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Chronic Thrombin Exposure Results in an Increase in Apolipoprotein-E Levels

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Abstract

Studies have shown that individuals with both a history of traumatic brain injury and inheritance of apolipoprotein E-4 (ApoE4) allele are associated with a poor neurologic outcome and an increased risk for Alzheimer's disease. We assessed the hypothesis that thrombin released during brain injury causes an increase in apolipoprotein-E levels and such increase in the levels of apolipoprotein-E4 isoform may have amyloidogenic effects. Rats received either thrombin (100 nm, 0.25 μ l/hr, 28 days) or vehicle via intracerebroventricular (i.c.v.) infusion. Thrombin treatment increased apolipoprotein-E levels in hippocampus as compared to vehicle treatment ($P < 0.001$). Infusion of human apolipoprotein-E4 (0.6 ng/hr, i.c.v., 56 days) into rats resulted in β -amyloid deposition and increased the number of GFAP-positive astrocytes. ApoE4 infusion also resulted in significant spatial memory deficits. These findings suggest that thrombin released during brain injury may contribute to an increase in apolipoprotein-E levels. Such increase in Apolipoprotein-E4 isoform facilitates β -amyloid deposition and cognitive deficits.

Keywords

Alzheimer's disease; brain injury; apolipoprotein-E4; spatial memory; β -amyloid; cognitive deficits

A major genetic risk factor for sporadic Alzheimer's disease is inheritance of the epsilon-4 allele of apolipoprotein E (Corder et al., 1993; Poirier et al., 1993a; Rebeck et al., 1993; Saunders et al., 1993; Crutcher, 2004). ApoE-4 is also associated with an earlier age of onset of the disease (Corder et al., 1993) and an increased amyloid plaque burden (Mortimer et al., 1991; Schemmel et al., 1993; Sadowski et al., 2004). There is significant evidence suggesting that the interactions between apoE and A β are critical for the development of A β deposits (Strittmatter et al., 1993). Apolipoprotein-E (ApoE) is a 34-kDa lipoprotein synthesized within the CNS that regulates plasma lipid transport and clearance. It is involved in nerve regeneration after injury (Mahley, 1988; Boyles et al., 1989; LeBlanc and Poduslo, 1990).

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Traumatic brain injury (e.g., hemorrhagic stroke, head injury), wherein neurons are exposed to high thrombin levels, is a significant risk factor for AD (Mayeaux et al., 1995). Moreover, this risk increases several times in individuals possessing the ApoE-4 allele relative to people inheriting ApoE-2 and ApoE-3 alleles (Mortimer et al., 1991; Mayeaux et al., 1995; Teasdale et al., 1997). Also, AD risk is significantly greater in ApoE-4 carriers, who have experienced head trauma than in those without a history of head trauma (Kutner et al., 2000). The E4 iso-form is also associated with neurologic deficits in the survivors of traumatic brain injury (Wisniewski et al., 1994; Teasdale et al., 1997; Friedman, 1999) and is a negative risk factor for recovery from head trauma (Friedman et al., 1999; Graham et al., 1999, 2000; Kutner et al., 2000). Aged football players possessing ApoE-4 iso-form have shown significantly lower cognitive performance than E2- or E3-carriers (Kutner et al., 2000). These observations suggest a significant interaction between traumatic brain injury and ApoE4 allele.

We observed previously that chronic exposure to thrombin, which is released at a high concentration after hemorrhagic stroke or traumatic brain injury, produces significant cognitive deficits that can be prevented by direct and specific inhibition of thrombin by hirudin (Mhatre et al., 2004) or thrombin receptor (PAR-1) antagonist (unpublished observations). Thrombin-induced cognitive deficits were associated with ventricular enlargement, increased number of reactive glial fibrillary acidic protein-positive astrocytes and TUNEL-positive cells, and alterations in the phosphorylation state of cytoskeletal proteins suggesting neuronal injury (Mhatre et al., 2004).

