Immunohistochemical Characterization of Alterations in the Distribution of Amyloid Precursor Proteins and β-Amyloid Peptide after Experimental Brain Injury in the Rat

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Recent reports suggest a relationship between traumatic brain injury and the precocious development of neurodegenerative cascades, including diffuse deposits of β-amyloid peptides (Aβ) in the injured brain. Because the lateral fluid-percussion (FP) model of experimental brain injury produces clinically relevant neuropathological sequelae in the rat brain, we used this model together with a series of antibodies specific for amyloid precursor proteins (APPs), APP-like proteins (APLPs), or Aβ to identify acute neurodegenerative changes after brain trauma. Male Sprague–Dawley rats were anesthetized and subjected to lateral FP brain injury of moderate to high severity. At 1 hr, 2 hr, 48 hr, 1 week, or 2 weeks after injury, animals were killed and their brains were removed for immunohistochemical analysis. APP/APLP immunoreactivity increased in specific brain regions as early as 1 hr after injury and persisted for at least 2 weeks. Axons in the thalamus and subcortical white matter showed the greatest APP/APLP accumulation. Injured cortex, striatum, cingulum, and hippocampus also demonstrated significant axonal accumulations of APP/APLP. Accumulation of APP/APLPs occurred primarily ipsilateral to the injury, although bilateral changes were observed in some brain regions. No deposition of Aβ was observed in any brain region at any time point examined. These results demonstrate a pattern of widespread axonal pathology after lateral FP brain injury in the rat, characterized by intra-axonal accumulations of APP/APLP immunoreactivity in the absence of plaque-like deposits of Aβ in the traumatized brain.

Key words: amyloid precursor proteins; amyloid precursor protein-like proteins; β-amyloid; Alzheimer's disease; lateral fluid-percussion brain injury

Environmental factors, including traumatic brain injury (TBI), are thought to be involved in determining the onset and progression of Alzheimer's disease (AD) (Mortimer et al., 1991), perhaps in combination with the genetic background of the patient (Mayeux et al., 1995; Nicoll et al., 1995). Senile plaques in the brains of AD patients are composed predominantly of fibrils of β-amyloid peptides (Aβs) (for review, see Cordell, 1994; Selkoe, 1994). Traditional stains for amyloid used in postmortem examinations of brains from boxers with dementia pugilistica did not reveal senile plaques (Corsellis et al., 1973). Re-examination of these brains using antibodies to Aβ demonstrated the presence of diffuse Aβ plaques (Roberts et al., 1990; Tokuda et al., 1991), suggesting a direct relationship between multiple incidents of head trauma and development of neuropathological changes associated with AD. Aβs are derived from amyloid precursor proteins (APPs), whereas APP-like proteins (APLPs) resemble APPs but do not contain the Aβ domain (Sandbrink et al., 1994). Postmortem evaluation of the neuropathological changes after a single incident of severe TBI has revealed widespread deposition of Aβ and increased APP (and presumably APLP) immunoreactivity (Roberts et al., 1991, 1994; Graham et al., 1995). Furthermore, APP immunoreactivity has been reported to be an early marker for axonal injury (Gentleman et al., 1993b; Sherriff et al., 1994b).

Alterations in neuronal APP immunoreactivity, beginning 24 hr after injury, have been reported after a weight-drop model of experimental TBI (Lewen et al., 1995). Increased APP immunoreactivity has been reported in neurons and glia after experimental lesions of rat brain induced by stab injury (Otsuka et al., 1991) or injection of excitotoxic compounds (Siman et al., 1989; Kawarabayashi et al., 1991; Wallace et al., 1991; Nakamura et al., 1992; Topper et al., 1995) or colchicine (Siman et al., 1989; Shigematsu and McGeer, 1992) into the brain. Changes in APP were seen as early as 30 min after lesion (Otsuka et al., 1991) and persisted for several weeks (Siman et al., 1989; Kawarabayashi et al., 1991; Nakamura et al., 1992). Similar increases in APP immunoreactivity have been observed after experimental ischemia in rats (Stephenson et al., 1992; Kalaria et al., 1993) and gerbils (Wakita et al., 1992; Tomimoto et al., 1994). Alterations in Aβ were not described in these studies.

Our laboratory has established a model of experimental lateral fluid-percussion (FP) brain injury in the rat (McIntosh et al., 1989) that produces reproducible and clinically relevant histopathological changes, including extensive neuronal loss and

Received Oct. 4, 1995; revised Oct. 24, 1995; accepted Oct. 26, 1995.

This research was supported in part by NINDS Grants NS26818 and NS08803, National Institute on Aging Grants AG10124 and AG11542, and a Veterans Administration Merit Review Grant. We thank Dr. S. Gandy, Cornell University, for kindly providing antibody 369w, and Drs. K. Kim and H. Wisniewski, New York State Institute for Basic Research in Developmental Disabilities, for kindly providing antibody 4G8. We also thank K. Pool for expert technical assistance and Dr. M. L. Schmidt for advice on photomicroscopy. This study was conducted in accordance with the animal welfare guidelines set forth in *Guide for the Care and Use of Laboratory Animals*, U.S. Department of Health and Human Services, Pub. No. 85-23, 1985.

