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Brief Communication

Apolipoprotein E4 Influences Amyloid Deposition But Not Cell Loss after Traumatic Brain Injury in a Mouse Model of Alzheimer's Disease

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The ϵ4 allele of apolipoprotein E (*APOE*) and traumatic brain injury (TBI) are both risk factors for the development of Alzheimer's disease (AD). These factors may act synergistically, in that *APOE4*+ individuals are more likely to develop dementia after TBI. Because the mechanism underlying these effects is unclear, we questioned whether *APOE4* and TBI interact either through effects on amyloid-β (Aβ) or by enhancing cell death/tissue injury. We assessed the effects of TBI in PDAPP mice (transgenic mice that develop AD-like pathology) expressing human *APOE3* (PDAPP:E3), human *APOE4* (PDAPP:E4), or no *APOE* (PDAPP:E−/−). Mice were subjected to a unilateral cortical impact injury at 9–10 months of age and allowed to survive for 3 months. Aβ load, hippocampal/cortical volumes, and hippocampal CA3 cell loss were quantified using stereological methods. All of the groups contained mice with Aβ-immunoreactive deposits (56% PDAPP:E4, 20% PDAPP:E3, 75% PDAPP:E−/−), but thioflavine-S-positive Aβ (amyloid) was present only in the molecular layer of the dentate gyrus in the PDAPP:E4 mice (44%). In contrast, our previous studies showed that in the absence of TBI, PDAPP:E3 and PDAPP:E4 mice have little to no Aβ deposition at this age. After TBI, all of the Aβ deposits present in PDAPP:E3 and PDAPP:E−/− mice were diffuse plaques. In contrast to the effect of *APOE4* on amyloid, PDAPP:E3, PDAPP:E4, and PDAPP:E−/− mice did not differ in the amount of brain tissue or cell loss. These data support the hypothesis that *APOE4* influences the neurodegenerative cascade after TBI via an effect on Aβ.

Key words: Alzheimer's disease; amyloid; APP; traumatic brain injury; apoE; hippocampus

The ϵ4 allele of apolipoprotein E (*APOE*) and traumatic brain injury (TBI) are both risk factors for the development of Alzheimer's disease (AD). It was first found that *APOE4* was a risk factor for AD in 1993 (Strittmatter et al., 1993), and numerous studies have confirmed this association. Several studies have found that individuals who sustained moderate to severe head injury are more likely to develop dementia and AD (Mayeux et al., 1993, 1995; Plassman et al., 2000). These risk factors appear to act synergistically, in that individuals who are *APOE4*+ are even more likely to develop dementia if they sustain TBI at some time in their life. For example, *APOE4*+ individuals were 10 times more likely to develop AD after TBI than those who were *APOE4*−, whereas *APOE4* in the absence of injury was associated with only twice the risk (Mayeux et al., 1995; Tang et al., 1996).

Although the mechanisms underlying these effects are unclear, some evidence suggests that both *APOE4* and TBI may influence the risk of AD via interactions with the amyloid-β (Aβ) peptide. For example, Aβ deposition can be found in ~30% of people who die shortly after TBI (Roberts et al., 1991, 1994); a significant percentage of these patients are *APOE4*+ (Nicoll et al., 1995, 1996). In addition, analysis of CSF from TBI patients revealed elevated levels of $A\beta_{1-42}$ for up to a week after TBI, in comparison with both controls and AD patients (Raby et al., 1998; Emmerling et al., 2000). In an evaluation of the effect of *APOE3*, *APOE4*, or *APOE*−/− in transgenic (TG) mice after TBI, greater mortality was observed in *APOE4* mice, whereas *APOE3* mice exhibited better neurological function between 3 and 11 d after TBI (Sabo et al., 2000). Although *APOE3* mice had less tissue loss after TBI than *APOE*−/− or wild-type mice, there was no significant difference in tissue loss comparing *APOE3* with *APOE4* mice. The mice studied did not express human amyloid precursor protein, so Aβ deposition did not occur (Sabo et al., 2000).