Emmerling et al. (2000) observed previously that the levels of A β 1–42 increase in a positive correlation with apolipoprotein-E levels in the cerebrospinal fluid of patients after severe brain injury. Despite the positive correlation between apolipoprotein-E and A β 1–42 levels observed in these patients, it is not yet clear whether the increase in apolipoprotein-E levels after brain injury in ApoE4-carriers contributes to A β deposition. The present study is designed to determine whether chronic exposure to thrombin as observed after brain injury alters apolipoprotein-E levels and whether such alterations in apolipoprotein-E levels in ApoE4-carriers contribute to β -amyloid deposition.

MATERIALS AND METHODS

Study A

Effect of Thrombin on Apolipoprotein-E Levels—The goal of Study A was to determine whether chronic exposure to thrombin contributes to an increase in apoE levels. Six- to eight-month-old Sprague-Dawley male rats (Harlan, Indianapolis, IN) were divided into two groups. Group 1 ($n = 6$) received vehicle (0.35% bovine serum albumin [BSA] in saline, 0.25 μ l/h intracerebroventricular [i.c.v.] for 28 days) via an implanted osmotic pump. Group 2 ($n = 6$) received thrombin (25 nM, 0.25 μ l/h, i.c.v. for 28 days), whereas Group 3 ($n = 6$) received thrombin (100 nM, 0.25 μ l/h, i.c.v. for 28 days). All experiments were carried out in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals.

Rats were implanted with cannulae terminating in the lateral ventricle under anesthesia (Mhatre et al., 2004). The coordinates of the cannulae relative to bregma were: Anterior posterior (AP), -0.9 ; Lateral, -1.5 ; Ventral, -3.5 . Either BSA or thrombin (25 or 100 nM) was delivered at a flow rate of 0.25 μ l/hr for 28 days by subcutaneously implanted osmotic minipump connected to the cannulae. Neurologic examination of all the animals was carried out periodically by an observer blinded to the group assignment. Each rat was assigned a score of 0–4, where 0 = no observable neurologic deficit; 1 = failure to extend the left forepaw; 2 = circling to the left; 3 = falling to the left; and 4 = cannot walk spontaneously.

Immunoblot Analysis for Apolipoprotein-E—At the end of thrombin treatment (28 days), rats were decapitated and hippocampus was dissected. Hippocampus ($n = 6/\text{group}$) was homogenized in the lysis buffer plus protease inhibitors. The homogenates were mixed with sample buffer and heated at 95°C for five min. Equal amounts of protein ($15\text{ }\mu\text{g}/\text{lane}$) were fractionated by 10% SDS PAGE. The electrophoretically separated proteins were then transferred to a nitrocellulose membrane (Millipore, Bedford, MA). The membranes were blocked with 5% nonfat dry milk in Tris buffer saline for 1 hr at room temperature. The membranes were incubated overnight at 4°C with a primary antibody for apolipoprotein E (1:200, AB947, Chemicon, Temecula, CA), washed and subsequently incubated with sheep anti-mouse IgG (1:500, AAM10, Serotec Ltd., Oxford, UK). ApoE and β -actin-immunoreactive bands were visualized with chemiluminescence detection reagents (Amersham, Piscataway, NJ).

Study B

Effect of Human Apolipoprotein-E Infusion on Amyloid Deposition—This experiment sought to test the association between the human apoE4 isoform and amyloid deposition. We infused apoE isoforms at a low concentration of apoE 0.6 ng/hr, 56 days. Six- to eight-month-old Sprague-Dawley male rats (Harlan) were divided into three groups. Group 1 ($n = 9$) received vehicle (0.35% BSA, $0.25\text{ }\mu\text{l}/\text{h}$ i.c.v. for 56 days) via an implanted osmotic pump. These pumps are designed for 28 days treatment. Old pumps were replaced with new pumps containing the same solution on Day 29. Group 2 ($n = 9$) received continuous i.c.v. infusion of human apoE3 (0.6 ng/hr, i.c.v. for 56 days). Group 3 ($n = 8$) received human apoE4 (0.6 ng/hr, i.c.v. for 56 days). The concentration of apoE4 and apoE3 isoforms used in vivo was found to be non-toxic to primary cultured rat cortical neurons. Neurologic examination of all the animals was carried out as described under thrombin treatment, by an observer blinded to the group assignment periodically after the surgery.