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degeneration accompanied by gliosis in cortical and subcortical brain structures (Cortez et al., 1989; Smith et al., 1991; Lowenstein et al., 1992; Hicks et al., in press; Soares et al., in press). Because both clinical and experimental studies suggest a relationship between TBI and AD-like neurodegenerative changes, the present study was undertaken to characterize the temporal and regional post-traumatic onset and progression of AD-like neuropathology after experimental lateral FP brain injury in the rat, including acute changes in APP/APLP and Aβ immunoreactivity.

MATERIALS AND METHODS

Surgical preparation. Male Sprague–Dawley rats (340–400 gm) were prepared for lateral FP brain injury ($n = 20$) or sham injury ($n = 10$) as described previously (McIntosh et al., 1989). Briefly, anesthetized animals (sodium pentobarbital, 60 mg/kg, i.p.) were placed in a stereotaxic frame, and the scalp and left temporal muscle were reflected. A 5 mm craniectomy was made, centered between lambda and bregma over the left parietal cortex, with the dura remaining intact at this site. A plastic, female Luer-Lok fitting was set in the craniectomy site and held in place with dental cement. Sham-injured (control) animals received identical anesthesia and surgery but did not receive the FP injury.

Lateral FP brain injury. The lateral FP brain-injury device was a Plexiglas cylinder filled with saline, closed at one end by a Plexiglas plunger and at the other by a male Luer-Lok fitting. Ninety minutes after anesthesia administration, the animal was attached to the device via paired Luer-Lok fittings. A pendulum, set at the appropriate height to produce a moderate to severe level of injury (2.4–3.0 atmospheres), was allowed to strike the plunger once, causing a rapid, high-pressure injection of saline into the closed cranial cavity. A pressure transducer connected to the device enabled measurement of the force of the injury in atmospheres, and these data were recorded on a computer monitor using Enhanced Graphics Acquisition Analysis Software (RC Electronics, Goleta, CA).

Immunohistochemistry. At 1 hr, 2 hr, 48 hr, 1 week, and 2 weeks after lateral FP brain injury, animals ($n = 4$ injured, $n = 2$ shams at each time point) were killed and their brains were prepared for immunohistochemistry. Animals were anesthetized lethally with sodium pentobarbital and perfused transcardially with heparinized saline for 1–2 min. Next, brains were fixed by immersion in 70% ethanol in 150 mM NaCl, which optimizes immunolabeling with the antibodies used in this study (Arai et al., 1990). Each brain was removed from the cranium, immersed in the fixative overnight, and then cut into coronal slices 3–5 mm thick and processed for paraffin embedding in an automated tissue processor (Shandon Hypercenter XP, Shandon Scientific Instruments, Cheshire, UK). Serial sections (6 μm) were cut on a Leitz rotary microtome (Leica, Malvern, PA) and mounted on poly-L-lysine-coated slides. Figure 1 shows the range of brain levels examined, encompassing the rostral–caudal extent of tissue damage. Primary antibodies recognizing APPs were LN39, a mouse monoclonal antibody (mAb) specific for the first 100 N-terminal amino acids in APP (Arai et al., 1991; Standaert et al., 1991), and 369w, a rabbit polyclonal antibody to the APP C-terminal amino acid domain (Buxbaum et al., 1990; Gandy et al., 1992; Ouimet et al., 1994). Because APLPs are closely homologous with the APPs, except for the Aβ domain, these anti-APP antibodies are presumed to recognize both APPs and APLPs (referred to here as APP/APLP immunoreactivity). Primary antibodies recognizing Aβ were 2332, a rabbit polyclonal antibody to amino acids 1–17 (Schmidt et al., 1994a,b), and 4G8, a mouse mAb to amino acids 1–24 (specifically 17–24) of Aβ (Kim et al., 1990). Antibody dilutions and descriptions are summarized in Table 1. Sections were incubated with primary antibody overnight at 4°C and then incubated at room temperature for 1 hr each with the appropriate secondary and tertiary antibodies, followed by enzymatic development with 3,3′-diaminobenzidine as described previously (Shin et al., 1993). Antibodies were diluted in 0.1 M Tris buffer with 2% serum, and tissue sections were washed in this buffer. Sections were counterstained with hematoxylin, dehydrated, and coverslipped. Ethanol-fixed, paraffin-embedded sections from confirmed human AD brains served as positive controls for labeling procedures. Omission of primary antibody or application of control serum instead of primary antibody on selected sections of rat tissue provided a negative control. Additional rat-brain sections were stained with toluidine blue for verification of and comparison with previously established patterns of post-traumatic neuronal loss (Cortez et al., 1989; Smith et al., 1991; Hicks et al., in press; Soares et al., in press). To verify the axonal location of APP/APLP accumulation, selected tissue sections were double-labeled for 68 kDa neurofilament proteins (polyclonal antibody anti-NFL) and APP/APLPs (mAb 22C11; Boehringer Mannheim, Indianapolis, IN) and visualized with fluorescein isothiocyanate (FITC) and Texas red fluorescent secondary antibodies, respectively. Light and fluorescence microscopy was performed using a Nikon Microphot SA with a UFX-DX camera system (Optical Apparatus, Ardmore, PA).