Because the mechanism by which the *APOE* genotype and TBI interact to influence dementia remains unresolved, we questioned whether *APOE4* and TBI interact through effects on Aβ, enhancement of cell death or tissue injury, or both. We used a well characterized model of TBI (Smith et al., 1995) and assessed the histological outcome in PDAPP mice (a TG mouse that develops AD-like pathology) that either lack apoE or express human *APOE3* or *APOE4*.

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Table 1. Histological analysis after TBI

	PDAPP:E3	PDAPP:E4	PDAPP:E−/−	$p < 0.05$
Sample size	10	9	4	
Percentage of cortical loss	24.9	21.4	21.9	No
Percentage of hippocampal loss	9.2	9.3	8.5	No
Percentage of CA3 inferior blade neuron loss	35.7	30.0	26.6	No
Percentage of group with Aβ-IR deposits	20	55.6	75	No
Percentage of group with molecular layer Aβ-IR deposits	0	44.4	0	Yes
Contralateral to TBI: hippocampal Aβ load (%)	0.1	0.9	13.8	Yes
Ipsilateral to TBI: hippocampal Aβ load (%)	0.1	2.3	10.3	Yes
Contralateral to TBI: % of total Aβ in molecular layer	0	28.1	0	Yes
Ipsilateral to TBI: % of total Aβ in molecular layer	0	29.8	0	Yes

MATERIALS AND METHODS

PDAPP mice expressing amyloid precursor protein (APP) with a mutation that causes familial AD (APP^{V717F}) (Games et al., 1995) but lacks the mouse *APOE* gene (PDAPP+/+, *APOE*−/−) (Bales et al., 1997) were bred with mice that express human *APOE* under control of the glial fibrillary acidic protein (GFAP) promoter (*GFAP-APOE3* and *GFAP-APOE4*) (Sun et al., 1998). The *GFAP-APOE3* and *GFAP-APOE4* mice were all on a C57BL/6 mouse *APOE*−/− background. The breeding produced three types of TG mice (Holtzman et al., 2000): PDAPP+/−, *APOE*−/− (PDAPP:E−/−), PDAPP+/−, *APOE3*+/− (PDAPP:E3), and PDAPP+/−, *APOE4*+/− (PDAPP:E4). Human *APOE3* and *APOE4* TG mouse lines used in this experiment were matched for APOE levels (Sun et al., 1998). The mice were subjected to a controlled cortical impact injury (left hemisphere) (Smith et al., 1995) at 9–10 months of age, while under pentobarbital (65 mg/kg) anesthesia. This injury results in underlying cortical damage with shrinkage of the hippocampus and CA3 cell loss. Mortality rates immediately after TBI were similar in all groups (~25%), resulting in sample sizes of $n = 4$ PDAPP:E−/−, $n = 10$ PDAPP:E3, and $n = 9$ PDAPP:E4. At 12–13 months (3 months after injury), all mice were killed; their brains were removed, fixed, frozen, and sliced into 50 μm coronal sections from the genu of the corpus callosum through the caudal extent of the hippocampus (Holtzman et al., 2000). Three sets of sections, each containing every sixth slice, were collected from each brain. The sections were then mounted and stained with pan anti-Aβ antibody (Biosource, Camarillo, CA), cresyl violet, or 4′,6′-diamidino-2-phenylindole (DAPI). Animals with Aβ-immunoreactive (IR) deposits were further analyzed using the Cavalieri point-counting method with stereological software (Stereo Investigator; MicroBrightField Inc., Colchester, VT) to quantify the area covered by Aβ-IR deposition (Aβ load) as described previously (Holtzman et al., 2000).

To obtain volume estimates of the hippocampus and cortex, cresyl violet-stained sections were analyzed. Hippocampal measurements were taken from every sixth section through the entire structure; cortical measurements were taken from the first three anterior sections containing hippocampal tissue. The cortical region of interest was defined as cortical tissue dorsal to the superior extent of the thalamus. The volumes of the hippocampus and defined cortical region were determined using the Stereo Investigator software. The percentage of hippocampal and cortical tissue lost (ipsilateral vs contralateral to injury) was calculated for each group.

