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Impairments in spatial memory retention of GFAP-apoE4 female mice

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Abstract

The human apolipoprotein E isoforms, apoE2, apoE3, and apoE4, have differential effects on brain function. Compared to apoE3, apoE4 increases the risk of age-related cognitive decline in humans and female mice expressing apoE in neurons. Here we show impaired spatial memory retention in female mice expressing apoE4 in astrocytes compared to those expressing apoE3 in astrocytes or lacking apoE. Thus, apoE4 impairs cognition whether expressed in neurons or astrocytes.

Keywords

water maze; passive avoidance; rotorod; apolipoprotein E; transgenic mice

Human apolipoprotein E is a protein encoded by three distinct alleles: $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$. The major resulting proteins, apoE2, apoE3 and apoE4 play an important role in the redistribution and metabolism of lipoproteins and cholesterol. In the brain, apoE is involved in development, regeneration, neurite extension and neuroprotection [11]. ApoE is associated with the pathological hallmarks of Alzheimer's disease (AD) and the severity of AD pathology is influenced by *APOE* genotype. ApoE4 also has detrimental effects on AD-like pathology in patients with other conditions, including progressive nuclear palsy [20] and Down's syndrome [4]. The detrimental effects of apoE4 are not limited to AD. Compared to apoE3, apoE4 also increases the risk of developing cognitive impairments after neurotrauma, ischemia, cardiopulmonary bypass surgery, human immunodeficiency virus infection, and cognitive impairments that occur with the normal aging and in the context of Parkinson's disease (for review, [15]). Various mechanisms have been proposed to mediate the differential effects of apoE isoforms on brain function, including effects on cholesterol transport, the metabolism amyloid β peptide, cell signaling by lipoprotein receptors, on proteolysis of apoE, and androgen receptor function (for review, [15]). Most synthesis of apoE in the brain takes place in glial cells, in particular astrocytes. However, neurons are also able to generate apoE, particularly following injury [1,5,8,21,22]. In addition, apoE can be secreted from astrocytes and be taken up by neurons. The cellular source of apoE might be important for isoform-dependent effects of apoE on brain function. Proteolytic apoE fragments and tangle-like inclusions were seen

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when apoE was expressed in neurons in culture or in neurons in transgenic mice but not when it was expressed in non-neuronal cells in culture or non-neuronal cells in transgenic mice [10]. Consistent with these data, a role for astrocytic apoE has been suggested in mitochondrial dysfunction and neurofibrillary tangle formation, while a role for neuronal apoE has been suggested in amyloid plaque formation [22].

ApoE4 has been identified as a risk factor for Alzheimer's Disease (AD), particularly in women, when compared to apoE3 [6]. Hartman and colleagues showed that 11–14 month-old transgenic male mice expressing human apoE4 in astrocytes under control of the glial fibrillary acidic protein (GFAP) promoter showed impairments in radial arm maze performance when compared to wild-type and mouse apoE deficient mice (*Apoe*^{-/-}) [9]. In contrast, these impairments were not seen in mice expressing human apoE3 in astrocytes [9]. No cognitive impairments were seen in 10–13 month-old GFAP-apoE4 male mice in spatial learning and memory in the water maze [9]. Consistent with these data, no impairments in water maze performance were seen in 6 or 18-month-old apoE4 male mice expressing human apoE4 in neurons [17,18]. In contrast, 6-month-old female mice expressing apoE4 in neurons or astrocytes under control of the neuron specific enolase (NSE) or GFAP promoter, respectively, showed impairments in spatial memory retention in the probe trial following three day of hidden platform training in the water maze compared to sex- and age-matched *Apoe*^{-/-} mice [17,18]. Such impairments were not seen in 6-month-old NSE-apoE3 female mice [14,16–18]. The sex-dependency of the effects of apoE4 on cognitive function might involve androgen and androgen receptor-mediated signaling [15,16]. While in earlier studies 6-month-old GFAP-apoE4 female mice and nontransgenic sex-matched *Apoe*^{-/-} littermates were compared behaviorally, 6-month-old GFAP-apoE3 and apoE4 female mice were not compared side-by-side and potential cognitive impairments in earlier probe trials during the hidden platform training were not assessed. In the current study, to determine whether there are isoform-dependent effects of apoE expressed in astrocytes on cognitive function in female mice, we compared cognitive performance of six-month-old female GFAP-apoE3, GFAP-apoE4 and *Apoe*^{-/-} littermate controls.