Table 1. Summary of antibodies used for immunohistochemistry

Antibody	Epitope Protein/ amino acids	Type	Dilution	Reference
LN39	APP/1–100	M	1:20	Arai et al. (1991) Standaert et al. (1991)
369w	APP/645–694[a]	P	1:2500	Buxbaum et al. (1990) Gandy et al. (1992) Ouimet et al. (1994)
2332	Aβ/1–17	P	1:8000	Schmidt et al. (1994a) Schmidt et al. (1994b)
4G8	Aβ/17–24	M	1:1000	Kim et al. (1990)

M, mAb; P, polyclonal antibody. [a]Amino acid sequence of APP_{695} isoform. Because of high homology between APPs and APLPs, LN39 and 369w are presumed to recognize both sets of proteins.

RESULTS

Toluidine-blue staining revealed the formation of a glial-lined cavity in the injured cortex attributable to focal neurodegenerative events during the first 2 weeks after lateral FP brain injury in the rat (Fig. 1*D*), as described previously (Cortez et al., 1989; Smith et al., 1991; Hicks et al., in press; Soares et al., in press). APP/APLP immunohistochemistry revealed an additional pattern of diffuse neuronal damage that evolved during this time course that involved brain regions remote from the injury site. Bilateral changes in APP/APLP distribution occurred as early as 1 hr after lateral FP brain injury in the rat. Although bilateral alterations in APP/APLP immunoreactivity were seen in some brain regions, accumulation of APP/APLP was much greater in the hemisphere ipsilateral to the injury. Maximal APP/APLP immunoreactivity was observed at 48 hr after injury. By 1 week after injury, the extent and intensity of APP/APLP immunoreactivity in each region had begun to decline. By 2 weeks after injury, hemosiderin deposits were found in regions that previously showed extensive APP/APLP accumulations, and APP/APLP immunoreactivity no longer was observed in most contralateral brain regions. These results are summarized in Table 2 and described in further detail below, according to brain region. Both APP/APLP antibodies (LN39 and 369w) showed immunoreactivity in identical brain regions, although in serial sections identical structures were not labeled consistently with both antibodies.

Because of the morphology of APP/APLP-positive structures, their abundance in white matter, and double-labeling experiments with APP/APLP and 68 kDa neurofilament antibodies (Fig. 2), we conclude that a vast majority of post-traumatic immunoreactive APP/APLP accumulations occurred in axonal swellings. Nevertheless, APP/APLP also may accumulate in dendrites. Indeed, APP/APLP immunoreactivity was increased slightly in a small number of neuronal perikarya in heavily immunoreactive areas of the thalamus and injured cortex at 48 hr and 1 week after injury.

Thalamus

In the medial thalamus at 1 hr after injury, accumulations of APP/APLP were detected bilaterally in early axonal swellings