In most animals, the inferior blade of the CA3 hippocampal field exhibited a focal lesion ipsilateral to the injury, also noted in rodent models of TBI (Nakagawa et al., 1999). CA3 neuronal counts were obtained using DAPI-stained sections through the entire extent of the hippocampus. A randomly selected subset of brains was analyzed ($n = 4$ PDAPP:E−/−; $n = 5$ PDAPP:E3; $n = 5$ PDAPP:E4), and measurements were taken from all sections that contained CA3 neurons. In each section, the inferior blade of CA3 was traced using the computer. Estimates of neuronal numbers were obtained with the optical fractionator technique using Stereo Investigator software. The area was traced with a 4× lens, and neurons were counted throughout the traced area using systematic random sampling with a 100× lens.

The data were analyzed using Statistica 6.0 (Statsoft Inc., Tulsa, OK), and α levels of $p < 0.05$ were set for significance. The frequency of Aβ deposition was analyzed using χ^2 analysis. The percentage of the hippocampus covered by Aβ-IR was analyzed with a two-way ANOVA that included one between-subjects factor (genotype: PDAPP:E3 vs PDAPP:E4 vs PDAPP:E−/−) and one within-subjects factor (hemisphere: ipsilateral vs contralateral to impact). Cortical and hippocampal volume estimate data were analyzed with two-way ANOVAs that included one between-subjects factor (genotype: PDAPP:E3 vs PDAPP:E4 vs PDAPP:E−/−) and one within-subjects factor (hemisphere: ipsilateral vs contralateral to impact). CA3 neuronal counts were analyzed with a two-way ANOVA that included one between-subjects factor (genotype: PDAPP:E3 vs PDAPP:E4 vs PDAPP:E−/−) and one within-subjects factor (hemisphere: ipsilateral vs contralateral to impact).

RESULTS

Aβ analysis

Frequency and pattern of Aβ deposition

We found previously that PDAPP mice expressing human *APOE3* or *APOE4* do not develop Aβ deposition until ~15 months of age, when PDAPP:E4 mice in particular begin depositing Aβ and amyloid (Holtzman et al., 2000; Fagan et al., 2002). In contrast, after TBI, we found that a high percentage of brain-injured PDAPP:E4 mice had Aβ deposition by 12–13 months of age. In the PDAPP:E4 mice, 55.6% had Aβ-IR deposits within the hippocampus and 44% had thioflavine-S-positive Aβ (fibrillar amyloid) in the molecular layer (ML) of the dentate gyrus (Table 1). Among the PDAPP:E3 mice, only 20% had hippocampal Aβ-IR deposits, all of which were diffuse plaques. No PDAPP:E3 mice had fibrillar amyloid deposition. Significantly more PDAPP:E4 mice had ML Aβ-IR deposits compared with PDAPP:E3 mice (χ^2, $p < 0.02$) (Fig. 1). This is notable because Aβ deposition in the ML of PDAPP mice coincides with the onset of fibrillar Aβ deposition and neuritic plaque formation (Holtzman et al., 2000; Fagan et al., 2002). Thus, only the PDAPP:E4 mice developed neuritic plaque formation after TBI at this age. Assessment of $A\beta_{40}$ and $A\beta_{42}$ immunostaining of PDAPP:E3 and PDAPP:E4 mice revealed the same pattern of staining as that seen with the pan-Aβ antibody (data not shown). Qualitatively, the same differences between PDAPP:E3 and PDAPP:E4 mice were noted with these antibodies. As in previous studies with PDAPP and other human APP TG mice, neurofibrillary tangles were not seen. Because PDAPP:E3 and PDAPP:E4 mice have little to no Aβ deposits at 12–13 months of age in the absence of TBI (Holtzman et al., 2000; Fagan et al., 2002), TBI appears to accelerate Aβ deposition in the form of amyloid in the presence of human *APOE4* to a greater extent than *APOE3*. Consistent with previous reports (Holtzman et al., 2000; Fagan et al., 2002), three of the four PDAPP:E−/− mice had hippocampal diffuse Aβ-IR deposits at 12 months of age; however, none were fibrillar.