GFAP-apoE mice, generated as described [16], were backcrossed onto the C57Bl6/J background for at least ten generations. The mice were kept on a 12:12 h light–dark schedule (lights on at 6AM) with lab chow (PicoLab Rodent Diet 20, #5053; PMI Nutrition International, St. Louis, MO) and water given *ad libitum*. Six-month-old GFAP-apoE3 (n = 9), GFAP-apoE4 (n = 8), and *Apoe*^{-/-} (n = 6) female mice were housed singly beginning 48 h prior to the first behavioral test. All procedures were according to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Oregon Health and Science University. To assess sensorimotor ability, the mice were tested using a rotorod (Hamilton-Kinder) over the course of three subsequent days with three trials each. Mice were placed on the elevated rod that rotated at 4 rpm. Every 15 s the rpm was increased by 4 rpm over a 3 s period. The fall latency was recorded by a timer and indicated when the mouse fell of the rod and broke the photo beams at the bottom of the chamber. All animals showed an effect of training over the 9 trials ($F = 5.998$, $p = 0.001$). GFAP-apoE4 mice performed worse than GFAP-apoE3 mice (Fig. 1C, $p < 0.05$) with an average fall latency of 41.57 ± 5.64 sec versus 58.89 ± 4.43 sec, respectively. *Apoe*^{-/-} mice had an average fall latency of 57.87 ± 4.84 sec, which was similar to that of GFAP-apoE3 mice ($p = 0.4501$) but not significantly different from apoE4 mice ($p = 0.0746$). In contrast to the comparable performance of 6-month-old GFAP-apoE3 and *Apoe*^{-/-} female mice, 6-month-old NSE-apoE3 female mice performed better on the rotorod than *Apoe*^{-/-} mice, reaching an average fall latency of 106 ± 19 sec versus 54 ± 7 sec ($p < 0.05$) [17]. These results indicate that the cell type expressing apoE is critically important in rotorod performance. When apoE is expressed in neurons, but not when it is expressed in astrocytes, apoE3 mice show better

rotorod performance than *ApoE*^{-/-} mice. When apoE is expressed in astrocytes, but not when it is expressed in neurons, apoE4 mice show worse rotorod performance than apoE3 mice.

A modified version of the Morris water maze [3,12] was used to assess spatial learning and memory. A circular pool (diameter 140 cm) was filled with opaque water (24°C) and mice were trained to locate a submerged platform in order to escape from the water (luminescence: 40 lx). First they were trained to locate a clearly marked platform (days 1 and 2). Subsequently, they were trained to locate a platform hidden beneath the surface of water made opaque using a white chalk (days 3–5). During the training with the hidden platform, the mice had to navigate to it using the available spatial cues. There were two daily sessions 3.5 h apart, each consisting of three 60 sec trials (with 10–15 min inter-trial intervals). Mice that failed to find the platform within 60 sec were led to the platform by the experimenter and allowed to stay on the platform for 3 sec. During the visible platform training, the platform was moved to a different quadrant of the pool for each session. During the hidden platform training, the platform location was kept constant for each group of mice. The mice were assigned to four groups using a randomized block design to avoid any potential quadrant bias. Mice were placed into the water facing the wall at the side of the pool in 9 different locations around the pool circumference, and the starting location was changed for each trial. The swimming patterns of the mice were recorded with the Noldus Ethovision video tracking system, set at 6 samples/sec. Distance moved to reach the platform, swim speeds, and cumulative distance to platform (distance to the platform location summed over all samples of a given trial) [7] were analyzed for the water maze learning curves. Probe trials assessed spatial memory retention 1 h after the last hidden platform training trial of each day of hidden training (total of 3 probe trials). The cumulative distance to the location where the platform was located during the hidden sessions was analyzed for the probe trials, as described. For the probe trials, mice were dropped in the quadrant opposite from the target quadrant.