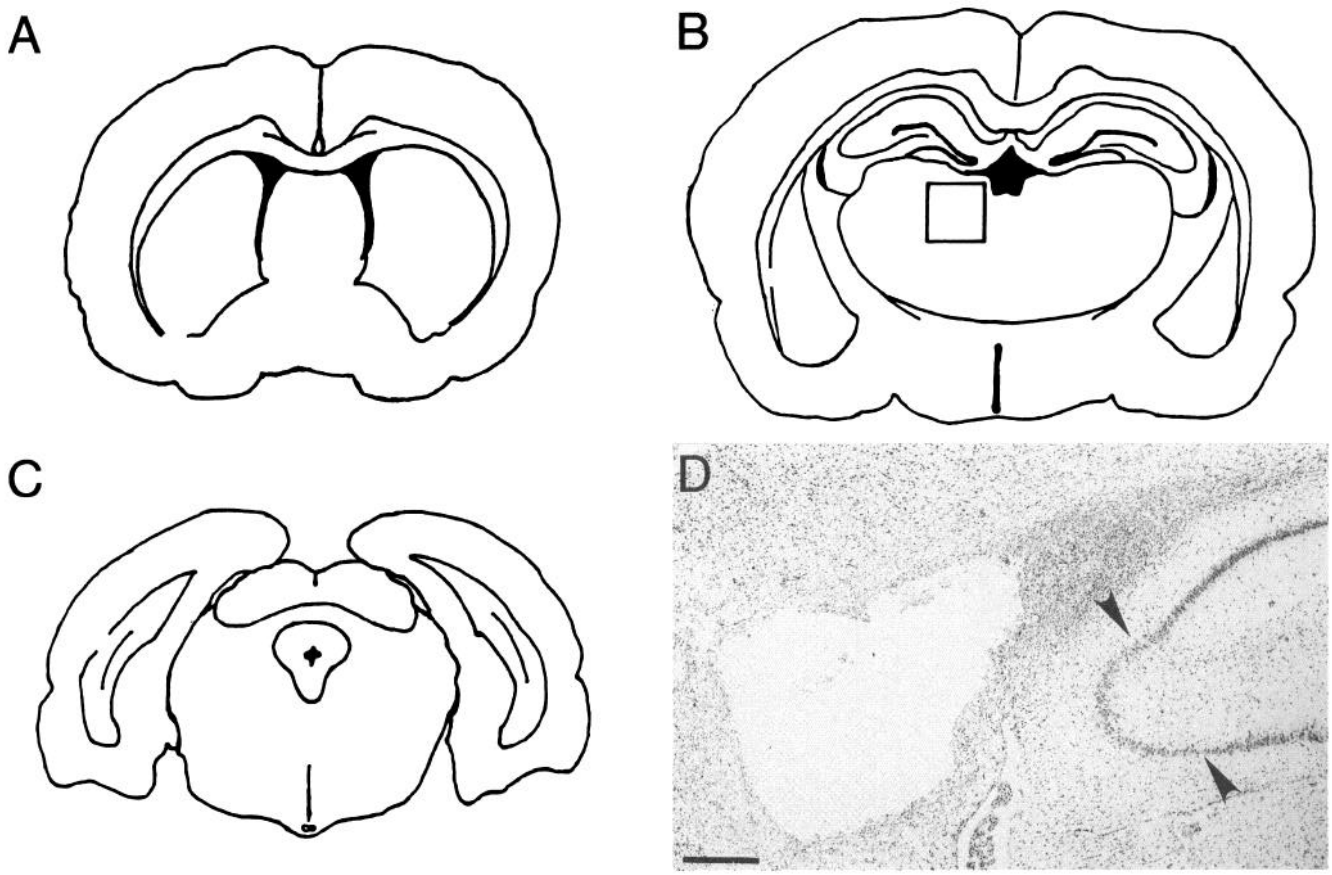

Figure 1. Extent of neuronal damage after lateral FP brain injury in the rat. Schematic drawings of (*A*) rostral, (*B*) central, and (*C*) caudal coronal brain levels that were examined. *Box* in *B* represents the region of maximal APP/APLP accumulation in the thalamus from which photos in Figure 2 were taken. *D*, Cortical and hippocampal neuronal loss 2 weeks after injury. Toluidine blue Nissl stain revealed a large cavity lined with glial cells in the cortex and marked loss of hippocampal pyramidal neurons in the portions of area CA3 indicated by *arrowheads.* Scale bar, 292 μm.

(Fig. 3*A*). By 2 hr after injury, APP/APLP-immunoreactive swellings in this region had increased greatly in size and number (Fig. 3*B*). By 48 hr after injury, APP/APLP-immunoreactive processes in the ipsilateral medial thalamus were stained robustly, having the more classic appearance of axonal swellings or retraction bulbs typical of trauma-induced axonal pathology (Fig. 3*C*). At 1 week after injury, APP/APLP-immunoreactive axons in the thalamus remained swollen as at 48 hr, but fewer axons were labeled. By 2 weeks after injury, APP/APLP immunoreactivity appeared in smaller or fragmented processes (Fig. 3*D*). APP/APLP accumulation occurred *bilaterally* in the medial thalamus at each time point observed. In addition, a band of APP/APLP-immunoreactive swollen axons in the lateral thalamus ipsilateral to the injury appeared only at 48 hr after injury (Fig. 4*A*).

Table 2. Rat brain regions showing APP/APLP immunoreactivity after lateral FP brain injury

	Time point after injury				
Brain region	1 hr	2 hr	48 hr	1 week	2 weeks
Thalamus	4/4	4/4	4/4	4/4	4/4
SWM	1/4	4/4	4/4	4/4	4/4
Striatum	0/3[a]	3/3[a]	4/4	3/3[a]	4/4
Cingulum	1/4	4/4	4/4	4/4	3/4
CP/SN	1/4	2/4	3/4	2/4	0/4
Fimbria	0/4	1/4	2/4	1/4	2/4
CC	1/4	1/4	1/4	1/4	0/4
DHC	0/4	0/4	2/4	2/4	1/4
Hippocampus	2/4	4/4	4/4	4/4	4/4
Cortex	3/4	4/4	4/4	4/4	4/4
Brainstem	0/4	1/4	3/4	2/4	0/4

Ratios indicate the number of injured animals showing detectable APP/APLP immunoreactivity in each region examined. [a]Sections from this level were examined only in three injured brains at each of these time points. SWM, Subcortical white matter; CP, cerebral peduncle; SN, substantia nigra; CC, corpus callosum; DHC, dorsal hippocampal commissure.