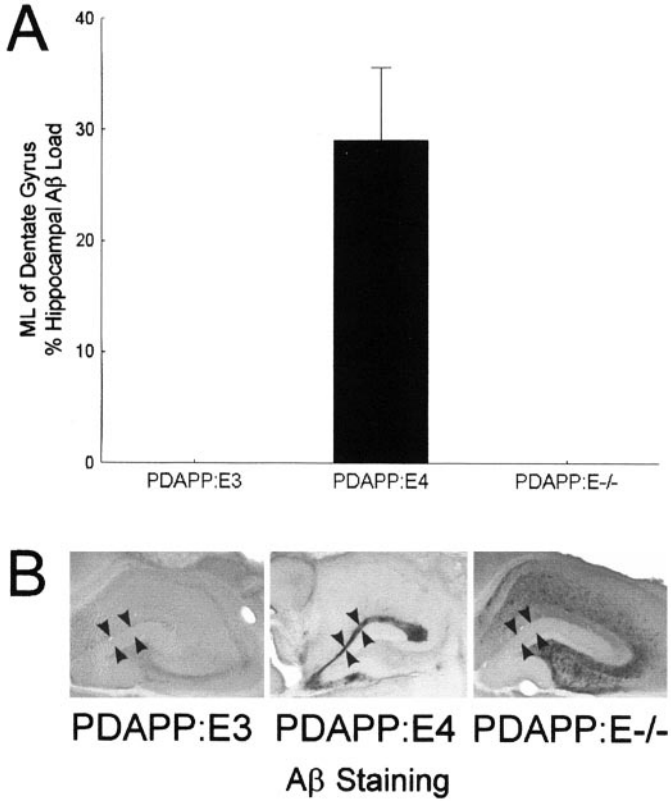

Figure 1. *A*, Almost one-third of the total hippocampal Aβ load was contained in the ML of the dentate gyrus in PDAPP:E4 mice. Localization of Aβ deposition in the ML is associated with the formation of fibrillar amyloid. In contrast, no ML Aβ-IR deposits were found in PDAPP:E3 or PDAPP:E−/− mice 3 months after TBI. *B*, Photomicrographs show Aβ staining in the hippocampus (*arrowheads* delineate the borders of the ML).

