormal clusters of LBP-like immunoreactive granules that were similar in appearance and distribution to the βAP-positive granules in βAP-transgenic mice (*1*). Seen with a light microscope, they resemble periodic acid–Schiff (PAS)–positive particles that have been described in hippocampus of aged C57BL/6 mice and aged nude mice (*4*).

In normal adult C57BL/6 mice, the clusters predominated in the hippocampus (Fig. 1A) and were located mainly in the stratum lacunosum moleculare of region CA1 and in the molecular layer of the dentate gyrus, although there was some variability in distribution among individuals. The clusters were also present in piriform cortex and cerebellum and were occasionally observed in the diencephalon, striatum, and amygdala. In adult mice, clusters usually ranged from 30 to 60 μm in diameter and consisted of 40 to 100 granules (Fig. 1A). Each granule was typically between 1 to 3 μm in diameter, although larger granules appeared with increasing frequency in older mice.

In addition to having LBP-like immunoreactivity, the granules appeared to be immunoreactive with polyclonal antisera directed against βAP, somatostatin, and neurotensin (*5*). However, when appropriate synthetic peptides were used to preabsorb the antibodies, staining of granules was not blocked even though staining of normally immunoreactive elements (such as somatostatin- and neurotensin-containing neurons) was eliminated, as was staining of βAP in concurrently processed brain tissue from individuals with Alzheimer's disease (Fig. 2, A, B, and C). Affinity purification of the antibody to LBP reduced immunoreactivity of the granules, while it enhanced the typical pattern of LBP-immunoreactivity in brain, which suggests that LBP epitopes also are not expressed in these clusters. Moreover, occasional batches of normal rabbit sera and control ascites fluid stained the granules. The granules also stained with monoclonal antibodies to synaptophysin and to laminin B2 chain, and a small percentage stained for phosphorylated neurofilaments (*5*), but we did not perform preabsorption controls with these antibodies. The granules did not stain with monoclonal antibodies that recognize βAP, APP, microtubule-associated protein (MAP2), and calbindin D28, nor

were they visualized with polyclonal antisera to glial fibrillary acidic protein (GFAP) and to vasoactive intestinal peptide (VIP) (*5*). The granules were not birefringent after staining with Congo Red, but some of them fluoresced after staining with thioflavin-S. The lesions were positive for PAS and Gomori's methenamine silver stain [Grocott's modification (*6*)], but were not detectable with hematoxylin and eosin, cresyl violet, thionin, Luxol fast blue, Bodian's Protargol, or acetylcholinesterase stains.

Combined immunocytochemistry for LBP and GFAP, as studied with a light microscope, suggested that these abnormal clusters consisted of intracytoplasmic inclusions that were localized primarily in astrocytic processes (Fig. 2D). Some of the inclusions were arranged around blood vessels in a pattern that suggested astrocytic endfeet. Ultrastructural examination of thin sections adjacent to immunostained semi-thin sections (7) disclosed that the granules were composed of unusual intracellular fibrillar material (Fig. 3A). In one instance, this material was found within an astrocytic soma (Fig. 3, B and C), and abnormal fibrillar accumulations were often located within processes that appeared to be astrocytic in origin. Synaptic contacts were not present on the membranes surrounding the inclusions. The fibrillar material excluded nearly all normal cellular organelles, including glial filaments. Amyloid-like fibrils were not observed in or near the granules. Immunoelectron microscopic analysis that used antibodies recognizing somatostatin revealed fibrillar inclusions but also many unlabeled and only peripherally labeled granules, which suggests that more inclusions might exist within a cluster than are seen with a light microscope.

Clusters were present in adult (7 to 9 months old) C57BL/6 mice of both sexes from two suppliers (*8*) and were more prevalent in female than in male retired breeders of the C57BL/6 strain. Clusters were more prevalent in mice supplied by Jackson Laboratory (C57BL/6J) than in those supplied by Charles River Laboratories (C57BL/6NCrlBR). No comparable clusters were found in adult BALB/c and C3H/He mice (*8*) (Fig. 1B). However, in C3H/He mice similar lesions were occasionally found in hippocampus, mostly among CA2 pyramidal neurons.

