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BRIEF COMMUNICATION

Voluntary Wheel Running Reduced the Effects of Acute Ethanol on Activity and Avoidance in C57BL/6J Mice

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MOLLENAUER, S., R. BRYSON, C. SPECK AND J. R. CHAMBERLIN. *Voluntary wheel running reduced the effects of acute ethanol on activity and avoidance in C57BL/6J mice.* PHARMACOL BIOCHEM BEHAV **39**(3) 821–824, 1991.—C57BL/6J mice were given five weeks of voluntary wheel running and then studied for behavioral impairment after an intoxicating dose of ethanol. Forty-four mice, 22 males and 22 females, were assigned to Wheel (free access to a running wheel in the home cage) or No Wheel conditions. At the end of the training period, animals were removed from the exercise cages and tested for noise avoidance after 2.4 g/kg ethanol (EtOH) or physiological saline (Sal). Mice could avoid 87.5-dB noise by entering and remaining in a randomly designated "safe corner." In unexercised animals, EtOH caused a strong suppression of locomotor activity and avoidance behavior: No Wheel EtOH mice differed significantly from No Wheel Sal mice on both measures. In exercised animals, EtOH failed to cause significant suppression: Wheel EtOH animals did not differ significantly from Wheel Sal animals on either measure. The present results suggest that prior exercise training may be effective in offsetting the effects of acute ethanol intoxication.

Exercise Voluntary wheel running Ethanol Noise avoidance Activity C57BL/6J mice

THE beneficial effects of exercise have been demonstrated in a number of systems, including cardiac and skeletal muscle (11,16). However, there has been relatively little research on the effects of prior exercise training on response to acute drug challenge, including ethanol (2). In one of the few investigations in this area, Ardies et al. (1) reported that exercise training caused more rapid clearance of blood ethanol. This finding suggests that prior exercise training might be effective in offsetting ethanol intoxication. In support of this idea, we recently found that five weeks of voluntary wheel running resulted in C57BL/6J mice showing significant resistance to the sleep-inducing effects of ethanol (8). Exercised animals took significantly longer to lose the righting reflex and showed significantly shorter sleep time after a sleep-inducing dose of ethanol (3.16 g/kg).

In the present research, we tested the hypothesis that prior exercise training would offset the effects of ethanol intoxication on noise avoidance behavior and activity in the C57BL/6J mouse. The C57BL/6J is an inbred strain that has been used widely in alcohol, hearing, and exercise research. Thus previous research has established its normal ethanol sensitivity (3,15), its physiological and behavioral responses to acoustic stimuli (10,17), and its predisposition to show high levels of wheel running and maintain normal body weight (7). Additionally, in previous work in our laboratory, we established appropriate parameters for noise avoidance conditioning in the C57BL/6J mouse (9). In this earlier work, we found that C57BL/6J mice rapidly learn to enter a corner of a square apparatus in order to terminate a noise of moderate intensity (87.5–90 dB).

In the present experiment, animals were given five weeks of voluntary wheel running and then compared to unexercised controls for noise avoidance after an intoxicating dose of ethanol (2.4 g/kg). Pilot work had established this dose as one that did not induce sleep but did produce significant intoxication as indicated by behavioral inhibition.

METHOD

Animals

The animals were 44 C57BL/6J mice, two months old at the beginning of the experiment. One animal died during the course of the experiment; one was lost as a consequence of apparatus failure. All animals were third-generation offspring bred from stock obtained from Jackson Laboratories, Bar Harbor, ME. The animals were separated by sex at approximately one month of

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age and were reared in same-sex litter groups, with no more than four per cage. They were maintained on a 12-h light/dark cycle adjusted so that all behavioral tests could be conducted during the active phase of the animals' cycle. The light phase began at 1:00 p.m., and all injections were administered between 8:00 and 10:00 a.m., with exercised and unexercised animals evenly represented across hours. The animals had ad lib access to water and mouse lab chow throughout the course of the experiment and until 30 minutes before ethanol injections.