Morris Water Maze

Rats in Study B were subjected to Morris water maze using a 150 cm diameter tank. Distal stationary cues were placed around the walls of the room. Rats were placed in a random start site, facing the tank wall. For every trial (during training and testing, 3 trials at every time point), the site of origin was in a different quadrant and the visible platform (5 mm above the surface of water) was also moved to a different quadrant. The distance between the platform and the site of origin always remained the same. Rats were allowed to swim for 70 sec. Latency to reach the platform was measured. Initially rats were trained till they achieved the criterion of reaching the platform in 20 sec or less. The following day and periodically after that, rats were tested for retrieval of the task.

Data Analysis

Latency from six trials for each experimental group was subjected to analysis of variance (ANOVA). Tukey-Kramer test for multiple comparisons was used as a post-hoc test. There was equal variance across all groups for the latency. Latency was analyzed separately over six trials.

Neuropathologic Examination

At the end of behavioral testing and the termination of the vehicle or ApoE-4 or -3 treatment, animals were terminally anesthetized, transcardially perfused with phosphate-buffered saline (PBS), pH 7.4, followed by 10% formalin and decapitated.

Staining for A β and Glial Fibrillary Acidic Protein

For immunohistochemical labeling of A β 1–42, serial brain sections were incubated in 0.5% H₂O₂ for 30 min. Sections were blocked with a blocking solution for 45 min, washed with phosphate buffered saline, incubated with a rabbit polyclonal antibody selective for A β 1–42 (AB5078P, 1:200, Chemicon, Temecula, CA) overnight at 4°C and subsequently with the biotinylated secondary antibody (1:300, BA-1000, Vector Laboratories) for 1 hr at room temperature. Staining was visualized with diaminobenzidine after incubation with streptavidin-peroxidase.

For GFAP labeling, a polyclonal antibody (rabbit) to GFAP (1:200, AB5804, Chemicon) was used. Negative immunohistochemical controls were treated in the same way but in the absence of primary antibody. The number of cells positive for GFAP were counted by an experimenter blind to the treatment in eight topographic regions measuring 0.23 mm² in interhemispheric cortex, lateral hemispheric cortex and in three fields in hippocampus at 20 $\times$ magnification in each of three equally spaced sections at the coordinates described from each rat ($n = 6$ animals/group). We used the Axion Vision program (Carl Zeiss, Thornwood, NY) to digitize the images. The average of the total number of GFAP-positive cells in the cortical and hippocampal areas (three sections each at -2 mm and at -4.8 mm from bregma) was used for statistical analysis.

RESULTS

All animals tolerated thrombin as well as apoE4 and apoE3 treatment based on the neurologic criteria.

Thrombin Treatment Significantly Increases Expression of Apolipoprotein-E

Immunoblot analysis of hippocampal homogenates from vehicle-treated, 25 nM thrombin-treated and 100 nM thrombin-treated rats showed a significant increase ($96 \pm 6\%$) in apolipoprotein E expression (34 kDa band) in thrombin (100 nM)-treated rats in comparison to vehicle-treated animals ($P < 0.001$, $n = 4$ –5 per group, ANOVA) (Fig. 1). Rats treated with a lower concentration of thrombin (25 nM) did not show any significant change in the expression of apolipoprotein-E (data not shown).

Infusion of Human ApoE-4 Results in Cognitive Deficits in Variable Maze Task

Cognitive function of rats was evaluated using a spatial memory task in a variable platform Morris water maze task after infusion with apolipoprotein-E4 or -E3. Figure 2A shows the average latency in the acquisition trials. All the rats reached the criterion for the variable platform maze task in three acquisition trials, though rats that received apolipoprotein-E4 isoform took longer time than the rats from other groups to locate the platform in the first acquisition trial (P -value NS, Fig. 2A). During retrieval, apoE-4 infusion induced overall greater latency in escaping to the platform in comparison to vehicle infused and apoE3-treated animals ($P < 0.001$, on Days 6, 12, 18, and 56 of treatment, $P < 0.05$ on Day 30 of treatment in comparison to the vehicle-treated group, $n = 8$ –9/group) (Fig. 2B). ApoE-3-infused animals showed some impairment in comparison to the vehicle-treated animals ($P < 0.05$ on Days 12, 18, 30, and 56 of treatment in comparison to the vehicle-treated group) but performed better than the animals that received apoE-4 (Fig. 2B).

