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Enhanced ethanol-, but not cocaine-induced, conditioned place preference in *Apoe*$^{-/-}$ mice

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Abstract

Apolipoprotein (apo) E is a glycoprotein that is most commonly associated with cardiovascular and Alzheimer's disease risk. Recent data showing that apoE mRNA expression is reduced in the frontal cortex of alcoholics raise the possibility that apoE may also be related to the rewarding properties of ethanol. In this study, we examined whether *Apoe* deletion affects the rewarding properties of ethanol in mice. Male and female wild-type (WT; C57BL/6J) and apoE knockout (*Apoe*$^{-/-}$; C57BL/6J-Apoe^{tm1Unc}) mice underwent an unbiased place conditioning procedure with ethanol (2 g/kg) or cocaine (5 mg/kg). Female mice were also tested for ethanol intake in a two-bottle choice procedure. *Apoe*$^{-/-}$ mice showed greater ethanol-induced conditioned place preference (CPP). In contrast, cocaine-induced CPP and ethanol intake were similar between the genotypes. These findings suggest that apoE normally reduces the conditioned rewarding properties of ethanol but not of cocaine. While the exact mechanisms underlying these effects of apoE are unknown, these data support a possible role for apoE in modulating the conditioned rewarding properties of ethanol.

Keywords: Conditioned place preference; Place conditioning; Ethanol; Cocaine; Apolipoprotein E; apoE; Locomotor activity; Knockout mice; C57BL/6J

1. Introduction

Apolipoprotein (apo) E is a glycoprotein important for the metabolism and redistribution of lipoproteins and cholesterol. The literature involving this protein traditionally focuses on different human apoE isoforms and their associated risk for cardiovascular disease and Alzheimer's disease (for review, see Mahley and Rall, 2000). More recent findings have shown that apoE can also influence other biological processes including immunoregulation (Mahley and Rall, 2000), neuronal repair, cytoskeletal function (Nathan et al., 1994) and stress responses (Raber et al., 2000).

Mice lacking the single rodent form of apoE have been developed, via homologous recombination, to study the functional importance of this protein (Piedrahita et al., 1992). While no gross physical (Raber et al., 1998) or central nervous system development abnormalities have been reported in *Apoe* knockout (*Apoe*$^{-/-}$) mice, deletion of *Apoe* has been shown to induce many age-related behavioral abnormalities. In particular, age-related cognitive and neurodegenerative effects are observed after 5–6 months of age in *Apoe*$^{-/-}$ mice (Masliah et al., 1995, 1997; Oitzl et al., 1997). *Apoe*$^{-/-}$ mice also demonstrate age-dependent increases in food and water intake (Raber et al., 2000) and hypothalamic–pituitary–adrenal (HPA) axis dysfunction that is associated with greater anxiety, decreased open-field activity and increased food and water intake (Raber et al., 2000).

Apoe deletion also alters the distribution of cholesterol in the synaptic plasma membrane (Igbavboa et al., 1997), an effect that can alter the function of several membrane receptor proteins, including the $GABA_A$ receptor (Sooksawate and Simmonds, 2001). Specifically, *Apoe*$^{-/-}$ mice show a higher distribution of cholesterol in the synaptic plasma membrane, which is specific to the exofacial leaflet. This effect is observed prior to the onset of the age-related dysfunctions in *Apoe*$^{-/-}$ mice that were described earlier (Igbavboa et al., 1997). Interestingly, chronic ethanol consumption similarly results in an increase in cholesterol

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content of the exofacial leaflet of the synaptic plasma membrane (Wood et al., 1990).

It was recently reported that ApoE mRNA expression is reduced in the frontal cortex of alcoholics (Lewohl et al., 2000). This was determined by using microarrays to compare mRNA expression levels in postmortem samples from alcoholics (intakes of more than 80 g of ethanol per day for more than 30 years) with samples from teetotalers and social drinkers (intakes averaging less than 20 g per day). The causal nature of the relationship between decreased apoE mRNA observed in alcoholics and the effects of ethanol is unknown. It is possible that repeated ethanol exposure results in decreased expression of apoE. This conclusion is supported by findings that *Apoe* deletion and chronic ethanol consumption share similar effects on membrane cholesterol (Igbavboa et al., 1997; Wood et al., 1990). However, it is also possible that decreased apoE contributes to alcoholism and does so by modifying the hedonic value of ethanol. The experiments contained here were designed to determine if existing changes in apoE expression can alter the conditioned reinforcing properties of ethanol.

The experiments described here were intended to investigate the role of apoE in effects of ethanol independent of the age-related cognitive impairments and neurodegenerative effects that result from *Apoe* deletion. Therefore, in the present experiments, mice were tested at ages prior to the reported occurrence of these effects. To this end, $Apoe^{-/-}$ and wild-type (WT) C57BL/6J mice were compared in ethanol-induced conditioned place preference (CPP), a well-established model of ethanol reward. To determine if the observed effect generalized to other drugs of abuse, we also tested both genotypes for cocaine-induced CPP. Given that apoE is involved in metabolic processes, we also assessed blood ethanol concentrations (BECs). Finally, because $Apoe^{-/-}$ mice showed stronger ethanol-induced CPP, ethanol drinking was examined using a two-bottle choice procedure to determine the generality of effects on ethanol-related behaviors.