Exercise Conditions

Beginning at two months of age, equal numbers of male and female mice were randomly assigned to Wheel or No Wheel conditions, with body weight balanced across conditions. Each animal was individually housed in a rectangular acrylic home cage, 25 × 45 cm and 25 cm deep, with a wire cloth floor and wire cloth lid. Half the cages were equipped with small rodent running wheels (Ward Co.) measuring 18 cm in diameter. The cages were housed in large sound-attenuated chambers equipped with fans and low-watt lighting to maintain the light-dark cycle; males and females were housed in separate chambers. Chambers were opened for servicing at 12:00 p.m. each day, and animals were not otherwise disturbed. In preliminary work monitoring number of wheel revolutions by computer, we obtained levels of activity comparable to previous reports (7,13) and confirmed that these animals show highly reliable wheel running, averaging approximately 500 revolutions per hour by the fifth week of exercise training.

Avoidance Apparatus

The avoidance apparatus was 32 × 32 cm square and 16 cm deep, made of white acrylic plastic, and covered with a fine mesh lid; it was housed inside a sound-attenuated chamber with a window in the lid. The apparatus was equipped with sixteen photobeams spaced at 2-cm intervals along each of the four sidewalls; these beams provided a grid of 256 intersections. Interruptions of these beams were read by a PC with software that 1) defined the safe area, a corner measuring 12 cm on a side, 2) controlled noise delivery, and 3) recorded the animal's activity and position in the apparatus, including the safe area.

Noise Stimulus

The computer-controlled noise stimulus was delivered through a piezo tweeter suspended 37 cm above the floor of the apparatus. The noise stimulus was centered at 10 kHz and constrained between 7 kHz and 14 kHz. The frequency for the noise was based on earlier research with C57BL/6J mice showing robust behavioral response in this frequency range (18,19). The properties of the noise stimulus and the calibration procedures have been described in more detail previously (9).

Procedure

After five weeks in Wheel or No Wheel conditions, animals were randomly assigned to ethanol (EtOH) or saline (Sal) conditions and tested for noise avoidance following an intraperitoneal injection of 2.4 g/kg (30% w/v) ethanol or normal saline in a volume of 0.01 ml/kg. Each animal was removed from its chamber 30 minutes before injection and placed in an individual holding cage. After injection, the animal was returned to the holding cage and then tested for avoidance 15 minutes after injection. The animal was placed in the center of the apparatus, and the chamber was closed. After a 2-minute habituation period, the noise stimulus was delivered for seven minutes, and the animal could terminate the noise by entering and remaining in a randomly designated corner. The computer controlled the noise stimulus and recorded both the animal's activity and time accumulated in the designated corner.

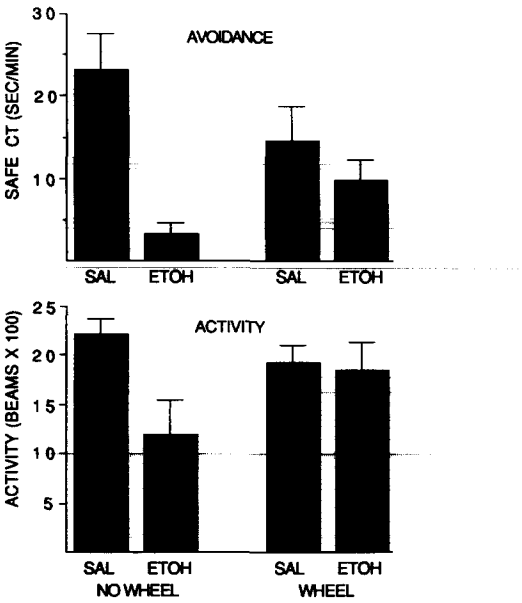

FIG. 1. Upper panel: Mean avoidance, or safe corner time (CT), per minute of noise exposure, ± SEM, after physiological saline (SAL) or an intoxicating dose of ethanol (2.4 g/kg) (ETOH). Lower panel: Mean activity, or number of beam crossings, ± SEM, after SAL or ETOH. Animals were tested after 5 weeks of voluntary exercise (Wheel) or no exercise (No Wheel).

RESULTS

The effects of wheel running on avoidance behavior and locomotor activity are summarized in Fig. 1. The data for each measure were analyzed by ANOVA with three factors: wheel, drug, and gender. The avoidance data, i.e., mean corner time (CT) or time in the designated safe corner during the seven minutes of noise presentation, are presented in the upper panel of the figure. As the figure shows, ethanol caused significant impairment of avoidance behavior, $F(1,34) = 16.27$, $p < 0.01$. The figure also shows an interaction between exercise and drug conditions, $F(1,34) = 5.09$, $p < 0.05$, in which the drug effect was especially strong for the No Wheel animals; No Wheel EtOH animals averaged less than five seconds per minute in the safe corner, and differed significantly from No Wheel Sal controls, $F(1,34) = 20.07$, $p < 0.01$. In contrast, Wheel EtOH animals did not differ significantly from Wheel Sal animals, $F(1,34) = 1.60$. The main effects of wheel and gender were not significant, nor did gender interact significantly with wheel and drug.