White matter

Although the thalamus showed the earliest changes in APP/APLP distribution, white matter tracts throughout the brain contained numerous APP/APLP-positive axonal swellings beginning at 2 hr and persisting up to 2 weeks after injury in most regions (see Table 2). Ipsilateral subcortical white matter, especially at the level of the entorhinal cortex and subiculum, revealed the most numerous and robust APP/APLP accumulation, with classic retraction bulb profiles at 48 hr (Fig. 4*B*) and 1 week after injury. Axons in the striatum (Fig. 4*C*) and cingulum (Fig. 4*D,E*) also contained APP/APLP accumulations bilaterally. In the contralateral hemisphere, the cerebral peduncle and adjacent substantia nigra revealed APP/APLP-immunoreactive swellings. APP/APLP-immunoreactive axons were observed less frequently in the

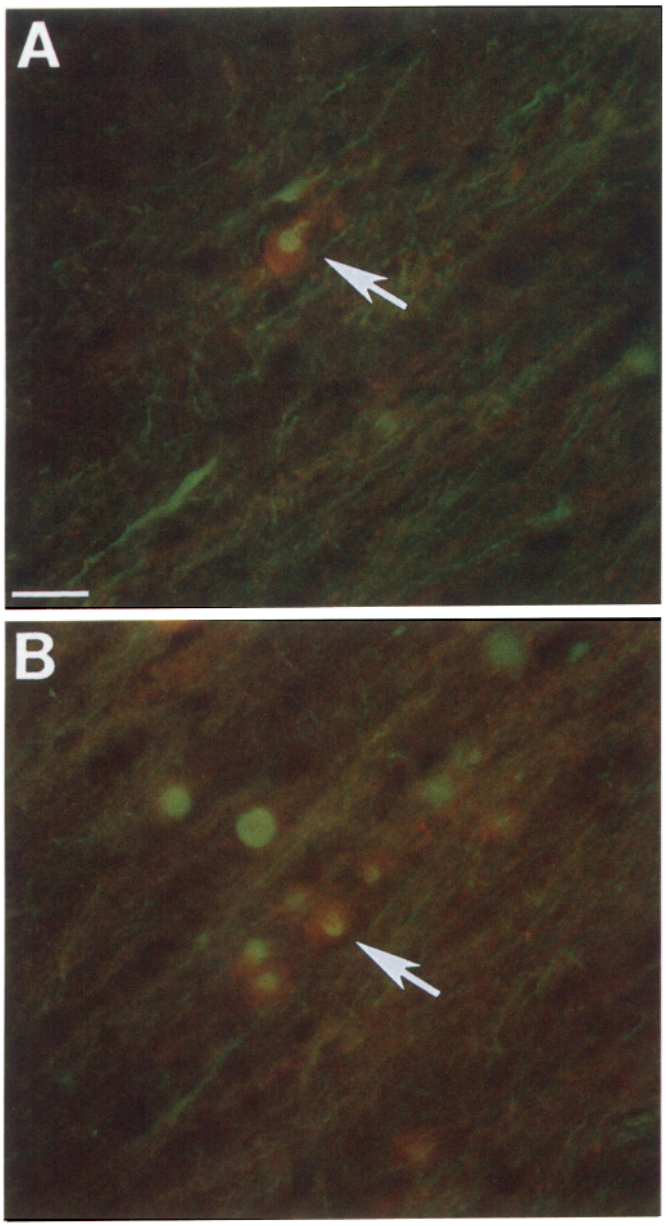

Figure 2. Axonal swellings double-labeled for APP/APLP (*red*) and 68 kDa neurofilament proteins (*green*). Double-exposure photomicrographs with Texas red and FITC fluorescent filters show swellings in the (*A*) lateral thalamus (ipsilateral) and (*B*) subcortical white matter (ipsilateral) 48 hr after lateral FP brain injury in the rat, in which a core of neurofilament is surrounded by APP/APLP (*arrows*). Scale bar, 19 μm (same magnification in both panels).

ipsilateral fimbria and bilateral corpus callosum and dorsal hippocampal commissure.

Hippocampus

APP/APLP accumulation occurred in the ipsilateral hippocampus in areas CA3 (Fig. 4*F*) and CA3c and the dentate hilus as early as 2 hr after injury. Notably, extensive neuronal loss occurs in these areas after lateral FP brain injury (Fig. 1*D*). At 2 weeks after injury, APP/APLP immunoreactivity in the molecular layer of the dentate gyrus of the ipsilateral hippocampus revealed a unique pattern of labeling that may represent fragmented axons or dendritic or synaptic accumulation of APP/APLP (Fig. 4*G*).

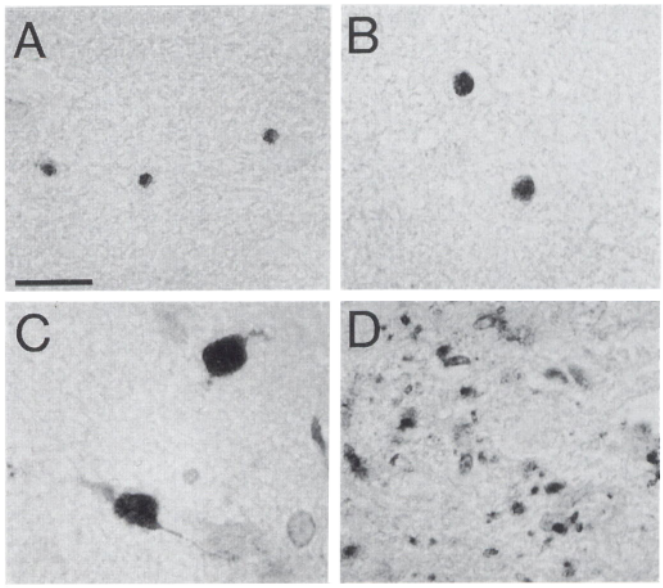

Figure 3. APP/APLP immunoreactivity in the thalamus from 1 hr to 2 weeks after lateral FP brain injury in the rat. *A*, APP/APLP accumulates in neuronal processes as early as 1 hr after injury with changes in size, morphology, and distribution over time: 2 hr (*B*), 48 hr (*C*), and 2 weeks (*D*) after injury. All sections shown were labeled with LN39. Scale bar, 14 μm (same magnification in all panels).

