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The Role of Apolipoprotein E and Ethanol Exposure in Age-Related Changes in Choline Acetyltransferase and Brain-Derived Neurotrophic Factor Expression in the Mouse Hippocampus

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Abstract

Disruption of apolipoprotein E (APOE) is responsible for age-dependent neurodegeneration and cognitive impairment. Elderly individuals are more sensitive than young individuals to the effects of ethanol (EtOH), particularly those affecting cognition. We investigated the role of APOE deficiency and EtOH exposure on age-dependent alterations in choline acetyltransferase (ChAT) and brain-derived neurotrophic factor (BDNF) mRNA and protein expression in the mouse hippocampus. Three-month-old (young) and 12-month-old (aged) *ApoE*-knockout (*ApoE*-KO) and wild-type (WT) mice were treated with saline or 2 g/kg EtOH, and the bilateral hippocampus was collected after 60 min for real-time PCR and western blotting analyses. ChAT ($P < 0.01$) and BDNF ($P < 0.01$) expression were significantly decreased in both young and aged saline- and EtOH-treated *ApoE*-KO mice versus young and aged saline- and EtOH-treated WT mice. Aged saline- and EtOH-treated *ApoE*-KO mice exhibited greater differences in ChAT and BDNF expression ($P < 0.01$) than young saline- and EtOH-treated *ApoE*-KO mice. Aged EtOH-treated WT mice also exhibited larger decreases in BDNF expression ($P < 0.01$)—but not in ChAT expression—than young EtOH-treated WT mice. EtOH decreased ChAT and BDNF expression in both young ($P < 0.01$) and aged ($P < 0.01$) *ApoE*-KO mice versus EtOH-free *ApoE*-KO mice of the same age. EtOH also decreased BDNF expression in aged ($P < 0.01$) WT mice versus EtOH-free aged WT mice. In summary, these results suggest that APOE deficiency and EtOH exposure cause age-dependent decreases in ChAT and BDNF in the hippocampus. Importantly, the decreases in ChAT and BDNF were greater in aged EtOH-treated mice, particularly those lacking APOE, raising the possibility that APOE-deficient individuals who consume alcohol may be at greater risk of memory deficit.

Keywords *ApoE*-KO mice · Aging · Ethanol · Choline acetyltransferase · Brain-derived neurotrophic factor

Introduction

Apolipoprotein E (APOE) is a cholesterol carrier in the brain with roles in lipid transport and injury repair (Chen et al. 1997; Mahley and Rall 2000). This protein has recently been identified as a risk factor for several neurodegenerative diseases (Giau et al. 2015). Compared with healthy mice, *ApoE*-knockout (*ApoE*-KO) mice exhibit specific synapse loss in cholinergic (http://topics.sciencedirect.com/topics/page/Cholinergic), noradrenergic (http://topics.sciencedirect.com/topics/page/Norepinephrine), and serotinergic projections to relevant brain regions (Chapman and Michaelson 1998) and perform worse in memory tasks (Gordon et al. 1995; Dodart et al. 2000). APOE isoforms differentially regulate maturation and the secretion of brain-derived neurotrophic factor (BDNF) from primary human astrocytes (Sen et al. 2017). Disruption of BDNF contributes to age-related cognitive decline (Linnarsson et al. 1997). Both BDNF and APOE are involved in neuronal processes, such as cell growth, resilience to noxious stimuli, and synaptic plasticity, and their disruption is considered to be a risk factor for Alzheimer's disease (AD; Hashimoto et al. 2009; Richter-Schmidinger et al. 2011; Lim et al. 2015). Aging is the main risk factor for diseases, including cancer, diabetes, heart disease, and AD (Niccoli and Partridge 2012). Age-related cognitive deficits are partly

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explained by changes in neural plasticity and synaptic activity (Seidler et al. 2010). Therefore, we examined *ApoE*-KO mice to elucidate the age-dependent role of APOE in choline acetyltransferase (ChAT) and BDNF expression in the hippocampus.