Human ApoE-4 Infusion Results in Amyloid Deposition in Rats

Figure 3 illustrates immunolabeling for A β of cortical sections of apoE4-infused rats. Cortical sections at a distance of 2 mm posterior to bregma (1.1 mm posterior to the infusion site) of the rats that received apoE4 infusion showed numerous A β deposits surrounded by numerous

microglial cells. Rats with apoE3 infusion showed few diffuse A β deposits (data not shown), whereas vehicle-treated rats did not exhibit any A β deposits.

Human apoE4-Infusion Significantly Increases the Number of GFAP-Positive Cells in Rat Brain

The number of GFAP-positive cells/microscopic field was significantly increased in all the cortical layers and hippocampus of apoE4-treated rats compared to that in vehicle-treated and apoE3-treated rats (vehicle, 0.9 ± 0.7 GFAP-positive cells/field; apoE3, 1.6 ± 0.9 GFAP-positive cells/field; apoE4, 6.1 ± 1.1 GFAP-positive cells/field; $P < 0.001$ [apoE4 vs. BSA and vs. apoE3 in the cortex]) (Fig. 4).

DISCUSSION

A novel and important finding of this study is that chronic thrombin exposure as observed after traumatic brain injury results in an increase in apoE levels in rat hippocampus. It is possible that thrombin-induced neuronal injury (Mhatre et al., 2004) may have contributed to an increase in apoE levels, as apoE is considered to play a role in neuronal repair, synaptic remodeling, and the clearance of cholesterol and lipid debris from the site of injury (Poirier et al., 1993a,b; White et al., 2001). Thus, alterations in apoE may occur, at least initially, as a part of brain's protective response to injury (White et al., 2001).

There are previous reports suggesting upregulation of apoE and changes in its localization after traumatic brain injury (Horseburgh et al., 1997; Schauwecker et al., 1998; Emmerling et al., 2000). There is also significant evidence in the literature stating that variations in apoE occur very rapidly after ischemia, reperfusion or any other kinds of traumatic brain injury (Ali et al., 1996) and these changes in apoE are accompanied by increased expression of amyloid precursor protein (Emmerling et al., 2000). These researchers have also shown that the levels of $\pm$ -amyloid peptide (A β 1–42) increase in the cerebrospinal fluid of patients after traumatic brain injury and remain elevated for some time after the injury and this increase in β -amyloid (A β 1–42) levels in CSF is best correlated with increase in possible markers for neuronal and axonal injury and in apoE levels. This suggests that neurotoxicity associated with increased apoE levels may be crucial to the observed elevation of A β in the CSF of TBI patients. These observations and the data from the present study suggest that the alterations in apoE levels in individuals with apoE4 isoform could have significance in the increased risk for AD after brain injury.

Our model of chronic human apoE-infusion in rats may have some advantage over transgenic mouse models as human apoE does not replace mouse apoE in this model. Some gene-targeted mice expressing human apoE3 have shown increased diet-induced hyperlipidemia, demonstrating that, in mice, human apoE3 is less efficient at clearing lipoproteins than wild-type mouse apoE (Sullivan et al., 1997).

In the present study, exogenously chronically administered human apoE3 did not seem to be neuroprotective as was observed in previous in vitro studies (Barger and Harmon, 1997). On the contrary, we observed cognitive impairment in both apoE3 and apoE4-treated rats that was more pronounced in apoE4-treated rats.