A clear age-related increase in the number of clusters was found in hippocampus of male C57BL/6 mice (*8*) (Fig. 4). Distinct lesions were virtually absent in these mice up to 6 months of age, but increased markedly thereafter. Although 24- to 31-month-old mice did not show more clusters compared to 18-month-old mice as a group, there was greater variability in the number of clusters in the oldest group compared to 18-month-old mice. The individual with the greatest number of clusters was 30 months of age, and larger granules (>3 μm) occurred more frequently in older animals. BALB/cByJ mice (*8*) showed no clusters at 2 months of age, and only a few small clusters were detected in the hippocampus of 21-month-old BALB/cByJ mice.

The inclusions in C57BL/6 mice do not resemble the extracellular amyloid deposits that have been reported in transgenic mice that overexpress the entire APP-751 transgene (*2*). They also do not resemble glial inclusions in human brain, as they differ in size, conventional staining properties, ultrastructural appearance, and anatomical distribution from corpora amylacea, Rosenthal fibers, and other glial inclusions (*9*). Staining properties of the murine inclusions suggest the presence of glycosaminoglycans.

The βAP-transgenic mice that develop similar granular clusters after 6 months of age have a C57BL/6 genetic background (*1, 10*). If these clusters in transgenic and C57BL/6 mice are identical, the background strains of transgenic hybrids may be the major determinant of the presence of inclusions. Our data indicate that the naturally

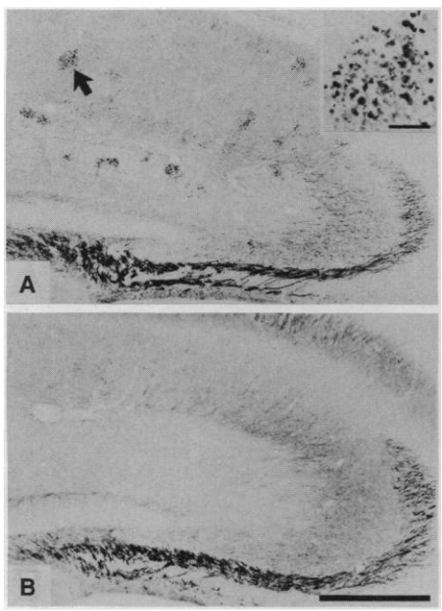


**Fig. 1.** Light micrograph of LBP-like immunoreactivity in 25-μm-thick sections through the dorsal hippocampus of (**A**) a normal 9-month-old female C57BL/6 mouse and (**B**) a normal 9-month-old female BALB/c mouse. Inset in (A) shows a cluster (arrow) at higher magnification. Scale bars: Inset (A) 20 μm; (B) 300 μm.

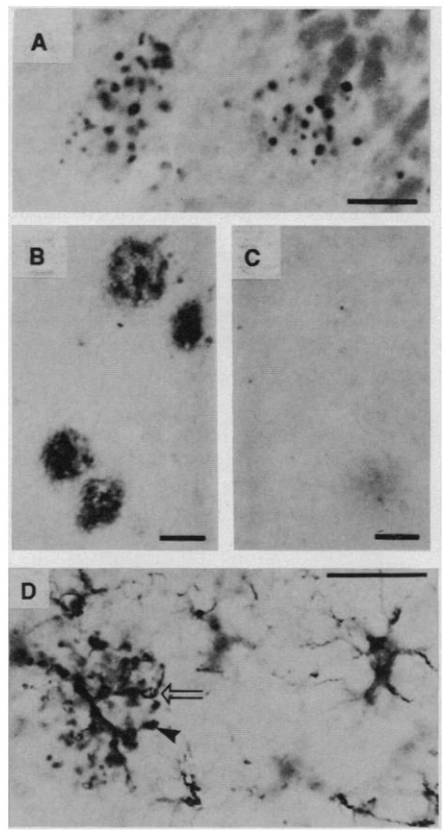


**Fig. 2.** Immunostained tissue from the dorsal hippocampus of mice and an individual with Alzheimer's disease. (**A**) Immunostaining of normal adult C57BL/6 mouse tissue with a polyclonal antibody to βAP that had been preabsorbed with a synthetic peptide of residues 1–28 of βAP [βAP (1–28)]. (**B**) Human tissue from an individual with Alzheimer's disease stained with βAP antiserum. (**C**) Human tissue from the same individual with Alzheimer's disease stained with βAP antiserum that had been preabsorbed with βAP (1–28). (**D**) Immunostaining of dorsal hippocampal tissue from normal adult C57BL/6 mouse with antibodies to LBP and to GFAP. Antibody to GFAP alone did not stain the granules themselves. Immunoreactive astrocytic inclusion (arrowhead); centrally unstained inclusion (open arrow). Scale bars: (A) 20 μm; (B) and (C) 50 μm; (D) 30 μm.