Since there was an effect of genotype on the swim speeds during the sessions with the visible platform (*ApoE*^{-/-}: 16.9 ± 0.3 cm/sec; GFAP-apoE3: 14.9 ± 0.3 cm/sec; GFAP-apoE4: 14.4 ± 0.4 cm/sec, $F = 7.053$, $p < 0.005$), distance moved to reach the platform was used as performance measure for the learning curves as it is not affected by velocity. All groups successfully learned to find the platform in the sessions with the visible (effect of session, $F = 20.408$, $p < 0.0001$) and hidden platform (effect of session, $F = 22.452$, $p < 0.0001$). However, there were no genotype differences in distance moved during the visible or hidden sessions. Similarly, there were no genotype differences in cumulative distance to the platform during the visible or hidden sessions. These data are consistent with the comparable water maze learning curves of 6-month-old NSE-apoE3, NSE-apoE4, and *ApoE*^{-/-} female mice [16,18].

The probe trials showed that all mice improved on the use of a spatial strategy to navigate to the target quadrant between probe trials (ANOVA, effect of probe, $F = 7.078$, $p < 0.005$ (Fig. 1A)). However, GFAP-apoE3 mice performed better than GFAP-apoE4 mice since they swam closer to the platform location over the three probe trials (ANOVA, probe x genotype interaction, $F = 4.923$, $p < 0.05$ (Fig. 1A)). In contrast, there were no differences in this measure between GFAP-apoE3 and *ApoE*^{-/-} mice (ANOVA, effect of genotype, $F = 0.088$, $p = 0.772$), as, in contrast to GFAP-apoE3 mice, *ApoE*^{-/-} mice did not improve their performance in the second probe trial compared to the first probe trial. However, in the third probe trial *ApoE*^{-/-} mice swam closer to the platform location than GFAP-apoE4 ($p < 0.005$), but not than GFAP-apoE3 mice ($p = 0.248$). Similarly, summing the cumulative distance over the three probe trials revealed that GFAP-apoE4 mice swam further from the platform location than GFAP-apoE3 mice ($p < 0.05$) and *ApoE*^{-/-} mice ($p < 0.02$, Fig. 1B). In contrast, GFAP-apoE3 mice performed similar as *ApoE*^{-/-} on this measure ($p > 0.1$). The poorer performance of GFAP-apoE4 than *ApoE*^{-/-} female mice in spatial memory retention in the water maze probe trials is

consistent with a pathogenic gain of function of apoE4 also seen when apoE4 is expressed in neurons of female mice [16–18].

Finally, emotional learning and memory was assessed using the passive avoidance test. Each mouse was placed in a lighted compartment of a chamber also containing a dark compartment (Hamilton-Kinder, Poway, CA). After 5 sec of acclimation, the bright house light turned on and the connecting gate to the dark compartment opened. The mouse preferring the darkened left side, steps quickly through the gate to enter the dark compartment. Upon doing so, the mouse received a brief and slight foot shock (0.3 mA for 3 sec). Each mouse was trained until it met a learning criterion of three consecutive 120-sec trials without entering the dark compartment, or up to 10 trials, whichever came first. There were no genotype difference in trials to criterion (Fig. 1D). After 24 h, each mouse was again placed in the light compartment, and the time to re-enter the dark compartment measured up to 300 seconds. There were no genotype differences in reentering the dark compartment 24 h later (Fig. 1E). These results are in accordance with comparable passive avoidance memory retention in 6-month-old NSE-apoE3, NSE-apoE3, and *ApoE*^{-/-} mice [17]. As both water maze and passive avoidance learning and memory are sensitive to hippocampal lesions [13,19], these data suggest that apoE4 does not cause a general hippocampus-dependent cognitive impairment.