2. Materials and methods

2.1. Animals

One hundred thirty-five mice, 63 $Apoe^{-/-}$ (C57BL/6J-Apoe^{tm1Unc}; 35 males and 28 females) and 72 WT (C57BL/6J; 36 males and 36 females) mice were used in the present experiments. Mice were offspring of homozygous breeding pairs that were backcrossed to C57BL/6J for 10 generations and obtained from the Jackson Laboratory (Bar Harbor, ME). Homozygous breeding pairs were used because of the large number of mice required for the experiments for which the low yield of heterozygous breeding was too cost, space and time prohibitive. Animals were housed three to four to a cage with continuous access to food and water. After the animals were allowed to acclimate to the colony for 10–14 days, experiments were carried out during the light phase of a 12-h light–dark cycle (lights on at 7 a.m.). Animals were handled only for cage changing prior to the start of the first experiment. Animals were 2–3 months of age at the beginning of the experiments and tested for a maximum of 7 weeks. No gross motor abnormalities were observed in $Apoe^{-/-}$ mice. The NIH Principles of Laboratory Animal Care were followed in conducting these studies and the protocol was approved by the OHSU IACUC.

2.2. Apparatus

The place conditioning chambers consisted of 12 identical acrylic and aluminum boxes (30 × 15 × 15 cm) enclosed in separate light and sound attenuating chambers. Locomotor activity and the location of the mouse within the box were determined by six infrared beams located 2.2 cm above the floor of the chamber at 5-cm intervals and recorded with a 10-ms resolution by a computer. The floor consisted of interchangeable halves, which were made of two distinct textures. Specifically, hole floors were made from perforated 16-gauge stainless steel with 6.4 mm round holes on 9.5 mm staggered centers. Grid floors were made of 2.3 mm stainless steel rods mounted 6.4 mm apart on acrylic rails. These floor textures were chosen based on previous findings demonstrating that drug-naive mice spend equal time on both floors (Cunningham, 1995), which allows use of an unbiased method of assessing CPP.

2.3. Procedure

The place conditioning procedures were similar to those used previously to successfully produce CPP with ethanol and cocaine in several different mouse strains (e.g., Cunningham, 1995; Cunningham et al., 1999). Animals were randomly assigned to one of two conditioning groups (GRID+ and GRID−) and subjected to an unbiased Pavlovian conditioning procedure. Each experiment consisted of three phases: habituation, conditioning and preference testing. On the first day of the experiment, animals were injected intraperitoneally with saline just prior to being placed in the conditioning chamber on a smooth paper floor for 5 min to habituate them to the experimental apparatus and injection procedures. During the conditioning phase, animals were injected with saline or a test drug (ethanol or cocaine) on alternating days just before being placed in the conditioning chamber where both sides of the floor were the same. Details specific to the individual test drugs are provided below. Animals in the GRID+ conditioning group were placed on the grid floor on drug injection days and the hole floor on saline injection days. Conversely, animals in the GRID− conditioning group were placed on the hole floor on drug injection days and the grid floor on saline injection days. The order of drug and saline exposure was counterbalanced within groups. During preference test sessions, animals were injected with saline and placed into the

conditioning chambers for 60 min where the box floor was half hole and half grid. Animals were placed in the middle of the box facing the rear so that the left two feet were on the left floor and the right two feet were on the right floor. The left/right position of the floors was counterbalanced within groups. Experimental sessions were conducted 5 days per week.

2.3.1. Ethanol place conditioning

Forty *Apoe*$^{-/-}$ (23 males and 17 females) and 48 WT (24 males and 24 females) mice were used in the ethanol CPP experiment. A 95% ethanol stock solution was diluted to 20% v/v with saline. A dose of 2 g/kg was administered intraperitoneally with an injection volume of 12.5 ml/kg. After completion of the habituation phase (Day 1), the conditioning phase began in which animals were injected with saline or ethanol on alternating days just prior to being placed in the conditioning chamber for 5 min. After four conditioning trials (4 CS+, 4 CS−; Days 2–9), a 60-min preference test was conducted (Day 10), which was followed by two additional conditioning trials (2 CS+, 2 CS−; Days 11–14) and a second preference test 72 h later (Day 15). Test 1 was carried out after only four conditioning trials in an attempt to capture any possible enhancement at a point before WT mice were expected to express a preference. The second test was conducted after a 72-h delay due to logistical constraints. This ethanol dose and trial duration were used because they have been shown to induce an intermediate level of CPP in many inbred strains (Cunningham et al., 1996). However, it should be noted that C57BL/6J mice do not readily express place preference for ethanol (Cunningham et al., 1992).

2.3.2. Cocaine place conditioning procedure

Twenty-three *Apoe*$^{-/-}$ (11 females and 12 males) and 24 WT (12 females and 12 males) mice were used in the cocaine CPP experiment. After habituation (Day 1), the conditioning phase began in which animals were injected with saline or cocaine on alternating days just prior to being placed in the conditioning chamber for 60 min. Cocaine (cocaine HCl) was mixed in saline (0.5 mg/ml) and was administered intraperitoneally at a dose of 5 mg/kg with an injection volume of 10.0 ml/kg. After four conditioning trials (4 CS+, 4 CS−; Days 2–9) a 60-min preference test was conducted (Day 10). This dose, trial duration and number of trials were chosen based on previous findings demonstrating that cocaine doses between 1 and 30 mg appear equally effective across a wide range of trial durations in C57BL/6J (Cunningham et al., 1999). This particular dose (5 mg/kg) was chosen to minimize the possibility of stereotypy (Cook et al., 1998).