ApoE is considered to be required for the conversion of A β from a soluble form to a pathologic/toxic form(s) with high beta-sheet content. In vitro all apoE isoforms have been reported to increase the β -sheet content of A β peptides by promoting fibril formation (Golabeck et al., 1996; Sanan et al., 1994). In vitro binding experiments, using human apoE and synthetic A β peptides, have consistently shown that these two proteins can form an SDS-resistant complex (Strittmatter et al., 1993; Ma et al., 1994; Castao et al., 1995). Data from apoE knockout/

A β PP (V717F) mice has shown that the presence of apoE is necessary for cerebral amyloid formation (Fagan and Holtzman, 2000). Blockade of apolipoprotein-E/ β -amyloid binding mitigates β -amyloid toxicity and fibril formation in vitro. This also reduces β -amyloid plaques in aging APP (V717F+/-) transgenic mice (Sadowski et al., 2004). Consistent with these studies, we observed significant A β deposition in the cortex of apoE4-treated and few diffuse deposits in apoE3-treated rats in comparison to vehicle-treated rats. This provides a clear evidence of the important role played by apoE-4 isoform in β -amyloid deposition. ApoE4-infused rats showed significant A β deposition indicating its amyloidogenic function. Amyloid deposition after infusion of human apoE4 in rat brain is a novel finding in context to neurodegeneration and the reported increased risk for Alzheimer's disease after traumatic brain injury in apoE4-carriers.

In the present study, apoE-4 infusion in rats also results in an increase in the number of GFAP-positive astrocytes in the cortex. Aged Tg-ApoE4 mice have shown a significantly higher glial inflammatory response evident by an increase in GFAP, TGF- β , and TNF- β expression in response to a neurotoxic stimulus in comparison to the aged wild-type mice (Harry, 2000). ApoE4-induced neuroinflammation as suggested by Harry (2000) could contribute to neurotoxicity and increased cognitive deficits as observed in this study. In other words, apoE isoform-specifically may determine the response to traumatic brain injury, which may be mediated by an increase in apoE levels associated with the injury in ApoE4-carriers.

In conclusion, vascular factors such as thrombin released during traumatic brain injury may play an important role in the upregulation of apoE levels after brain injury. The increase in apoE levels after hemorrhagic stroke or brain injury may initiate a cascade of events leading to A β deposition, neuroinflammation, and cognitive deficits. ApoE isoform-specifically affects neuroinflammation and cognitive deficits. Increased neuroinflammation in ApoE4-carriers may alter the neurologic outcome after hemorrhagic stroke or traumatic head injury. Our data suggests that thrombin released during brain injury may play an important role in this sequence of events and therefore, the inhibition of thrombin may be considered as one of the strategies to improve the neurologic outcome after brain injury.

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References

- Ali SM, Dunn E, Oostveen JA, Hall ED, Carter DB. Induction of apolipoprotein E mRNA in the hippocampus of the gerbil after transient global ischemia. *Brain Res Mol Brain Res* 1996;38:37-44. [PubMed: 8737665]
- Barger SW, Harmon AD. Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. *Nature* 1997;388:878-81. [PubMed: 9278049]
- Boyles JK, Zoellner CD, Anderson LJ, Kosik LM, Pitas RE, Weisgraber KH, Hui DY, Mahley RW, Gebicke-Haerter PJ, Ignatius MJ. A role for apolipoprotein E, apolipoprotein A-I, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. *J Clin Invest* 1989;83:1015-31. [PubMed: 2493483]
- Castao EM, Prelli F, Pras M, Frangione B. Apolipoprotein E carboxyl-terminal fragments are complexed to amyloids A and L. *J Biol Chem* 1995;270:17610-17615. [PubMed: 7615568]
- Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993;261:921-923. [PubMed: 8346443]