The increased susceptibility of ϵ 4-carrying women and female mice expressing apoE4 compared the ϵ 4-carrying men and male mice expressing apoE4 might be due to the ability of androgens and androgen-receptor-mediated signaling to protect against the detrimental effects of apoE4 on cognition. Brief periods of androgen treatment antagonized cognitive impairments in adult apoE4 female mice and acute androgen receptor blockade caused impairments in apoE4, but apoE3, male mice [16]. In addition, cytosolic androgen receptor binding in the cortex of apoE4 mice was lower than that in the cortex of apoE3 and *ApoE*^{-/-} mice [16]. Finally, reducing the levels of circulating androgens in male mice by castration caused apoE-isoform-dependent effects on cognitive function [14]. As castration of apoE4 male mice did not recapitulate the severity of cognitive effects seen in age-matched apoE4 female mice, effects of sex steroids on sex differences in brain organization might play a role [2].

In conclusion, our data show 6-month-old GFAP-apoE4 female mice show poorer spatial memory retention than age- and sex-matched GFAP-apoE3 and *ApoE*^{-/-} mice. These data support that the detrimental effects of apoE4 on spatial memory retention in the water maze is independent of the cellular source of apoE.

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Literature References

1. Bao F, Arai H, Matsushita S, Higuchi S, Sasaki H. Expression of apolipoprotein E in normal and diverse neurodegenerative disease brain. *Neuro Report* 1996;7:1733–1739.
2. Beatty WW. Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences. *Horm Behav* 1979;12:112–163. [PubMed: 573741]
3. Benice T, Rizk A, Pfankuch T, Kohama S, Raber J. Sex-differences in age-related cognitive decline in C57BL/6J mice associated with increased brain microtubule-associated protein 2 and synaptophysin immunoreactivity. *Neuroscience* 2006;137:413–423. [PubMed: 16330151]
4. Del Bo R, Comi GP, Bresolin N, Castelli E, Conti E, Degiuli A, Ausenda CD, Scarlato G. The apolipoprotein E ϵ 4 allele causes a faster decline of cognitive performances in Down's syndrome subjects. *J Neurol Sci* 1997;145:87–91. [PubMed: 9073034]