Several investigations have studied the effects of ethanol (EtOH) on memory acquisition (White et al. 2000; Acheson et al. 2001). EtOH consumption increases the risk of AD in individuals with the *ApoE* e4 allele (Anttila et al. 2004; Kivipelto et al. 2008). Elderly individuals are more sensitive than young individuals to the effects of EtOH, particularly those on cognition (Swartzwelder et al. 1995). It has also been reported that spatial memory in aging adult rats is increasingly affected by EtOH (Rajendran and Spear 2004). Numerous studies have presented evidence of a link between the modulation of brain morphology and APOE expression (Cohen et al. 2001; den Heijer et al. 2002; Love et al. 2006; Espeseth et al. 2008), which suggests that APOE plays a pivotal role in aging. Disruption of APOE has an impact on longevity (Ang et al. 2008; Bonomini et al. 2010) and is involved in several age-related diseases (Bonomini et al. 2010). In addition, *ApoE*-KO mice exhibit an age-related decline in presynaptic terminals in the hippocampus (Buttini et al. 2002).

To date, no data have been published on the effects of APOE and EtOH on ChAT and BDNF expression in the hippocampus of aged mice. Thus, we evaluated the role of APOE deficiency and EtOH exposure in age-related changes in ChAT and BDNF expression in the mouse hippocampus. We compared 3-month-old (young) and 12-month-old (aged) *ApoE*-KO mice, with C57BL/6J (wild-type [WT]) mice to investigate ChAT and BDNF mRNA and protein expression in the hippocampus after saline or EtOH (2 g/kg) treatment using real-time PCR and western-blotting (WB) analyses.

Materials and Methods

Animals

All experimental mice had the C57BL/6J genetic background. Breeding pairs of *ApoE*-KO mice were used to generate the experimental groups. Two 8-week-old breeding pairs of *ApoE*-KO mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and bred at the animal facility of Kagawa University (Takamatsu, Japan). WT C57BL/6J mice were also bred at our animal facility. All experiments were conducted with male mice. All mice were housed under a controlled environmental regimen of 21 ± 3 °C, 50–70% humidity, and 12:12-h light:dark cycle, and they all had access to food and water ad libitum. All animal experiments were approved by the Kagawa University Animal Investigation Committee.

Experimental Groups

ApoE-KO and WT mice were each divided into four experimental groups: (1) young saline; (2) young EtOH (2 g/kg); (3) aged saline; (4) aged EtOH (2 g/kg). EtOH was administered at a concentration chosen to produce physiologically relevant blood concentrations, reaching a peak of approximately 20 mM at 30 min and then gradually declining (Jamal et al. 2016). For the aged groups we used *ApoE*-KO mice aged 12 months due to their short life span (Moghadasian et al. 2001). In addition, *ApoE*-KO mice aged 12–15 months may exhibit an age-related decline in presynaptic terminals in the hippocampus (Buttini et al. 2002). EtOH was dissolved in 0.9% saline and administered via intraperitoneal (IP) injection in a volume of 10 ml/kg. The bilateral hippocampus was collected 60 min after the IP injection of EtOH (20%, w/v) or saline.

Brain Tissues

Mice were killed by cervical decapitation. The bilateral hippocampus (approx. 15 mg) was immediately dissected on ice, and one-half of the hippocampus was placed in a 2-ml tube for RNA extraction, and the other half was placed in a 2-ml tube for protein analysis. The tubes were stored at −70 °C until use.

Quantitative Real-Time PCR Analysis

The hippocampus was homogenized using a Polytron® homogenizer (Kinematica AG, Lucerne, Switzerland) in 0.4 ml of Isogen (Nippon Gene Co., Ltd., Tokyo, Japan). An additional 0.4 ml of Isogen was added to yield a total sample volume of 0.8 ml. Total RNA was extracted using Isogen and a spin column (Nippon Gene Co., Ltd.). RNA was transcribed into cDNA using the PrimeScript™ RT Master Mix (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. Briefly, the reaction was conducted at 37 °C for 15 min in a total volume of 10 μl and then inactivated at 85 °C for 5 s. cDNA diluted five times was used as a template, and real-time PCR was performed using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus; Takara Bio Inc.). Specific primers were used to amplify ChAT (forward [F]: 5′-TGGA TGAAACATACCTGATGAGCAA-3′; reverse [R]: 5′-CGTGAAAGCTGGAGATGCAGAA-3′), BDNF (F: 5′-GGTATCCAAAGGCCAACTGA-3′; R: 5′-CTTA TGAATCGCCAGCCAAT-3′), and β-actin (F: 5′-CATC CGTAAAGACCTCTATGCCAAC-3′; R: 5′-ATGG AGCCACCGATCCACA-3′). Amplification was performed in 96-well plates using a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with an initial denaturation step at 95°C for 5 s followed by 40 cycles of 95°C for 5 s, 60°C for 40 s, and 60°C for 1 min. Melting curves were calculated at the end of cycling to ensure the

amplification of a single PCR product. The data were normalized to the internal standard β-actin.