Cortex

APP/APLP-immunoreactive swellings were observed consistently in and around the degenerating injured cortical regions from 2 hr to 2 weeks after injury (Fig. 4*H*).

Brainstem

Occasional accumulations of APP/APLP were observed throughout the brainstem from 2 hr to 1 week after injury.

Aβ

Immunohistochemical labeling of additional sections from these same brains with two different antibodies specific for Aβ did not reveal any extracellular deposits of Aβ at the time points examined (Fig. 4*I*). Furthermore, sham-operated control brains revealed no APP/APLP accumulations or Aβ deposits at any time point, and the brains of two naive rats (anesthesia only, no surgery or injury) also were free of such deposits.

DISCUSSION

Immunohistochemistry with antibodies against the N and C termini of APP/APLP revealed marked accumulation of APP/APLP in damaged axons of the thalamus as early as 1 hr after lateral FP brain injury. Additional brain regions (subcortical white matter, striatum, cingulum, hippocampus, and injured cortex) showed significant APP/APLP immunoreactivity by 2 hr after injury, and immunohistochemical staining for APP/APLP in these regions persisted for up to 2 weeks (the latest time point analyzed in this study). Throughout the affected brain regions, these APP/APLP-immunoreactive swellings enlarged progressively with time and then seemed to undergo fragmentation. Although changes in APP/APLP were most overt in axons ipsilateral to the injury site, marked increases in APP/APLP immunoreactivity occurred bilaterally in the thalamus, cingulum, and dorsal hippocampal commissure and contralaterally in the cerebral peduncle and substantia nigra. In contrast to changes in the injured hemisphere,