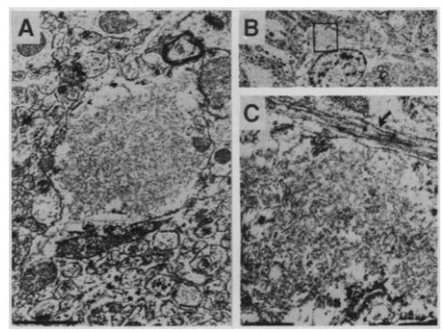


**Fig. 3.** (**A**) Electron micrograph of a granule from a C57BL/6 mouse showing unusual fibrillar material largely free of normal cellular organelles. (**B** and **C**) Similar fibrillar material found in an astrocytic soma. Boxed area in (B) is shown at higher magnification in (C). Arrow in (C) points to glial filaments that bypass fibrillar material. Scale bars: (A) 1 μm; (C) 0.5 μm.

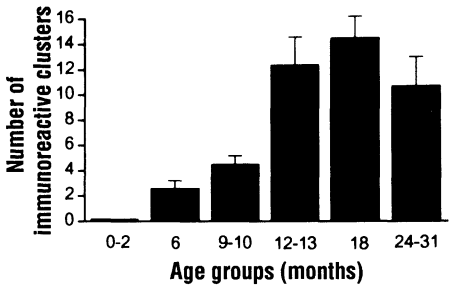


**Fig. 4.** Age-related increase in number of inclusions in brains of male C57BL/6 mice. LBP-like immunoreactive clusters were quantified in 25-μm coronal sections through the dorsal hippocampus. For each animal, the number of clusters per unilateral hippocampus was determined in several sections and subsequently averaged. Numbers of mice were 6, 5, 4, 9, 9, and 17 at ages 0 to 2, 6, 9 to 10, 12 to 13, 18, and 24 to 31 months, respectively. Error bars represent SEM; ANOVA for age effect, $F(5,44) = 5.26$, $P < 0.001$.

occurring lesions in C57BL/6 mice do not contain amyloid. Even though the βAP-transgene is expressed in low amounts (*1*), it may influence the development or composition of these abnormal inclusions.

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7. In preparation for electron microscopy, mice were perfused as described (*5*) or with 4% paraformaldehyde, 0.1% glutaraldehyde, and 15% saturated picric acid. Some sections were stained with antibodies to LBP or somatostatin, after which immunoreactive clusters were excised for electron microscopy. In other sections, blocks of tissue were removed without prior immunostaining. The samples were dehydrated, embedded in Epon, trimmed, and sectioned. Thick sections (adjacent to thin sections) were deplasticized in 4% sodium ethoxide and immunostained for LBP to localize clusters of granules, which were then studied in adjacent thin sections with a Hitachi H-600 electron microscope.
8. For strain comparisons, mice (7 to 9 months of age, retired breeders) were supplied by Jackson Laboratory (Bar Harbor, ME) and Charles River Laboratories (Wilmington, MA). To study the age-related appearance of the lesions, C57BL/6J mice were obtained from Jackson Laboratory at weaning and aged at the Gerontology Research Center, National Institute on Aging, National Institutes of Health. Young and old BALB/cByJ mice were obtained directly from Jackson Laboratory.
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We reported (*1*) the presence of amyloid-like deposits in the hippocampus of transgenic mice that harbor a gene encoding the 42–amino acid amyloid fragment of the amyloid precursor protein. At the time of publication, transgene-positive mice showed the phenotype, while transgene-negative control mice did not. It was brought to our attention by D. L. Price that similar deposits had recently been identified in nontransgenic C57BL/6 mice. Because of the possibility of an endogenous genetic contribution to the described phenotype, we extended our analysis to additional, and older, transgene-negative littermates. We found that a number of them also had amyloid-like structures in the hippocampus. It thus appears that the two lines of transgenic mice described in our paper represent sub-strains prone to formation of these structures. Mice from other lines of transgenics with the same genetic background (B6D2F1 × ICR) appear to have these deposits much less frequently.

This new information raises questions about the extent to which the transgene contributed to the amyloid-like phenotype in these mice. Work is under way to show whether a transgene-dependent phenotype can be induced in other mouse strains that are not predisposed to this phenomenon. Until then, data in (*1*) cannot be used to support the conclusion that a β/A4 transgene causes formation of amyloid-like deposits in mouse brain.

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