Western Blotting

The hippocampus was homogenized using a Polytron® homogenizer in 0.4 ml of RIPA lysis buffer (Santa Cruz Biotechnology, Inc., Dallas, TX, USA). An additional 0.4 ml of RIPA lysis buffer supplemented with phenylmethylsulfonyl fluoride, sodium orthovanadate, and protease inhibitor cocktail (8 μl each; Santa Cruz Biotechnology, Inc.) was added to yield a total sample volume of 0.8 ml. After centrifugation at 10,000 g at 4 °C for 15 min, the supernatant was used for WB analysis. The protein content of the supernatant was determined using the Bradford assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA), with bovine serum albumin (Sigma-Aldrich Corp., St. Louis, MO, USA) as the standard. Samples were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis with molecular weight markers (Bio-Rad Laboratories, Inc.) and then transferred to polyvinylidene difluoride membranes. The membranes were incubated first with primary antibodies against ChAT (1:500; Chemicon International, Inc., Temecula, CA, USA), BDNF (1:1000; Santa Cruz Biotechnology, Inc.), and β-actin (1:2000; Wako Pure Chemical Industries, Ltd., Osaka, Japan) and then with corresponding horseradish peroxidase-linked secondary antibodies. Band intensities were evaluated using an LAS-1000plus Lumino-Imaging Analyzer (Fujix

Ltd., Tokyo, Japan). The relative protein expressions were normalized to those of β-actin in each sample.

Statistics

All values were expressed as the mean ± standard error of the mean; P values of < 0.05 were considered to be significant. The data were analyzed with SigmaPlot (Systat Software Inc., San Jose, CA, USA) using three-way analysis of variance. Post-hoc tests were carried out where appropriate using the Tukey–Kramer's test.

Results

The effect of APOE deficiency, age, and EtOH on ChAT mRNA levels in the hippocampus is shown in Fig. 1. In saline-and EtOH-treated animals, the expression of ChAT mRNA was significantly decreased in APOE-deficient mice (young mice: $P < 0.001$; aged mice: $P < 0.001$) compared with WT mice of the same age. Also in both the saline and EtOH groups, aging had a significant effect on ChAT, as shown by the reduction in mRNA levels (all P levels, aged $ApoE$-KO mice: $P < 0.001$) compared with young $ApoE$-KO mice. EtOH administration caused a significant reduction in ChAT mRNA expression in both young and aged $ApoE$-KO mice (all P values, real-time PCR: <0.01) compared with saline-treated $ApoE$-KO animals of the same age.

Fig. 1 The effects of apolipoprotein E (*APOE*), age, and alcohol (*EtOH*) on choline acetyltransferase (*ChAT*) mRNA expression in saline- ($n = 5$) and EtOH-treated mice ($n = 5$). †$P < 0.01$ vs. young (3 months old) saline wild-type (WT) mice; ‡$P < 0.01$ vs. old (12 months old) saline WT mice; §$P < 0.001$ vs. young EtOH WT mice; #$P < 0.01$ vs. old EtOH WT mice; ±$P < 0.01$ vs. young saline $ApoE$-knockout (*ApoE*-KO) mice; μ$P < 0.01$ vs. young EtOH $ApoE$-KO mice; *$P < 0.01$ vs. young and old saline $ApoE$-KO mice. *dF* Degrees of freedom, *ANOVA* analysis of variance. Data are shown as the mean ± standard error of the mean (SEM)

Result of three-Way ANOVA of the above fig

|	dF	F
Animal	1, 32	91.520***
Group	1, 32	95.803***
Age	1, 32	26.609***
Interactions		
Animal x group	1, 32	4.036*
Animal x age	1, 32	5.569*
Group x age	1, 32	0.277
Animal x group x age	1, 32	0.778

***P <0.001; *P <0.05

The representative WB results for ChAT protein and the effect of APOE deficiency, age, and EtOH on ChAT protein levels in the hippocampus are shown in Fig. 2. In saline-and EtOH-treated animals, ChAT protein levels were significantly decreased in APOE-deficient mice (young mice: $P < 0.01$; aged mice: $P < 0.01$) compared with WT mice of the same age. Aging had a significant effect on ChAT levels, as evident by the reduction in protein (all P values, aged $ApoE$-KO mice: $P < 0.01$) levels compared with young $ApoE$-KO mice in both the saline and EtOH groups. EtOH administration caused a significant reduction in ChAT protein in both young and aged $ApoE$-KO mice (all P values, protein: ≤ 0.01) compared with saline-treated $ApoE$-KO animals of the same age.