5. Diedrich JF, Minnigan H, Carp RI, Whitaker JN, Race R, Frey W, Haase AT. Neuropathological changes in scrapie and Alzheimer's disease are associated with increased expression of apolipoprotein E and cathepsin D in astrocytes. *J Virol* 1991;65:4759–4768. [PubMed: 1870200]
6. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. *J Am Med Assoc* 1997;278:1349–1356.
7. Gallagher M, Burwell R, Burchinal M. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci* 1993;107:1–16.
8. Han SH, Einstein G, Weisgraber KH, Strittmatter WJ, Saunders AM, Pericak-Vance M, Roses AD, Schmechel DE. Apolipoprotein E is localized to the cytoplasm of human cortical neurons: A light and electron microscopic study. *J Neuropathol Exp Neurol* 1994;53:535–544. [PubMed: 8083695]
9. Hartman RE, Wozniak DF, Nardi A, Olney JW, Sartorius L, Holtzman DM. Behavioral phenotyping of GFAP-apoE3 and -apoE4 transgenic mice: apoE4 mice show profound working memory impairments in the absence of Alzheimer's-like neuropathology. *Exp Neurol* 2001;170:326–344. [PubMed: 11476599]
10. Huang Y, Qin Lu X, Wyss-Coray T, Brecht W, Sanan D, Mahley RW. Apolipoprotein E fragments present in Alzheimer's disease brain induce neurofibrillary tangle-like intracellular inclusions in neurons. *Proc Natl Acad Sci USA* 2001;98:8838–8843.
11. Mahley R, Huang Y. Apolipoprotein E: From atherosclerosis to Alzheimer's disease and beyond. *Curr Opin Lipidol* 1999;10:207–217. [PubMed: 10431657]
12. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 1984;11:47–60. [PubMed: 6471907]
13. Morris R, Garrud P, Rawlins J, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. *Nature* 1982;297:681–683. [PubMed: 7088155]
14. Pfankuch T, Rizk A, Olsen R, Poage C, Raber J. Role of circulating androgen levels in effects of apoE4 on cognitive function. *Cognitive Brain Res* 2005;1053:88–96.
15. Raber J. Differential gene actions of polymorphic alleles at the APOE locus; potential role of androgens and androgen receptor-mediated signaling. *Science of Aging (SAGE) Knowledge Environment KE*. 2004posted March 17, 2004
16. Raber J, Bongers G, LeFevour A, Buttini M, Mucke L. Androgens protect against apolipoprotein E4-induced cognitive deficits. *J Neurosci* 2002;22:5204–5209. [PubMed: 12077215]
17. Raber J, Wong D, Buttini M, Orth M, Bellosta S, Pitas RE, Mahley RW, Mucke L. Isoform-specific effects of human apolipoprotein E on brain function revealed in *ApoE* knockout mice: increased susceptibility of females. *Proc Natl Acad Sci USA* 1998;95:10914–10919. [PubMed: 9724804]
18. Raber J, Wong D, Yu GQ, Buttini M, Mahley RW, Pitas RE, Mucke L. Alzheimer's disease: Apolipoprotein E and cognitive performance. *Nature* 2000;404:352–354. [PubMed: 10746713]
19. Stubble-Weatherly L, Harding J, Wright J. Effects of discrete kainic acid-induced hippocampal lesions on spatial and contextual learning and memory in rats. *Brain Res* 1996;716:29–38. [PubMed: 8738217]
20. Tsuboi Y, Joseph K, Cookson N, Dickson D. APOE E4 is a determinant for Alzheimer pathology in progressive supranuclear palsy. *Neurology* 2003;60:240–245. [PubMed: 12552038]
21. Xu PT, Gilbert JR, Qui H, Ervin J, Rothrock-Christian TR, Hulette C, Schmechel DE. Specific regional transcription of apolipoprotein E in human brain neurons. *Am J Pathol* 1999;154:1–11. [PubMed: 9916910]
22. Xu Q, Walker BAD, Kanegawa T, Mahley RW, Y H. Profile and regulation of apolipoprotein E (apoE) expression in the CNS in mice with targeting of green fluorescent protein gene to the apoe locus. *J Neurosci* 2006;26:4985–4994. [PubMed: 16687490]

Figure 1.

GFAP-apoE4 female mice showed poorer spatial learning and memory in the water maze than GFAP-apoE3 or littermate control *ApoE*^{-/-} female mice. **A.** Across the probe trials, GFAP-apoE4 female mice ($n = 8$) swam further away from the platform location ($p < 0.05$) than GFAP-apoE3 female mice ($n = 9$). While there was no overall significant difference in the cumulative distance to the target across the three probe trials of GFAP-apoE4 and *ApoE*^{-/-} female ($n = 6$) mice, *ApoE*^{-/-} female mice showed a lower cumulative distance in the third probe trial than GFAP-apoE4 female mice ($p < 0.005$). **B.** The total cumulative distance over the three probe trials showed that GFAP-apoE4 female mice swam further away from the platform location than GFAP-apoE3 or *ApoE*^{-/-} female mice ($p < 0.05$). **C.** GFAP-apoE4 female mice fell off the rotating rod sooner than GFAP-apoE3, but not than *ApoE*^{-/-}, female mice ($p < 0.05$). **D.** GFAP-apoE4, GFAP-apoE3, and *ApoE*^{-/-} female mice required a comparable number of passive avoidance learning trials to reach the criterion. **E.** GFAP-apoE4, GFAP-apoE3, and *ApoE*^{-/-} female mice showed comparable passive avoidance memory retention 24 h following training. All values are shown as mean $\pm$ SEM, * $p < 0.05$, ** $p < 0.005$. $n = 6$ *ApoE*^{-/-} mice, $n = 9$ GFAP-apoE3, and $n = 8$ GFAP-apoE4 female mice.