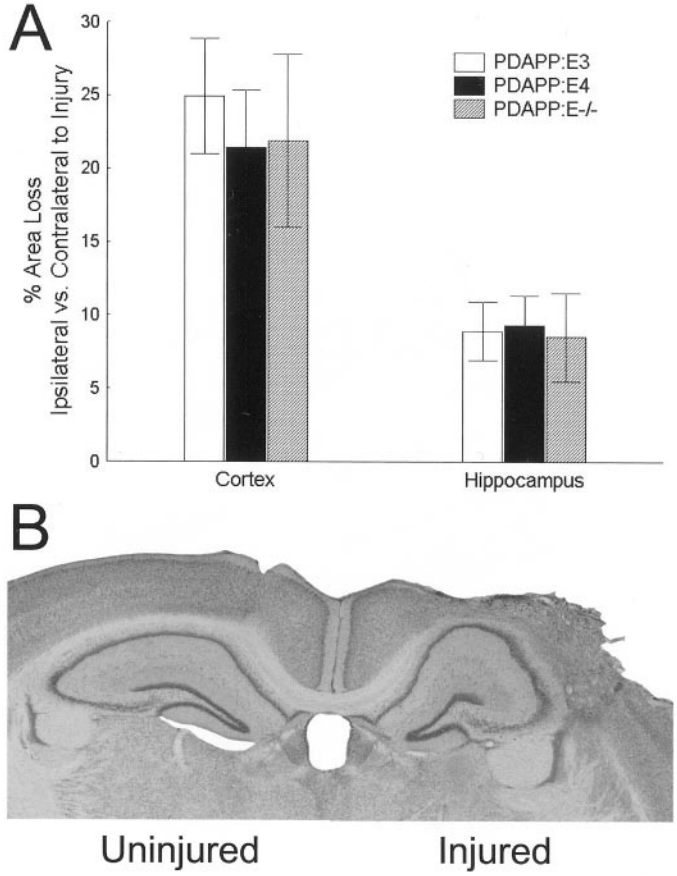

Figure 2. *A*, No group differences were found for percentage of area loss in either the cortex or the hippocampus. *B*, Photomicrograph shows a cresyl violet-stained brain section with atrophy of the cortex and hippocampus in the injured hemisphere 3 months after TBI.

Hippocampal Aβ load

Analysis of all three groups revealed no significant difference between the two hemispheres in the amount of Aβ immunoreactivity. There was a significant main effect of genotype in that, consistent with previous reports (Holtzman et al., 2000; Fagan et al., 2002), PDAPP:E−/− mice had a significantly greater Aβ load than PDAPP:E3 or PDAPP:E4 mice ($p < 0.0001$). However, the Aβ deposits present in PDAPP:E−/− mice consisted of only thioflavine-S-negative, diffuse Aβ (i.e., nonfibrillar, nonamyloid deposits). The amount of diffuse Aβ in PDAPP:E−/− mice after TBI was not clearly increased compared with PDAPP:E−/− mice in the absence of TBI (Holtzman et al., 2000; Fagan et al., 2002). A separate PDAPP:E3 versus PDAPP:E4 analysis revealed a significant main effect of genotype (PDAPP:E4 > PDAPP:E3; $p < 0.05$) but no significant hemisphere effect. Approximately 35% of the hippocampal Aβ load in PDAPP:E4 mice was contained within the ML, whereas no PDAPP:E3 or PDAPP:E−/− mice had ML deposition. Analysis of the percentage of Aβ deposition within the ML of the dentate gyrus revealed a significant main effect of genotype (PDAPP:E4 > PDAPP:E3 and PDAPP:E−/−; $p < 0.006$) (Fig. 1).

Cortical and hippocampal volume estimates

Cortex

After TBI, the cortical volume ipsilateral to impact was significantly less than the contralateral, nonimpacted hemisphere for all groups ($p < 0.0001$) (Fig. 2). A main effect of genotype revealed that PDAPP:E4 mice had slightly but significantly more cortical tissue bilaterally than PDAPP:E3 or PDAPP:E−/− mice, which did not differ ($p < 0.001$). The hemisphere–genotype interaction was not significant. The percentage of tissue loss in the cortex ipsilateral versus contralateral to injury revealed no significant genotype differences.

Hippocampus

After TBI, the hippocampus ipsilateral to impact was significantly smaller than the contralateral hippocampus for all groups ($p < 0.008$) (Fig. 2). A main effect of the genotype revealed that PDAPP:E3 mice had slightly but significantly less overall hippocampal tissue bilaterally than PDAPP:E4 or PDAPP:E−/− mice, which did not differ ($p < 0.001$). The hemisphere–genotype interaction was not significant. The percentage of tissue loss in the hippocampus ipsilateral versus contralateral to injury revealed no significant genotype differences.

Neuronal counts (CA3 inferior blade)

After TBI, the inferior blade of CA3 ipsilateral to injury had significantly fewer neurons (~35% less) than the uninjured hemisphere for all groups ($p < 0.0005$) (Fig. 3). There were no significant genotype main effects or interactions. The percentage of CA3 cell loss revealed no significant genotype differences.