The effect of APOE deficiency, age, and EtOH on BDNF mRNA levels in the hippocampus is shown in Fig. 3. In saline- and EtOH-treated animals, BDNF mRNA levels (young mice: $P < 0.001$; aged mice: $P < 0.001$) were significantly decreased in APOE-deficient mice compared with WT mice of the same age. Aging had a significant effect on BDNF, as evident by the reduction in mRNA levels (all P values, aged $ApoE$-KO mice: $P < 0.001$) compared with young $ApoE$-KO mice in both the saline and EtOH groups. BDNF also exhibited a significant

decline with aging (aged WT mice: $P < 0.001$) compared with young WT mice in the EtOH group. EtOH administration caused a significant reduction in BDNF mRNA in young and aged $ApoE$-KO mice and aged WT mice (all P values, real-time PCR: < 0.01) compared with saline-treated $ApoE$-KO and WT animals of the same age.

The representative WB results for BDNF protein and the effect of APOE deficiency, age, and EtOH on BDNF protein levels in the hippocampus are given in Fig. 4. In saline-and EtOH-treated animals, BDNF protein levels were significantly decreased in APOE-deficient mice (young mice: $P < 0.01$; aged mice: $P < 0.01$) compared with WT mice of the same age. Aging had a significant effect on BDNF, as evident by the reduction in protein levels (all P values, aged $ApoE$-KO mice: $P < 0.01$) compared with young $ApoE$-KO mice in both the saline and EtOH groups. BDNF also exhibited a significant decline with aging (aged WT mice: $P < 0.01$) relative to young WT mice in the EtOH group. EtOH administration caused a significant reduction in BDNF protein in young and aged $ApoE$-KO and aged WT mice (all P values, protein: < 0.01) compared with saline-treated $ApoE$-KO and WT animals of the same age.

Fig. 2 The effects of APOE, age and EtOH on ChAT protein expression in saline- ($n = 5$) and EtOH-treated mice ($n = 5$). †$P < 0.001$ vs. young saline WT mice; ‡$P < 0.01$ vs. old saline WT mice; §$P < 0.01$ vs. young EtOH WT mice; #$P < 0.01$ vs. old EtOH WT mice; ±$P < 0.001$ vs. young saline $ApoE$-KO mice; μ$P < 0.01$ vs. young EtOH $ApoE$-KO mice; *$P < 0.01$ vs. young and old saline $ApoE$-KO mice. Data are shown as the mean ± SEM
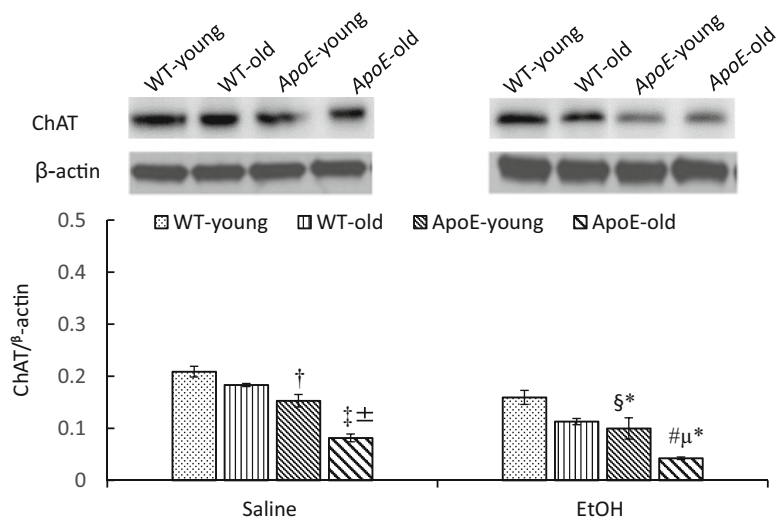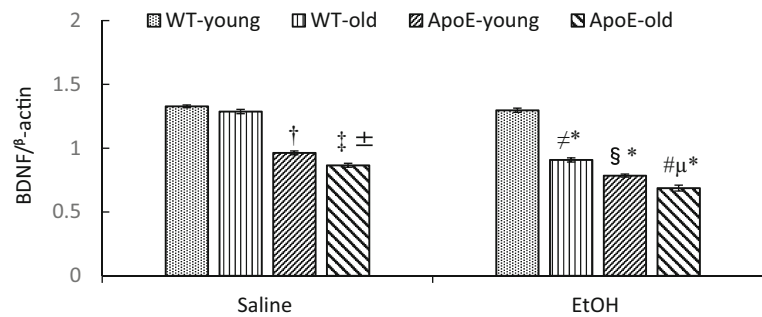