- Crutcher KA. Apolipoprotein E is a prime suspect, not just an accomplice, in Alzheimer's disease. *J Mol Neurosci* 2004;23:181–188. [PubMed: 15181246]
- Emmerling MR, Morganti-Kossmann MC, Kossmann T, Stahel PF, Watson MD, Evans LM, Mehta PD, Spiegel K, Kuo YM, Roher AE, Raby CA. Traumatic brain injury elevates the Alzheimer's amyloid peptide A beta 42 in human CSF: a possible role for nerve cell injury. *Ann N Y Acad Sci* 2000;903:118–122. [PubMed: 10818496]
- Fagan AM, Holtzman DM. Astrocyte lipoproteins, effects of apoE on neuronal function, and role of apoE in amyloid-beta deposition in vivo. Targeted replacement of the mouse apolipoprotein E gene with the common human APOE3 allele enhances diet-induced hypercholesterolemia and atherosclerosis. *Microsc Res Tech* 2000;50:297–304. [PubMed: 10936884]
- Friedman G, Froom P, Sazbon L, Grinblatt I, Shochina M, Tsenter J. Apolipoprotein E-epsilon 4 genotype predicts a poor outcome in survivors of traumatic brain injury. *Neurology* 1999;52:244–248. [PubMed: 9932938]
- Golabek AA, Soto C, Vogel T, Wisniewski T. The interaction between apolipoprotein E and Alzheimer's amyloid β -peptide is dependent on β -peptide conformation. *Biol Chem* 1996;271:10602–10606.
- Graham DI, McIntosh TK, Maxwell WL, Nicoll JA. Recent advances in neurotrauma. *J Neuropathol Exp Neurol* 2000;59:641–651. [PubMed: 10952055]
- Graham DI, Gentleman SM, Nicoll JA, Royston MC, McKenzie JE, Roberts GW, Mrak RE, Griffin WS. Is there a genetic basis for the deposition of beta-amyloid after fatal head injury? *Cell Mol Neurobiol Res* 1999;19:19–30.
- Harry GJ, Lefebvre d'Hellencourt C, Bruccoleri A, Schmechel D. Age-dependent cytokine responses: trimethyltin hippocampal injury in wild-type, APOE knockout, and APOE4 mice. *Brain Behav Immun* 2000;14:288–304. [PubMed: 11120597]
- Horseburgh K, Fitzpatrick M, Nilsen M, Nicoll JAR. Marked alterations in the cellular localization and levels of apolipoprotein E following acute subdural hematoma in rat. *Brain Res* 1997;763:103–10. [PubMed: 9272834]
- Kutner KC, Erlanger DM, Tsai J, Jordan B, Relkin NR. Lower cognitive performance of older football players possessing apolipoprotein E [epsilon]-4. *Neurosurgery* 2000;47:651–658. [PubMed: 10981753]
- LeBlanc AC, Poduslo JF. Regulation of apolipoprotein E gene expression after injury of the rat sciatic nerve. *J Neurosci Res* 1990;25:162–171. [PubMed: 2319626]
- Ma J, Yee A, Brewer HB Jr, Das S, Potter H. Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature* 1994;372:92–94. [PubMed: 7969426]
- Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988;240:622–630. [PubMed: 3283935]
- Mayeaux R, Ottman R, Maestre G, Ngai C, Tang MX, Ginsberg H, Chun M, Tycko B, Shelanski M. Synergistic effects of traumatic head injury and apolipoprotein-epsilon 4 in patients with Alzheimer's disease. *J Neurol Res* 1995;45:555–557.
- Mhatre M, Nguyen A, Kashani S, Pham T, Adesina A, Grammas P. Thrombin, a mediator of neurotoxicity and memory impairment. *Neurobiol Aging* 2004;25:783–793. [PubMed: 15165703]
- Mortimer JA, van Duijn CM, Chandra V, Fratiglioni L, Graves AB, Heyman A, Jorm AF, Kokmen E, Kondo K, Rocca WA, et al. Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. *Int J Epidemiol* 1991;20:S28–S35. [PubMed: 1833351]Suppl
- Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S. Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet* 1993a;342:697–699. [PubMed: 8103819]
- Poirier J, Baccichet A, Dea D, Gauthier S. Cholesterol synthesis and lipoprotein reuptake during synaptic remodeling in hippocampus in adult rats. *Neuroscience Res* 1993b;55:81–90.
- Rebeck GW, Reiter JS, Strickland DK, Hyman BT. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron* 1993;11:575–580. [PubMed: 8398148]
- Sadowski M, Pankiewicz J, Scholtzova H, Ripellino JA, Li Y, Schmidt SD, Mathews PM, Fryer JD, Holtzman DM, Sigurdsson EM, Wisniewski T. A synthetic peptide blocking the apolipoprotein E/