2.3.3. Blood ethanol concentrations

Twenty-three female *Apoe*$^{-/-}$ ($n=11$) and WT ($n=12$) mice were used to determine BECs. These mice had previously been used 2 weeks earlier in the ethanol place conditioning study described above. Male mice were not available because they were used in other experiments not included here. The same dose of ethanol used during ethanol CPP (2 g/kg) was administered intraperitoneally and 20 μl tail blood samples were taken after 30 and 60 min. Samples were analyzed using gas chromatography (Terdal and Crabbe, 1994).

2.3.4. Ethanol-drinking two-bottle choice procedure

Eleven female *Apoe*$^{-/-}$ and 12 female WT mice were used in the two-bottle choice drinking procedure. These mice were previously used in the BEC and ethanol CPP experiments described above. Mice were moved to individual hanging wire cages and given access to tap water through two 25-ml graduated cylinders fitted with drinking spouts. The mice were allowed to habituate to the housing and drinking conditions for 3 days before data were collected. Following the acclimation phase, water consumption was recorded daily for 4 days. During the choice phase of the experiment, one of the two water bottles was replaced with a bottle containing ethanol in increasing concentrations (3%, 6% and 10%, v/v). Left and right positions of the two bottles were reversed every 48 h to control for side preference. At these times, mice were weighed and bottles were cleaned and replaced on the cages.

Each concentration of ethanol was available for 4 days (3% and 6%) or 6 days (10%) and presented in ascending order over the experiment. Throughout the experiment, evaporation estimates were calculated from bottles placed on an empty cage with one containing water and the other containing the appropriate ethanol solution. Specifically, any change in the volume of a given solution compared to the previous day was deducted from consumption values of that solution for that day. Daily consumption from each tube (ml) was used to calculate a total consumption, ethanol dose and preference ratio (ethanol intake/total intake).

2.4. Data analysis

Conditioning activity data were analyzed using three-way ANOVAs with the factors genotype (*Apoe*$^{-/-}$ or WT), sex (male or female), and trial type (drug or saline). Place preference test data were analyzed with three-way ANOVAs and the factors genotype, sex and conditioning group (GRID+ or GRID−). Place conditioning test activity data were analyzed with two-way ANOVAs using the factors genotype and sex. Data from the ethanol place conditioning experiment consisted of two tests and were collected in two replications. Therefore, additional factors of test (1 or 2) and replication (1 or 2) were entered for all analyses of that experiment. BEC data were analyzed by two-way ANOVA with the factors genotype and time (30 and 60 min after ethanol injection). Ethanol drinking data were analyzed by two-way ANOVA with the factors genotype and ethanol concentration (0%, 3%, 6% or 10% v/v). Effect sizes were calculated as partial ω^2 (Keppel, 1991).

3. Results

3.1. Ethanol place conditioning

Two WT male and 1 *Apoe*$^{-/-}$ female mice were removed from the experiment due to injuries sustained in the home cage. The final number of mice in each group is shown in the figure captions.

3.1.1. Conditioning activity

Fig. 1 depicts mean activity rates (counts/min) averaged across the six CS+ (ethanol) and CS− (saline) conditioning trials. All groups showed general stimulation to ethanol compared to saline. However, female WT mice showed the greatest degree of stimulation. This conclusion was confirmed by a four-way ANOVA (Genotype × Sex × Trial Type × Replication) on activity during conditioning trials, which revealed significant main effects and two-way interactions of all factors except those involving replication ($.001 < P < .01$; $.06 <$ partial $\omega^2 < .45$). In addition, a significant three-way interaction of Genotype × Sex × Trial Type was observed [$F(1,81) = 7.4$; $P < .009$; partial $\omega^2 = .03$].

The Genotype × Sex × Trial Type interaction (Fig. 1) was due to a greater effect of sex on the response to ethanol in WT than in *Apoe*$^{-/-}$ mice. Specifically, female WT mice showed greater activation after ethanol than any other group. This observation was supported by follow-up analyses within each sex showing a significant Genotype × Trial Type interaction in female mice [$F(1,41) = 21.4$; $P < .001$; partial $\omega^2 = .20$], but not male mice. Pairwise Bonferroni corrected follow-ups of this two-way interaction demonstrated that female *Apoe*$^{-/-}$ mice were more active than female WT mice on ethanol trials ($P < .001$), but did not differ on saline trials.

3.1.2. Place preference test

Analysis of test data indicated the development of significant CPP [main effect of conditioning group: $F(1,73) = 22.2$; $P < .001$; partial $\omega^2 = .11$], and significantly stronger ethanol-induced CPP in *Apoe*$^{-/-}$ mice than WT mice (Fig. 2). This observation was supported by a five-way ANOVA (Genotype × Sex × Conditioning Group × Test × Replication) that revealed significant main effects of conditioning group and test [$F(1,73) = 5.4$; $P < .03$; partial $\omega^2 = .02$] and Genotype × Conditioning Group [$F(1,73) = 4.0$; $P < .05$; partial $\omega^2 = .02$] and Conditioning Group × Test interactions [$F(1,73) = 5.6$; $P < .02$; partial $\omega^2 = .03$] for time spent on the grid floor during the test. Follow-up analyses for each test demonstrated significant CPP at Test 1 ($P < .001$; Fig. 2A and C) and Test 2 ($P < .001$; Fig. 2B and D), which increased from Test 1 to Test 2. Moreover, across both tests, *Apoe*$^{-/-}$ mice showed a significant ethanol place preference ($P < .001$) whereas WT mice exhibited only a trend towards ethanol place preference ($P = .11$). Although inspection of Fig. 2 suggests that female mice may have stronger ethanol CPP than male mice, there were no statistically significant effects involving sex. The only effect that approached the conventional criteria for significance was the Sex × Conditioning Group interaction [$F(1,73) = 3.1$; $P = .08$].