Result of three-Way ANOVA of the above fig

	dF	F
Animal	1, 32	86.392***
Group	1, 32	46.954***
Age	1, 32	42.043***
Interactions		
Animal x group	1, 32	0.792
Animal x age	1, 32	3.325*
Group x age	1, 32	0.0457
Animal x group x age	1, 32	1.277

***$P < 0.001$; *$P < 0.05$

Fig. 3 The effects of APOE, age, and EtOH on brain-derived neurotrophic factor (*BDNF*) mRNA expression in saline- (*n* = 5) and EtOH-treated mice (*n* = 5). †*P* < 0.001 vs. young saline WT mice; ‡*P* < 0.001 vs. old saline WT mice; §*P* < 0.001 vs. young EtOH WT mice; #*P* < 0.001 vs. old EtOH WT mice; ±*P* < 0.001 vs. young saline *ApoE*-KO mice; μ*P* < 0.001 vs. young EtOH *ApoE*-KO mice; ≠*P* < 0.001 vs. young EtOH WT mice; *P* < 0.001 vs. young and old saline WT and *ApoE*-KO mice. Data are shown as the mean ± SEM
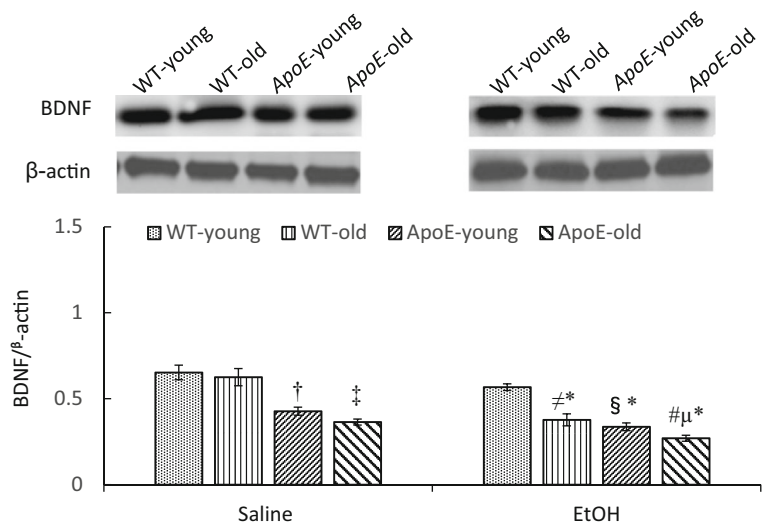

Result of three-Way ANOVA of the above fig

	dF	F
Animal	1, 32	1109.561***
Group	1, 32	282.836***
Age	1, 32	187.877***
Interactions		
Animal x group	1, 32	1.367
Animal x age	1, 32	26.615***
Group x age	1, 32	58.763***
Animal x group x age	1, 32	58.270***

***P <0.001

Fig. 4 The effects of APOE, age, and EtOH on BDNF protein expression in saline- (*n* = 5) and EtOH-treated mice (*n* = 5). †*P* < 0.01 vs. young saline WT mice; ‡*P* < 0.01 vs. old saline WT mice; §*P* < 0.01 vs. young EtOH WT mice; #*P* < 0.01 vs. old EtOH WT mice; ≠*P* < 0.01 vs. young EtOH WT mice; μ*P* < 0.001 vs. young EtOH *ApoE*-KO mice; *P* < 0.01 vs. young and old saline WT and *ApoE*-KO mice. Data are shown as the mean ± SEM