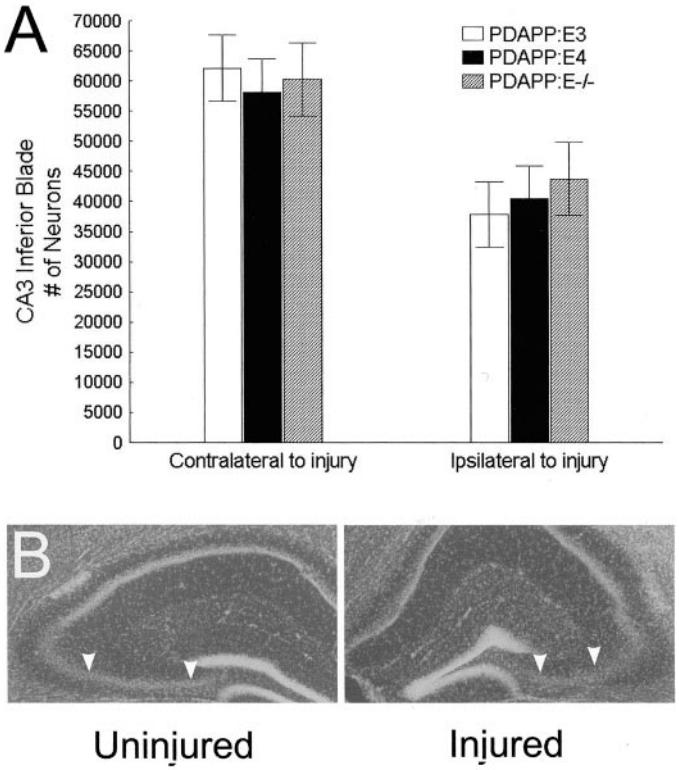

Figure 3. *A*, No group differences were found for neuronal loss within the inferior blade of CA3. *B*, Photomicrograph shows a DAPI-stained brain section revealing atrophy of the CA3 region (*arrowheads* delineate the borders of the CA3 inferior blade) 3 months after TBI.

DISCUSSION

Our previous work (Holtzman et al., 2000; Fagan et al., 2002) demonstrated that Aβ deposition in PDAPP mice that express human *APOE* normally does not begin until ~15 months of age, ≥6 months later than in animals expressing murine *apoE*. The appearance of Aβ-IR deposits by 12–13 months in the current study suggests that TBI accelerated the Aβ deposition process in the presence of human APOE4. Furthermore, only PDAPP:E4 mice had significant Aβ-IR deposits in the ML of the dentate gyrus within 3 months of TBI. These ML deposits are associated with thioflavine-S-positive staining, indicating the conversion of soluble Aβ to a β-sheet conformation and neuritic plaque formation. As in our previous studies, PDAPP:E−/− mice had higher levels of Aβ deposition than PDAPP:E3 or PDAPP:E4 mice, yet none of these deposits consisted of true amyloid. Thus, although the presence of APOE facilitates Aβ fibril formation, human APOE is likely also to play a role in Aβ clearance. Our results suggest that TBI and *APOE4* (compared with *APOE3*) interact to result in greater and earlier amyloid deposition. Overall, these data suggest that the association with *APOE4* and higher risk for cognitive impairment and AD after TBI may in part be attributable to APOE–Aβ interactions.

Human studies have shown that both short- and long-term recovery from TBI seem to be influenced by APOE. *APOE4*+ individuals scored significantly worse on neuropsychological tests 3 weeks after mild to moderate TBI than *APOE4*− individuals (Liberman et al., 2002), and *APOE4* was predictive of longer periods of unconsciousness and worse clinical outcome after TBI (Friedman et al., 1999). Furthermore, *APOE4*+ individuals were twice as likely as *APOE4*− individuals to be dead, comatose, or severely disabled 6 months after TBI (Teasdale et al., 1997). In addition to the poor general clinical outcome associated with *APOE4*, memory performance within 6 months of head injury was worse in *APOE4*+ patients compared with *APOE4*− patients (Crawford et al., 2002), whereas *APOE4* led to worse motor function after TBI (Lichtman et al., 2000). Mild, repetitive head injury also appears to interact with APOE. *APOE4*+ professional boxers had significantly worse neurological scores on a test of chronic brain injury that encompassed cognitive, motor, and behavioral domains than boxers who were *APOE4*− (Jordan et al., 1997). Similarly, older *APOE4*+ professional football players scored lower on cognitive tests than *APOE4*− players (Kutner et al., 2000).