Fig. 3 depicts mean activity rates during the two tests for each Genotype × Sex group. In general, WT mice were more active than *Apoe*$^{-/-}$ mice, and males were more active than females. A four-way ANOVA (Genotype × Sex × Test × Replication) supported these observations, yielding significant main effects of genotype [$F(1,81) = 27.8$; $P < .001$; partial $\omega^2 = .13$] and sex [$F(1,81) = 5.4$; $P < .03$; partial $\omega^2 = .02$]. In addition, significant main effects of test [$F(1,81) = 19.1$; $P < .001$; partial $\omega^2 = .09$] and replication

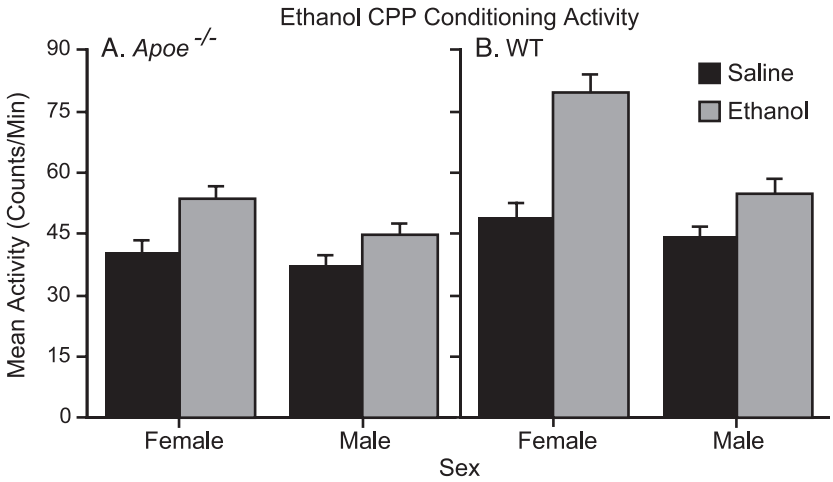

Fig. 1. Mean activity counts per minute (± S.E.M.) for each sex and genotype during CS+ (ethanol; 2 g/kg) and CS − (saline) conditioning trials of the ethanol CPP experiment. *Apoe*$^{-/-}$ (A; $n = 19$ female, $n = 24$ male) and WT (B; $n = 24$ female, $n = 22$ male) mice were given intraperitoneal injections of either ethanol or saline and were then immediately placed in apparatus on a specific floor texture for 5 min. Significant Genotype × Trial Type interaction in female mice ($P < .001$), but not males. Females differ on ethanol ($P < .001$), but not on saline trials.