Result of three-Way ANOVA of the above fig

	dF	F
Animal	1, 32	92.860***
Group	1, 32	36.787***
Age	1, 32	16.497***
Interactions		
Animal x group	1, 32	3.036
Animal x age	1, 32	1.046
Group x age	1, 32	3.816*
Animal x group x age	1, 32	3.447*

***P <0.001; *P <0.05

Discussion

In this study, we first investigated whether APOE deficiency causes age-related changes in ChAT and BDNF expression in the hippocampus in young and aged *ApoE*-KO and WT mice. Second, we examined whether the administration of EtOH to these mice augments the effect of age on ChAT and BDNF expression. To our knowledge, this study is the first to investigate the role of APOE deficiency and EtOH exposure on ChAT and BDNF expression in the hippocampus of aged mice. We hypothesized that EtOH exposure would elicit greater cholinergic and trophic effects in *ApoE*-KO mice in an age-dependent manner. Our results indeed showed that APOE deficiency and EtOH exposure decreased ChAT and BDNF mRNA and protein levels in an age-dependent manner. Importantly, the decreases in ChAT and BDNF mRNA and protein levels were greater in aged EtOH-treated mice, particularly those lacking APOE, than in their younger counterparts. Our findings highlight the contribution of APOE and EtOH to the decreased ChAT and BDNF expression evident in the hippocampus of aged mice.

Different APOE isoforms have differing effects on mouse brain functions in vivo that are influenced by age and sex (Raber et al. 1998). The brain is second only to the liver with respect to the abundance of APOE expression. APOE-deficient mice thus represent an attractive model for studying the relationship between APOE and the cholinergic system and trophic factors of the brain. Cholinergic neurons are assumed to undergo moderate degenerative changes during aging, resulting in cholinergic hypofunction (Muller et al. 1991; Schliebs and Arendt 2011). Disrupted BDNF expression is also related to declining hippocampal volume and memory in late adulthood (Erickson et al. 2010). This decrease in BDNF may result in a lack of trophic support for cholinergic neurons (Hock et al. 2000).

Consistent with these observations, we noted significant decreases in both ChAT and BDNF mRNA and protein levels in the hippocampus of EtOH-free *ApoE*-KO mice (Figs. 1, 2, 3, and 4). These decreases were more pronounced in aged *ApoE*-KO mice than in young *ApoE*-KO mice, but absent in WT mice irrespective of age. These findings represent plausible evidence of a link between APOE and ChAT and BDNF expression in aged mice. APOE deficiency is associated with the dysfunction of basal forebrain cholinergic neurons (Fisher et al. 1998), but the mechanism by which its deficiency in aging decreases ChAT and BDNF expression remains unclear. Cognitive decline is a major concern of the APOE-deficient population, and aging is associated with heightened cognitive disturbance (de Chaves and Narayanaswami 2008). *ApoE*-KO mice have been demonstrated to be prone to an age-dependent decrease in synaptic density in distinct areas of the brain (Mashlia et al. 1995), with the result being reduced

neurotransmitter release and poor performance in memory tasks (Raber et al. 1998; Buttini et al. 1999). Brain aging itself is linked to changes in the levels of neurotransmitters, such as acetylcholine (Baxter et al. 1999), neurotrophic factors, such as BDNF (Hwang et al. 2006), and dopamine receptor density (Roth and Joseph 1994). These findings lead us to believe that APOE deficiency causes significant decreases in ChAT and BDNF expression in the mouse hippocampus, especially in aged animals.