- beta-amyloid binding mitigates beta-amyloid toxicity and fibril formation in vitro and reduces beta-amyloid plaques in transgenic mice. *Am J Pathol* 2004;165:937–948. [PubMed: 15331417]
- Sanan DA, Weisgraber KH, Russell SJ, Mahley RW, Huang D, Saunders A, Schmechel D, Wisniewski T, Frangione B, Roses AD. Apolipoprotein E associates with beta amyloid peptide of Alzheimer's disease to form novel monofibrils. Isoform apoE4 associates more efficiently than apoE3. *J Clin Invest* 1994;94:860–869. [PubMed: 8040342]
- Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ, et al. Association of apolipoprotein E allele 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 1993;43:1467–1472. [PubMed: 8350998]
- Schauwecker PE, Cogen JP, Jiang T, Cheng HW, Collier TJ, McNeill TH. Differential regulation of astrocytic mRNAs in the rat striatum after lesions of the cortex or substantia nigra. *Exp Neurol* 1998;149:87–96. [PubMed: 9454618]
- Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci USA* 1993;90:9649–9653. [PubMed: 8415756]
- Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc Natl Acad Sci USA* 1993;90:1977–1981. [PubMed: 8446617]
- Sullivan PM, Mezdour H, Aratani Y, Knouff C, Najib J, Reddick RL, Quarfordt SH, Maeda N. Targeted replacement of the mouse apolipoprotein E gene with the common human APOE3 allele enhances diet-induced hypercholesterolemia and atherosclerosis. *J Biol Chem* 1997;272:17972–17980. [PubMed: 9218423]
- Teasdale GM, Nicoll JAR, Murray G, Fiddes M. Association of apolipoprotein E polymorphism with outcome after head injury. *Lancet* 1997;350:1069–1071. [PubMed: 10213549]
- White F, Nicoll JA, Horsburgh K. Alterations in ApoE and ApoJ in relation to degeneration and regeneration in a mouse model of entorhinal cortex lesion. *Exp Neurol* 2001;169:307–318. [PubMed: 11358444]
- Wisniewski T, Castano EM, Golabek A, Vogel T, Frangione B. Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. *Am J Pathol* 1994;145:1030–1035. [PubMed: 7977635]

Fig. 1.

A: Western blot analysis of ApoE from hippocampal homogenates of vehicle-treated (lanes 1–4), and 100 nM (0.6 pmol/day, 28 days) thrombin-treated (lanes 5–9) rats. **B:** The bar graph indicates mean ApoE band intensity normalized to β -actin $\pm$ SD, $n = 4-5$, $P < 0.001$.

Fig. 2.

A: Effect of ApoE isoforms on the training performance in Morris water maze (mean $\pm$ SEM escape latencies to a visible platform in the training trials). Rats received either 1) apoE4 (0.6 ng/hr, i.c.v. for 56 days, closed circles, $n = 8$), or 2) apoE3 (0.6 ng/hr, i.c.v. for 56 days, hollow circles, $n = 9$), or 3) BSA (0.35% in saline, 0.25 μ l/h i.c.v. for 56 days, triangles, $n = 9$). **B:** Latency to find a visible platform in the test trials. Test trials were conducted on the day after the initial training and every sixth day after that. * $P < 0.05$, ** $P < 0.001$.

Fig. 3. Immunohistochemical labeling using specific antibody to A β 42 (AB5078P) to detect amyloid deposits in the cerebral cortex after either infusion of vehicle or apoE4 (0.6 ng/hr, i.c.v., 56 days). Arrows indicate amyloid deposits. Magnification = 40 $\times$.

Fig. 4. GFAP-labeling after vehicle (0.35% BSA, 0.25 μ l/hr, 28 days) or apoE3 (0.6 ng/hr, i.c.v., 56 days) or apoE4 (0.6 ng/hr, i.c.v., 56 days) treatment. Arrows indicate GFAP-labeled reactive glial cells after apoE3 and apoE4 treatment. Magnification = 20 $\times$.