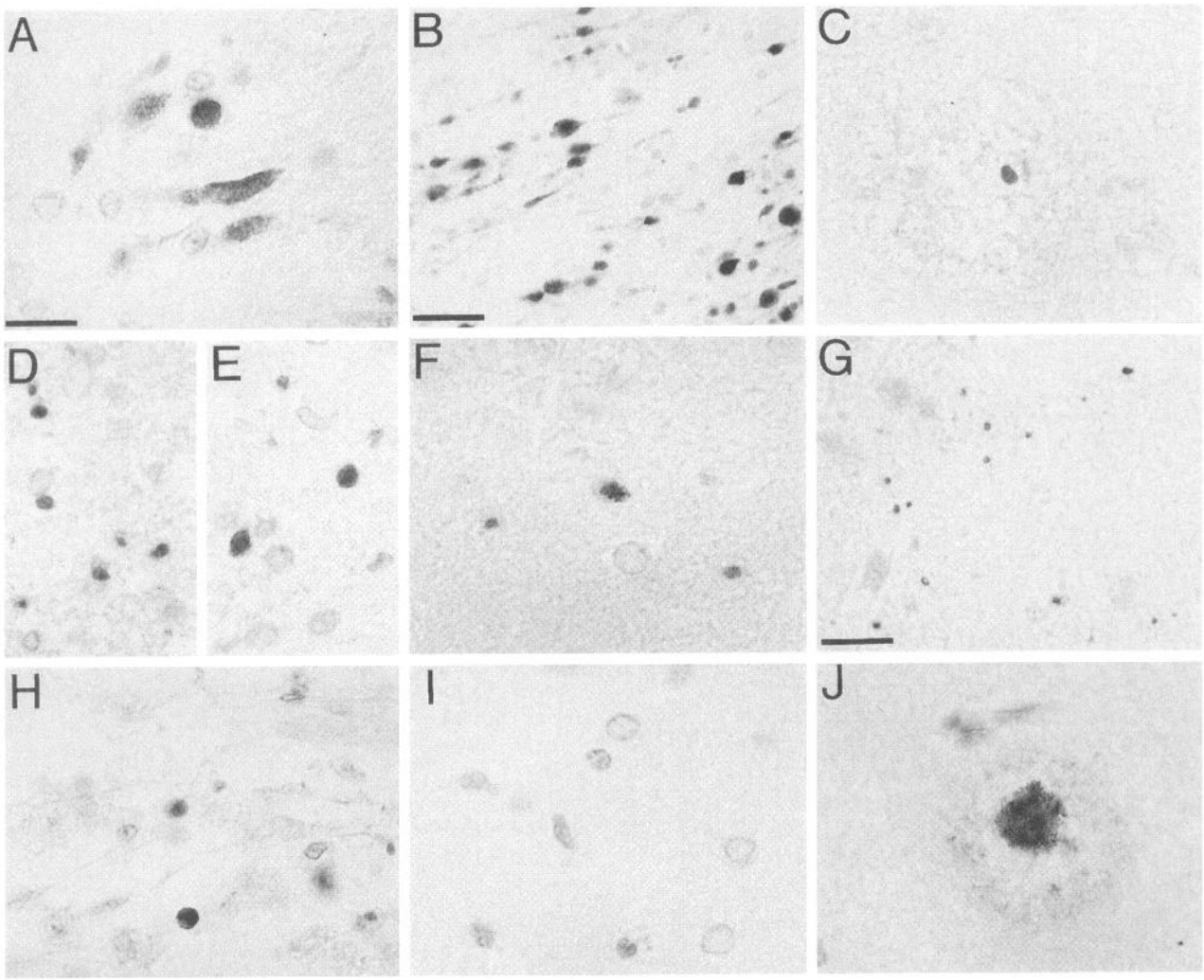

Figure 4. Additional brain regions showing accumulation of APP/APLP during the first 2 weeks after lateral FP brain injury in the rat. *A*, Ipsilateral lateral thalamus. *B*, Ipsilateral subcortical white matter. *C*, Ipsilateral striatum. Ipsilateral (*D*) and contralateral (*E*) cingulum. *F*, Ipsilateral CA3 stratum oriens. *G*, Molecular layer of the ipsilateral dentate gyrus of the hippocampus. *H*, Injured cortex. *I*, Absence of Aβ deposition after lateral FP brain injury in the rat; ipsilateral hippocampus from injured rat brain. *J*, Amyloid plaque in the hippocampus of an AD brain. *A, B, D, E*, At 48 hr after injury. *C, F*, At 2 hr after injury. *G–I*, At 2 weeks after injury. Sections pictured in *A–H* were labeled with LN39. Sections in *I* and *J* were labeled with 4G8. Scale bars: *A, C–F, H–J*, 14 μm; *B*, 36 μm; *G*, 12 μm.

contralateral APP/APLP changes resolved by 2 weeks after injury. Examination of sections from these same brains with two different anti-Aβ antibodies revealed no Aβ deposits at any time point examined.

The presence of APP/APLP immunoreactivity in swollen axons has been used recently as an early marker of diffuse axonal injury (DAI) (Gentleman et al., 1993b; Sherriff et al., 1994b). DAI encompasses specific neuropathological sequelae, including diffuse white matter damage, axonal degeneration, and traumatic coma, after specific types of TBI in humans (Strich, 1961) and nonhuman primates (Gennarelli et al., 1982b). The extent of DAI increases in parallel with the severity of TBI and correlates with measures of postinjury morbidity and mortality (Gennarelli et al., 1982a; Pilz, 1983; Adams et al., 1989; Povlishock, 1992; Povlishock et al., 1992; Blumbergs et al., 1994). Because APP undergoes fast axonal transport (Koo et al., 1990), intra-axonal accumulations of APP/APLP may serve as earlier, highly specific markers of DAI compared with other axonal proteins (e.g., neurofilament subunits or ubiquitin) (Yaghmai and Povlishock, 1992; Grady et al., 1993; Sherriff et al., 1994a).

Widespread axonal damage after experimental brain injury has been reported previously using neurofilament antibodies (Dixon et al., 1991; Povlishock, 1993; Foda and Marmarou, 1994). Very recently, axonal injury has been described after weight-drop injury in the rat using an antibody to APP/APLP (Lewen et al., 1995). In the present study, the time course and distribution of the axonal changes seen with APP/APLP antibodies suggest an ongoing secondary process of axonal damage for at least 2 weeks after TBI. Furthermore, traditional silver-staining techniques have revealed axonal changes in hallmark brain regions involved in DAI pathology (subcortical white matter, thalamus, striatum, and brainstem) after lateral FP brain injury in the rat (D.I. Graham, unpublished data), thus corroborating the present APP/APLP data. These observations indicate that lateral FP brain injury, considered previously to be a focal model of TBI, produces both focal and diffuse pathologies reminiscent of clinical TBI.

The mechanisms underlying Aβ plaque formation in humans and other species currently are unknown. In the present study, we hypothesized that severe closed head injury in the rat would induce pathological hallmarks of neurodegenerative cascades, given the evidence for Aβ deposition after TBI in humans. However, lateral FP brain injury in the rat did not lead to deposition of Aβ, despite alterations in the distribution of APP/APLP, and these findings are consistent with findings reported in studies of other types of brain injury in rodents. For example, rodent models of ischemia (Stephenson et al., 1992; Wakita et al., 1992; Kalaria et al., 1993), penetrating injury (Otsuka et al., 1991), ablation with neurotoxic compounds (Siman et al., 1989; Kawarabayashi et al., 1991; Wallace et al., 1991; Nakamura et al., 1992; Topper et al., 1995), and traumatic weight drop (Lewen et al., 1995) also have shown post-traumatic changes in APP/APLP, with no evidence of Aβ deposits or plaque-like lesions. Although a single incidence of TBI has been shown to cause amyloid deposition in human brains (Roberts et al., 1991, 1994), a single insult may be insufficient to induce cerebral amyloid deposition in experimental TBI models. It is important to note that the repeated head trauma experienced by boxers induces AD-like neuropathology that includes both diffuse amyloid plaques and neurofibrillary tangles (Corsellis et al., 1973; Roberts et al., 1990; Tokuda et al., 1991). To date, no reports have discussed the consequences of multiple incidences of TBI in experimental models.