Studies examining cholinergic functions and trophic factors in relation to EtOH consumption in elderly individuals are rare. Our findings as well as those of other researchers (Rose et al. 2013; Jamal et al. 2016) demonstrate that blood EtOH values in adult WT mice are similar in various other strains of mice. Although we did not measure blood EtOH concentrations in *ApoE*-KO mice, it has been shown in rats that the peak blood EtOH concentrations for equivalent doses of EtOH increase with age (Seitz et al. 1989). Several studies have examined the effects of aging on EtOH metabolism and toxicity in rats, and the results reveal that the EtOH elimination rate decreases with age (Ritzmann and Springer 1980; Seitz et al. 1989; Kim et al. 2003). As such, the levels of EtOH in the blood and brain of adult and aged mice are higher than those of young mice (Ritzmann and Springer 1980). In this study, we investigated whether acute EtOH administration in *ApoE*-KO and WT mice reduces ChAT and BDNF expression to a greater degree with age. Using real-time PCR and WB, we found that EtOH exposure decreased ChAT and BDNF expression in *ApoE*-KO mice in an age dependent manner (Figs. 1, 2, 3, and 4). Likewise, EtOH also decreased BDNF expression—but not ChAT expression—in aged WT mice relative to young WT mice. Importantly, this heightened decline in ChAT and BDNF expression was evident in both young and aged *ApoE*-KO mice compared with EtOH-treated WT mice of the same age, suggesting that the effects of EtOH and APOE are additive.

Aging increases an individual's sensitivity to and the effects of alcohol (Vestal et al. 1977; Spencer and Hutchison 1999). Some elderly people are impaired in many aspects of learning and memory (Bowles and Poon 1985; Eustache et al. 1995; Balota et al. 2000). Age-related cognitive decline is similar to the cognitive decline seen in chronic alcoholism (Bowles and Poon 1985; Eustache et al. 1995; Balota et al. 2000). Interestingly, our results show that the EtOH-related decline in ChAT and BDNF expression in aged mice of both genotypes was not parallel to that evident in young EtOH-treated mice, but exceeded it. Possible explanations for the age-related enhancement of the effects of EtOH include the increased rates of cellular deterioration that accompany normal aging (Yu 1996) and the pharmacokinetic induction of higher blood EtOH concentrations in older individuals compared with younger individuals (Vestal et al. 1977). Our data support the latter hypothesis

by demonstrating that EtOH has a greater effect on ChAT and BDNF levels in aged mice than in young mice.

Cholinergic neurons and trophic factors appear to be particularly vulnerable to EtOH exposure (Davis 2008; Ehrlich et al. 2012). In support of this, we observed a significant effect of EtOH on ChAT and BDNF levels in both young and aged *ApoE*-KO mice compared with EtOH-free mice of the same age (Figs. 1, 2, 3, and 4). However, in WT mice, EtOH had similar to no effects, indicating that EtOH exerts some negative effects on cholinergic neurons and trophic factors. This is, in part, consistent with the lower spatial performance of aged rats with long-term moderate EtOH consumption compared with abstinent aged adult rats (Baird et al. 1998). In our study, EtOH failed to change the levels of ChAT and BDNF in young WT mice compared with saline-treated young WT mice, consistent with the findings of our previous study (Jamal et al. 2013).

EtOH is known to have effects on memory and other cognitive functions in humans (Brown et al. 2010) and animals (Hoffmann and Matthews 2001). *ApoE*-KO mice exhibit a greater EtOH (2 g/kg)-induced conditioned place preference than WT mice (Bechtholt et al. 2004). We recently showed that EtOH (2 g/kg) caused spatial memory deficits in *ApoE*-KO mice, suggesting a possible contribution of APOE to cognitive decline, although WT mice exhibited a similar decline in spatial task performance (Jamal et al. 2012). Our data of decreases in ChAT and BDNF levels after EtOH exposure in *ApoE*-KO mice further support the notion that APOE and EtOH act synergistically to reduce ChAT and BDNF expression in the hippocampus. These findings raise the possibility that individuals with APOE deficiency who consume alcohol may be at greater risk of memory deficit. APOE deficiency is associated with many diseases, such as dyslipidemia, atherosclerosis, AD, and Parkinson's disease (Giau et al. 2015). Our data indicate that APOE deficiency, aging, and EtOH administration decrease cholinergic and trophic factor expression in the hippocampus of mice, which may be similar to APOE deficiency in humans.

In conclusion, our findings suggest that APOE deficiency, aging, and EtOH administration decrease ChAT and BDNF mRNA and protein levels in the mouse hippocampus. Importantly, heightened decreases in ChAT and BDNF levels were most evident in aged EtOH-treated mice, particularly those lacking APOE. Our results also suggest that APOE deficiency and EtOH exposure act in concert to decrease ChAT and BDNF levels in the mouse hippocampus, thereby providing support for the involvement of these two markers in age-related cognitive deficits.

Compliance with Ethical standards

Conflict of Interest The authors have no conflict of interest to declare

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