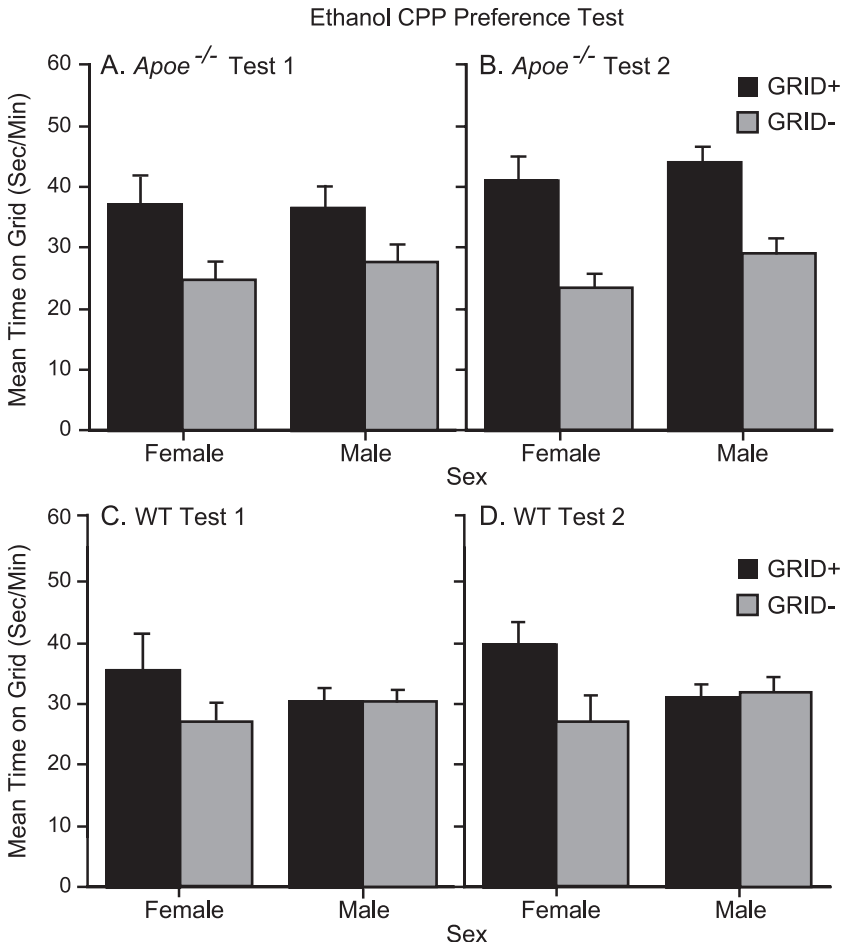

Fig. 2. Mean time sec/min (± S.E.M.) spent on the GRID floor for female and male *Apoe*$^{-/-}$ mice during the 60-min postconditioning Test 1 (A) and Test 2 (B) of the ethanol CPP experiment. Mean time sec/min (± S.E.M.) spent on the GRID floor during the 60-min postconditioning for female and male WT mice during Test 1 (C) and Test 2 (D) of the ethanol CPP experiment. Animals in the GRID+ condition were given ethanol paired with the grid floor during conditioning trials ($n=10$ female *Apoe*$^{-/-}$, $n=12$ male *Apoe*$^{-/-}$; $n=12$ WT female, $n=12$ WT male). Animals in the GRID− condition were given ethanol paired with the hole floor during conditioning trials ($n=9$ female *Apoe*$^{-/-}$, $n=12$ male *Apoe*$^{-/-}$; $n=12$ WT female, $n=10$ WT male). Significant Genotype × Conditioning Group interaction ($P<.001$). Significant effect of conditioning group in *Apoe*$^{-/-}$ ($P<.001$), but not in WT mice.

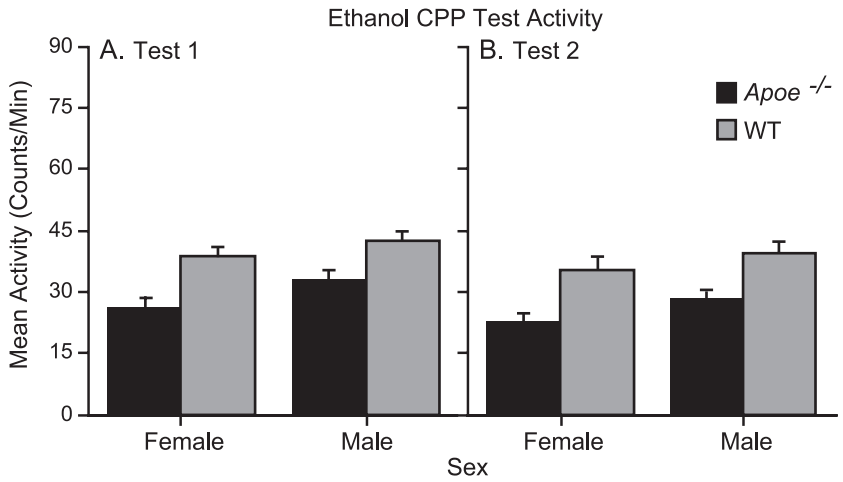

Fig. 3. Mean activity counts per minute (± S.E.M.) during place preference Test 1 (A) and Test 2 (B) for each genotype in the ethanol CPP experiment. *Apoe*$^{-/-}$ ($n=19$ female, $n=24$ male) and WT ($n=24$ female, $n=22$ male) mice were injected with saline (12.5 ml/kg) immediately before being placed in the apparatus on a half grid, half hole floor for 60 min. Significant main effects of genotype ($P<.001$) and sex ($P<.05$).

[$F(1,73) = 5.3$; $P < .03$; partial $\omega^2 = .02$] were observed, indicating that mice were more active in Test 1 than Test 2 and that mice in Replication 1 were generally less active than those in Replication 2 (data for replications not shown). However, replication did not interact significant with any other variable.

3.2. Cocaine place conditioning

Two WT males were removed from the experiment due to a procedural error.

3.2.1. Conditioning activity

During the conditioning phase, *Apoe*$^{-/-}$ mice were generally less active than WT mice (Fig. 4). Nevertheless, both genotypes showed a general activation to cocaine. These observations were supported by a three-way ANOVA (Genotype × Sex × Trial Type) that yielded significant main effects of genotype [$F(1,41) = 12.0$; $P < .001$; partial $\omega^2 = .11$] and Trial Type [$F(1,41) = 241.7$; $P < .001$; partial $\omega^2 = .73$]. No other effects were significant.

3.2.2. Place preference test

Fig. 5 shows mean time spent on the grid floor during the test session. In contrast to ethanol, cocaine produced a similar-sized CPP in both *Apoe*$^{-/-}$ (Fig. 5A) and WT (Fig. 5B) mice that did not differ between males and females. This conclusion was supported by a three-way ANOVA (Genotype × Sex × Conditioning Group) that revealed a significant main effect of conditioning group [$F(1,37) = 10.7$; $P < .003$; partial $\omega^2 = .10$], indicating that mice in the GRID+ condition spent significantly more time on the grid floor than mice in the GRID − condition. There were no significant effects involving genotype or sex. Furthermore, in contrast to the ethanol CPP study, no effects involving sex approached significance.

Fig. 6 depicts mean activity rates during the preference test. Two-way ANOVA (Genotype × Sex) yielded a significant main effect of genotype [$F(1,41) = 13.4$; $P < .001$; partial $\omega^2 = .12$], reflecting lower activity in *Apoe*$^{-/-}$ mice than in WT mice. There were no significant sex effects.

3.3. Blood ethanol concentrations

The mean (± S.E.M.) BECs for WT and *Apoe*$^{-/-}$ mice, respectively, were 208.8 ± 3.9 mg/dl and 192.2 ± 5.3 mg/dl at 30 min after ethanol injection, and 183.5 ± 2.1 mg/dl and 184.7 ± 2.5 mg/dl 60 min after ethanol injection. BEC data were analyzed by two-way ANOVA (Genotype × Time). A significant main effect of time [$F(1,19) = 21.7$; $P < .001$; partial $\omega^2 = .32$] confirmed that BECs were generally higher 30 min after injection than 60 min after injection. However, a significant Genotype × Time interaction [$F(1,19) = 6.5$; $P < .02$; partial $\omega^2 = .11$] was also observed. Bonferroni-corrected pairwise comparisons indicated that BECs of WT mice decreased significantly between 30 and 60 min ($P < .001$), while those of *Apoe*$^{-/-}$ mice did not. Additionally, WT mice had significantly higher BECs than *Apoe*$^{-/-}$ mice 30 min after injection ($P < .02$), but did not differ 60 min after injection.