Clinical and experimental TBI is also associated with accelerated Aβ deposition (Roberts et al., 1991), with an even greater effect observed in *APOE4*+ individuals on both parenchymal and vascular Aβ deposits (Nicoll et al., 1995, 1996; Macfarlane et al., 1999; Leclercq et al., 2002). Aβ deposition is also accelerated after seizure-induced neurodegeneration, even in young *APOE4*+ subjects (Gouras et al., 1997). In addition to human studies, the effects of TBI on Aβ and AD pathology have also been studied using TG mouse models of AD. Smith et al. (1998) reported that TBI in PDAPP mice resulted in an 84% loss of CA3 neurons compared with only a 36% loss in non-TG mice. Nakagawa et al. (1999, 2000) have reported that TBI in both young and old PDAPP mice induces atrophy and reduces Aβ deposition in the ipsilateral versus contralateral hippocampus. Aβ deposition after repetitive brain injury using different APP TG mice (Tg2576) and milder cortical impact has also been reported (Uryu et al., 2002). Our study extends these findings and demonstrates the amyloid-promoting effects of human *APOE4*.

How TBI results in an isoform-dependent increase in amyloid deposition is not clear. Both *in vitro* and *in vivo* studies demonstrate that APOE can interact with Aβ and influences the probability of whether Aβ will aggregate in a β-sheet conformation, resulting in neuritic toxicity (for review, see Wisniewski et al., 1997; Holtzman, 2001). The level of apoE plays a significant role in this effect, because mouse apoE regulates Aβ deposition in a gene dose-dependent manner *in vivo* (Bales et al., 1997). The effects of TBI on APOE–Aβ interactions may be secondary to an increase in APOE levels after TBI as well as alterations in APOE-dependent Aβ clearance. An increase in APOE levels has been noted after multiple types of brain injury coincident with glial activation (Teter, 2000). In addition to neuronal degeneration, there is cellular reorganization with increased gliosis and alterations in the vasculature. APOE can potentially interact with different apoE receptors as well as the extracellular matrix. Because both of these factors change in regions of injury, APOE-mediated Aβ clearance may be reduced after TBI, thereby favoring amyloid deposition. It is interesting that amyloid deposits were increased not unilaterally but bilaterally after TBI in the presence of APOE4. This suggests that mechanisms such as changes in *APOE* expression and alterations in APOE-dependent clearance are likely to occur bilaterally in this model of TBI.

The current study, in which the only known difference between the groups of PDAPP mice was the presence or absence of human APOE isoforms, provides evidence that isoform-specific APOE–Aβ interactions contribute to the premature development of AD pathology. Although the promotion of amyloid deposition per se is unlikely to lead to accelerated dementia, the neuritic dystrophy associated with amyloid as well as other events coincident with or downstream of amyloid formation in humans are likely to contribute to cognitive dysfunction. These processes include Aβ oligomer formation, tangle formation, cell loss, and

synaptic loss. Some *in vivo* studies have found that apoE influences aspects of brain function and plasticity after different forms of injury (Fagan et al., 1998; Sheng et al., 1998; Stone et al., 1998; Buttini et al., 1999; Genis et al., 2000; Sabo et al., 2000), including TBI (Chen et al., 1997), and it is possible that APOE influences the outcome after different forms of brain injury via more than one mechanism. Our data suggest that understanding the mechanism(s) by which TBI promotes APOE isoform-dependent amyloid deposition will lead to important insights into how accelerated Aβ-related AD-like changes occur and potential ways to prevent it.

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