Several additional factors may prevent Aβ deposition in the rat brain. Although cognitively normal humans develop amyloid plaques with age, even the most senescent rats do not develop Aβ deposits or senile plaques, despite alterations in APP gene expression (Higgins et al., 1990). Three amino acids differ between rat/mouse Aβ and Aβ in humans and other species that develop plaques as a function of age (Johnstone et al., 1991). These amino acids may play a critical role in Aβ deposition or fibril formation. Alternatively, the proteases that cleave Aβ from APP remain uncharacterized, and it is possible that rodent enzymes differ in some critical aspect from those in humans. Furthermore, the sequence, concentration, or distribution of other plaque-associated proteins, such as tau (Trojanowski and Lee, 1994), α-1-antichymotrypsin (Fraser et al., 1993), heparan sulfate proteoglycan (Snow et al., 1994), or apolipoprotein E (Strittmatter et al., 1993) may differ in rats versus humans, and these proteins could be essential cofactors required for Aβ-plaque formation. Indeed, allelic variation in the apolipoprotein E gene is a risk factor for the development of AD pathology in the general population (Strittmatter et al., 1993; Roses, 1994; Utermann, 1994) and may be an important determinant of which patients will develop AD-like pathology after TBI (Mayeux et al., 1995; Nicoll et al., 1995). Although the amino acid sequence of rat apolipoprotein E resembles human apolipoprotein E4, the isoform associated with AD pathology, the rat and human forms of this protein also differ at many residues (McLean et al., 1983). Future studies using transgenic mice that develop AD-like pathology (for review, see Higgins and Cordell, 1995) may provide insights into the role of TBI in the pathogenesis of AD. One such transgenic mouse expressing human APP developed spontaneous deposits of Aβ that form amyloid fibrils (Games et al., 1995).

Examination of alterations in cognitive function also may help elucidate the relationships between TBI and AD. Profound and prolonged impairments of spatial learning and memory occur after various models of experimental TBI (Lyeth et al., 1990; Smith et al., 1991; Hamm et al., 1992; Pierce et al., 1993, 1994). Clinical TBI also causes prolonged cognitive impairment (Brooks, 1972). Although the relationship of cognitive changes after experimental and clinical brain injury with the dementing syndrome of AD is unclear, the shared occurrence of retrograde and anterograde amnesia may provide opportunities for pharmacological intervention to evaluate treatment paradigms and discern mechanisms possibly relevant to both disorders.

Although disruption of axonal transport is the most likely explanation for the observations in the present study, other possible explanations cannot be excluded and merit further investigation. The axonal nature of these swellings is supported by the colocalization of neurofilament and APP/APLP in these swellings; however, the distribution of typical cytoskeletal markers after central nervous system injury should be interpreted cautiously. Acute alterations in microtubule-associated protein 2 (Taft et al., 1992; Hicks et al., 1995), neurofilament proteins (Posmantur et al., 1994; Saatman and McIntosh, 1994), and spectrin (Saatman et al., 1995) have been shown after experimental rat brain injury, and Hall et al. (1989) showed that rearrangement of cytoskeletal proteins can occur in the lamprey after axotomy. Furthermore, evidence indicating diffuse dendritic damage after TBI has been reported (Posmantur et al., 1995). The presence of APP/APLP or neurofilament alone in many swellings may indicate a differential time course for accumulation of each protein in damaged axons, which would be expected from their different rates of transport and the ability to detect swellings sooner with APP/APLP than with neurofilament antibodies.

Alternatively, APP/APLP and Aβ regulation may be influenced by other proteins or genes that are acutely upregulated after experimental brain injury, including immediate-early genes (Phillips and Belardo, 1992; Raghupathi et al., 1995), heat shock proteins (Raghupathi et al., 1995), and cytokines (Taupin et al., 1993; Shohami et al., 1994; Fan et al., 1995). Such alterations may trigger a proposed cascade of Aβ deposition in both head trauma and AD (Royston et al., 1992; Gentleman et al., 1993a). *In situ* hybridization analysis of APP/APLP mRNA levels and distribution may reveal specific temporal and regional post-traumatic relationships between these genes and proteins.

In conclusion, APP/APLP immunohistochemistry reveals widespread damage after lateral FP brain injury in the rat in the absence of AD-like deposits of Aβ. Changes in the distribution of APP/APLP appear as markers of axonal damage and diffuse brain injury and are not indicative of specific AD pathology. The reasons for this difference in neuropathology between TBI in humans versus rats is unclear, but they may reflect species differences in the proteins involved in plaque formation or a need for multiple incidents of TBI to induce Aβ deposition in the rat brain. Nevertheless, the rapid and prolonged accumulation of APP/APLP after lateral FP brain injury in the rat suggests that this model is useful for investigating the mechanisms and dynamics underlying APP/APLP accumulation, processing, and breakdown.

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