3.4. Ethanol drinking

Because *Apoe*$^{-/-}$ mice showed enhanced ethanol-induced CPP, we examined ethanol drinking as an additional measure of ethanol reinforcement. Data for each ethanol concentration were averaged over 4 or 6 days (see Materials

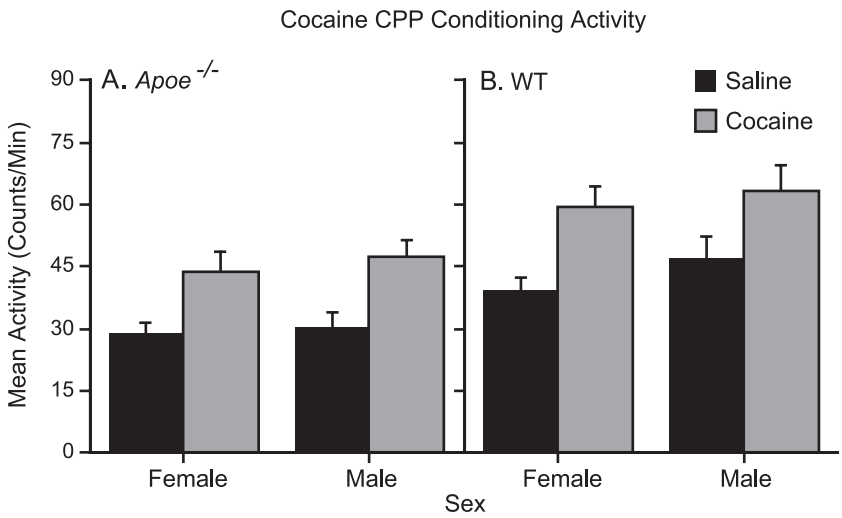

Fig. 4. Mean activity counts per minute (± S.E.M.) for each genotype during CS+ (cocaine; 5 mg/kg) and CS − (saline) trials of the cocaine CPP experiment. *Apoe*$^{-/-}$ (A; $n = 11$ female, $n = 12$ male) and WT (B; $n = 12$ female, $n = 10$ male) mice were given intraperitoneal injections of either cocaine or saline and were then immediately placed in apparatus on a specific floor texture for 60 min. Significant main effects of genotype ($P < .001$) and trial type ($P < .001$).

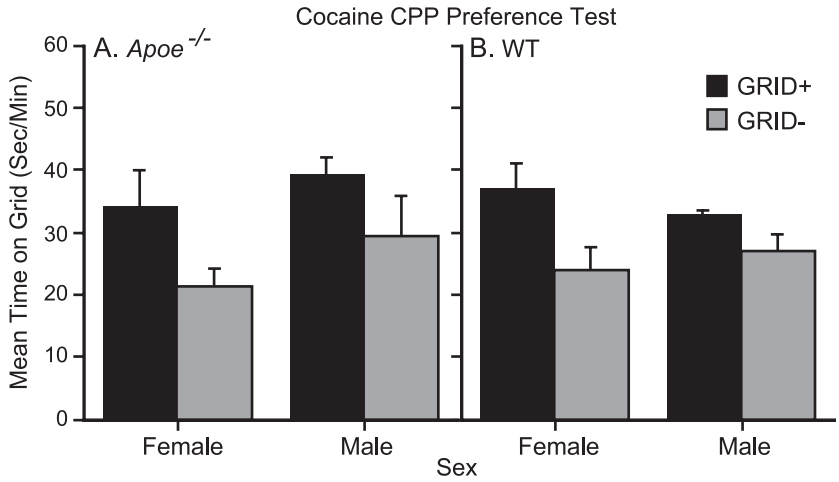

Fig. 5. Mean time sec/min (± S.E.M.) spent on the GRID floor by each sex for *Apoe*$^{-/-}$ (A) and WT (B) mice during the 60-min postconditioning test session in the cocaine CPP experiment. Animals in the GRID+ condition ($n=6$ female *Apoe*$^{-/-}$, $n=6$ male *Apoe*$^{-/-}$; $n=6$ WT female, $n=4$ WT male) were given cocaine paired with the grid floor during conditioning trials. Animals in the GRID− condition ($n=5$ female *Apoe*$^{-/-}$, $n=6$ male *Apoe*$^{-/-}$; $n=6$ WT female, $n=6$ WT male) were given cocaine paired with the hole floor during conditioning trials. Significant main effects of genotype ($P<.001$) and trial type ($P<.001$).

and methods). Group means for total consumption, ethanol preference and dose are listed in Table 1.