Locomotor activity, as measured in beam interruptions, is presented in the lower panel of Fig. 1. Again, ethanol caused a strong depression of behavior, $F(1,34) = 4.84$, $p < 0.05$, and exercise attenuated the effect. The interaction between wheel and drug was not significant in the activity data, $F(1,34) = 3.23$, $p = 0.08$, but planned comparisons showed a pattern of results closely paralleling the avoidance data. The No Wheel EtOH animals showed a substantial depression of activity, differing significantly from No Wheel Sal animals, $F(1,34) = 8.12$, $p < 0.01$, while Wheel EtOH animals did not differ from Wheel Sal ani-

mals ($F<1$). The ANOVA also showed an interaction between drug and gender, $F(1,34)=5.22$, $p<0.05$, in which the activity of male animals was depressed by ethanol, $F(1,34)=10.72$, $p<0.01$, but that of females was not ($F<1$).

The ANOVA of body weight indicated that male mice reliably weighed more than female mice (mean weight = 27.33 and 21.80 g, respectively), $F(1,38)=127.40$, $p<0.01$, but exercise had no significant effect on body weight, $F(1,38)=3.02$, nor did gender and exercise interact ($F<1$).

DISCUSSION

The present research provides further evidence that exercise training can offset acute ethanol intoxication. After five weeks of voluntary wheel running, C57BL/6J mice showed significant resistance to a dose of ethanol (2.4 g/kg) that was severely intoxicating but not sleep-inducing. When treated with this dose of ethanol, unexercised animals showed a marked suppression of locomotor activity and avoidance behavior, but exercised animals did not differ from saline controls. In previous work, we had found that C57BL/6J mice given five weeks of wheel running were significantly more resistant than unexercised controls to a sleep-inducing dose of ethanol (8). In both cases, five weeks of exercise training reduced the behavioral consequences of acute ethanol treatment.

An important feature of the present research is the fact that no further exercise was administered after ethanol injection; i.e., animals were not returned to the exercise chambers after ethanol injection but instead were placed in a holding cage until avoidance testing. Thus the present results suggest that prior exercise training can affect subsequent ethanol response.

Animal models of exercise training have most often addressed the question of whether chronic exercise can reverse or prevent deterioration associated with disease, aging, or chronic ethanol ingestion (4,12). Concerning the adverse effects of aging, research now suggests that exercise training has protective effects against physiological deterioration, provided it is introduced before old age (12). Likewise, Samorajski et al. (13) reported that voluntary wheel running attenuated impairment of memory for one-trial passive avoidance in middle-aged but not old-aged C57BL/6J mice. With respect to the adverse effects of chronic ethanol ingestion, Farrar et al. (4) reported that exercise training was able to reduce the deterioration in mitochondrial function of skeletal muscle caused by chronic dietary ethanol. The present results suggest further that exercise training may offset the behavioral impairment caused by acute ethanol intoxication. They also raise the interesting possibility that exercise could offset some of the behavioral impairment caused by chronic ethanol ingestion.

As to the mechanisms that might explain the present results, exercise may have affected several physiological factors, including ethanol clearance and distribution. Any change in body composition that would affect the distribution of ethanol to blood and brain could be expected to alter the behavioral effects of ethanol. The analysis of weight data in the present results showed that access to running wheels failed to cause significant differences in body weight. However, it could be that the exercised animals had different body compositions, with proportionately less body fat and more body water. The effect of this would be that exercised animals had proportionately more body water and thus a higher volume of distribution for ethanol, or an effectively lower dose (5). Another factor that might have contributed to the present results is accelerated clearance of ethanol. Intense exercise following ethanol administration has been shown to cause an increased rate of ethanol clearance (1,14), and several investigations have suggested that exercise training causes increased activity of the hepatic microsomal ethanol oxidizing system in rats (1,6). Thus one possible explanation of the present results is that wheel running caused an alteration in hepatic activity that, in turn, caused more rapid clearance of ethanol. We are currently studying the effects of voluntary wheel running on ethanol clearance.

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