3.4.1. Total consumption

Total consumption was similar between genotypes, but varied based on the ethanol concentration available. A two-way ANOVA (Genotype × Ethanol Concentration) confirmed this conclusion. This analysis resulted in a significant main effect of ethanol concentration [$F(3,63)=30.1$; $P<.001$; partial $\omega^2=.49$]. Generally, total consumption was significantly different between all available ethanol concentrations ($.001<P<.05$) with the exception of the comparison between 0% and 6% ethanol. A Genotype × Ethanol Concentration interaction was also observed [$F(3,63)=2.8$; $P<.05$; partial $\omega^2=.06$]. Follow-up comparisons at each ethanol concentration showed that the genotypes did not differ at any individual ethanol concentration. Further one-way ANOVAs at each genotype demonstrated that the interaction was due to the pattern of genetic differences across ethanol concentrations. Both WT and *Apoe*$^{-/-}$ mice showed less total consumption of fluid when 10% ethanol was available than all other ethanol concentrations (0%, 3% or 6% v/v; $.001<P<.005$). However, WT mice also drank significantly less fluid when 6% ethanol was available compared to when 3% ethanol was available ($P<.001$).

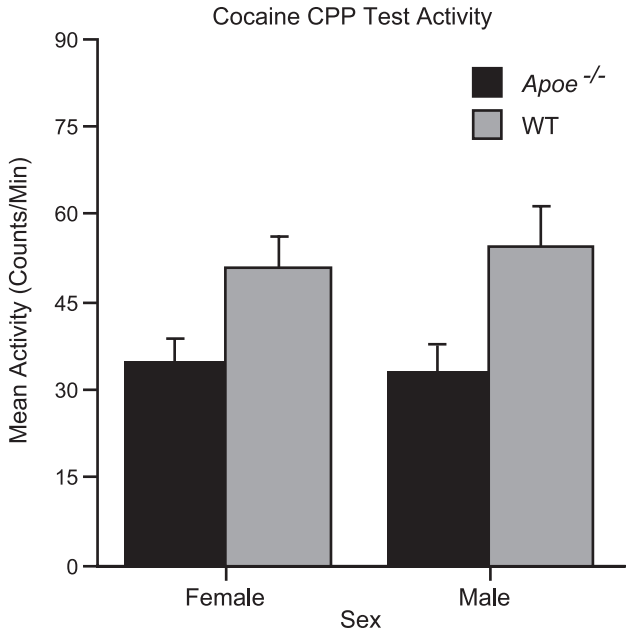

Fig. 6. Mean activity counts per minute (± S.E.M.) for each sex and genotype during the place preference test in the cocaine experiment. *Apoe*$^{-/-}$ ($n=11$ female, $n=12$ male) and WT ($n=12$ female, $n=10$ male) mice were injected with saline (10 ml/kg) immediately before being placed in the apparatus on a half grid, half hole floor. Significant main effect of genotype ($P<.001$).

Table 1
Group mean (±S.E.M.) WT and *Apoe*$^{-/-}$ mice during ethanol drinking

	Ethanol concentrations available (v/v)			
	0%	3%	6%	10%
Total consumption (ml/kg)				
Apoe$^{-/-}$ (n=11)	278.69±8.45	290.13±8.72	278.89±7.49	237.64±5.72
WT (n=12)	307.40±23.49	325.97±18.20	279.78±13.53	238.28±10.68
Ethanol dose (g/kg)				
WT	–	5.44±0.55	11.83±0.68	17.31±0.76
Apoe$^{-/-}$	–	4.55±0.37	11.23±0.68	16.27±0.91
Ethanol preference ratio				
Apoe$^{-/-}$	–	0.67±0.05	0.85±0.04	0.87±0.04
WT	–	0.70±0.04	0.89±0.03	0.93±0.03

3.4.2. Ethanol dose

Ethanol dose increased with increasing ethanol concentrations, but did not differ between genotypes. Dose data were analyzed using a two-way ANOVA with the factors genotype and ethanol concentration (3%, 6% or 10% v/v). This analysis revealed a significant main effect of ethanol concentration [$F(2,42)=371.0$; $P<.001$; partial $\omega^2=.91$]. Post hoc comparisons indicated that ethanol doses were significantly different across all possible comparisons of ethanol concentration ($P<.001$).

3.4.3. Ethanol preference ratio

The genotypes preferred ethanol equally and exhibited greater preferences as the ethanol solution became more concentrated. This conclusion was supported by a two-way ANOVA with the factors genotype and ethanol concentration. This analysis demonstrated a significant main effect of ethanol concentration [$F(2,42)=45.0$; $P<.001$; partial $\omega^2=.56$], but no effects of genotype. Follow-up analyses showed that ethanol preference ratios differed significantly across all possible comparisons with the preference ratio increasing with increasing ethanol concentrations ($.001<P<.04$).

4. Discussion

These studies illustrate that deletion of the apoE gene results in enhanced ethanol-induced CPP in mice. In fact, apoE-deficient mice showed a significant place preference under conditions where WT mice did not. Because *Apoe*$^{-/-}$ and WT mice did not differ in cocaine-induced CPP, it is possible that these effects are specific to the conditioned rewarding properties of ethanol-paired stimuli. That is, ethanol conditioned reward might normally be suppressed in the presence of apoE.

The mechanism through which the absence of apoE enhances ethanol-induced CPP is unknown. One possibility is that this effect may be linked to apoE's role in cholesterol transport. It has been shown that apoE-deficient mice exhibit increased cholesterol content in the exofacial leaflet of the synaptic plasma membrane (Igbavboa et al., 1997). Such alterations in membrane cholesterol content have been shown to alter the function of many membrane proteins. For example, the potency of GABA at the $GABA_A$ receptor is reduced (Sooksawate and Simmonds, 2001). This finding is of particular interest because ethanol-induced CPP is also enhanced with $GABA_A$ receptor blockade (Chester and Cunningham, 1999). Similarly, animals pretreated with $GABA_A$ antagonists on ethanol conditioning days demonstrated reduced ethanol-induced locomotor stimulation (Chester and Cunningham, 1999), a finding that parallels the lower activation seen in apoE-deficient mice. Our data cannot confirm that a GABA mechanism underlies our findings; however, this suggestion provides an interesting possibility for future investigation.

Apoe$^{-/-}$ mice demonstrated reduced locomotor stimulation by ethanol. This reduced locomotor stimulation in *Apoe*$^{-/-}$ mice does not support the psychomotor stimulant theory of reward, which predicts that animals showing greater drug-induced activation should express a stronger CPP (Wise and Bozarth, 1987). However, this finding is consistent with several previous studies showing a dissociation between ethanol's activating and rewarding effects in the CPP model (Cunningham, 1995; Cunningham et al., 2000; Cunningham and Ignatoff, 2000; Risinger et al., 1994; Risinger and Oakes, 1996). Additionally, the patterns of activity observed in WT mice, such that female C57BL/6 mice show greater stimulation to ethanol than males, is in agreement with the literature in that, female mice have generally been shown to exhibit greater ethanol-induced activation (Dudek et al., 1991). The mechanism underlying greater activation to ethanol in female mice is unknown. However, the observation in the present experiments that ethanol-induced locomotor activity was decreased by *Apoe* deletion, only in female mice, provides an interesting possible mechanism for these sex differences. Estrogen has been shown to up-regulate the expression of apoE mRNA (Stone et al., 1997), perhaps in doing so, the effects of ethanol are altered. Of particular relevance to the psychomotor stimulant theory of reward (Wise and Bozarth, 1987), this locomotor difference exists in the absence of significant sex differences in ethanol-induced CPP. Furthermore, contrary to the predictions of the psychomotor stimulant theory of reward, the trend toward stronger CPP in female mice is not associated with greater ethanol-induced activation. Indeed, female *Apoe*$^{-/-}$ mice show less ethanol induced activation compared to WT mice with a concomitant increase in the magnitude of ethanol CPP.

Apoe$^{-/-}$ mice also demonstrated lower activity levels during the ethanol preference test session. Previous research has suggested that lower activity levels during a CPP test might enhance the expression or retard the extinction of CPP (e.g., Cunningham, 1995; Vezina and Stewart, 1987). However, the finding of significant genetic differences in test session activity without a difference in cocaine-induced CPP makes it less likely that activity differences underlie the genetic difference in ethanol-induced CPP. Furthermore, similar dissociations between activity level during the conditioning test session and the expression of preference have been demonstrated (Cunningham et al., 1995).

Under the experimental conditions of this study, WT mice did not show significant ethanol-induced CPP. In general C57BL/6J mice do not readily express place preference for ethanol in an unbiased procedure (Cunningham et al., 1992). While C57BL/6J have been shown to acquire ethanol-induced place preference (Nocjar et al., 1999), the magnitude is weak compared to other strains (Cunningham et al., 1992, 1996). In addition, testing between Conditioning Trials 4 and 5 and conducting the second test after a 72-h delay may have offset the benefit of the additional conditioning trials due to extinction and decay of the CPP,

respectively. However, the relatively low level of CPP in WT mice may have allowed the effect of Apoe deletion to be detected. For example, use of a WT strain, such as DBA/2J, which expresses a robust ethanol-induced CPP (Cunningham et al., 1992), might have interfered with detection of the mutation's effect due to a ceiling effect.

Our conclusion that the effect of Apoe deletion on CPP may be specific to ethanol must be tempered by the fact that we tested cocaine using only one combination of conditioning parameters (i.e., a 5-mg/kg dose, a 60-min trial duration, and four conditioning trials). We cannot exclude that Apoe deletion would have been found to similarly alter cocaine-induced CPP using a different combination of parameters, especially parameters that produced a less robust CPP in WT C57BL/6 mice. The relative specificity of the effect reported here can only be confirmed in future studies that examine effects of Apoe deletion on place conditioning induced by cocaine and other drugs under a variety of parametric conditions.

Female *Apoe*$^{-/-}$ and WT mice did not differ in their consumption of ethanol in a two-bottle choice procedure. These findings are not consistent with previous studies suggesting a possible inverse relationship between ethanol drinking and CPP (e.g., DBA vs. C57; Cunningham et al., 1992; McClearn and Rodgers, 1959; HAP vs. LAP; Grahame et al., 2001). However, our results are consistent with the nonsignificant genetic correlation reported in a large group of BXD recombinant inbred strains derived from the C57BL/6J and DBA/2J strains (Phillips et al., 1998).

Although a small genetic difference in BEC was observed 30 min after ethanol injection, these data do not readily explain the difference in ethanol-induced place conditioning. *Apoe*$^{-/-}$ mice showed lower BECs but stronger ethanol-induced CPP compared to WT mice. Given the normally positive relationship between ethanol dose and CPP (Cunningham et al., 1992; Risinger and Oakes, 1996), lower BECs would be expected to yield weaker ethanol CPP, a direction opposite to that observed. However, one cannot rule out metabolic effects because a complete blood–ethanol elimination curve was not carried out and because of possible discrepancies between the tail–blood ethanol levels reported here and brain–blood ethanol levels.

In summary, these findings suggest that apoE can modulate the rewarding properties of ethanol, supporting a possible causal relationship between lower levels of apoE mRNA expression in the frontal cortex and alcoholism in humans. Because the neurobiological mechanisms underlying these effects are unknown, these data encourage further examination of apoE's role in ethanol reward.

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