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EtOH Self-Administration in Anticipation of Noise Stress in C57BL/6J Mice

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MOLLENAUER, S., R. BRYSON, M. ROBISON, J. SARDO AND C. COLEMAN. *EtOH self-administration in anticipation of noise stress in C57BL/6J mice.* PHARMACOL BIOCHEM BEHAV **46**(1) 35–38, 1993.—C57BL/6J mice were studied for self-administration of ethanol (EtOH) during a signal period that preceded delivery of an environmental stressor (noise) in the home cage. Animals were given 5 weeks of conditioning in which a 5-min period of 75-dB pulsed noise (SIGNAL) preceded a 20-min period of more intense, 90-dB pulsed noise (NOISE) five times daily. EtOH (10% w/v) was then provided in a choice procedure, and drink tube contacts were monitored by computer. Mice that had received the 5 weeks of SIGNAL and NOISE pairings showed an increase in EtOH-seeking behavior, as reflected in EtOH tube contacts during the SIGNAL period. The increase was significant as compared to contacts during baseline or QUIET periods and also as compared to contacts during the same period for control (Ctrl) mice that had received only the 75-dB SIGNAL during conditioning. A subsequent test for passive avoidance confirmed that the 75-dB SIGNAL was aversive for mice that had received noise conditioning but not for Ctrl mice. In sum, the results were in accord with a priori predictions that mice would not show increased EtOH tube contacts during occurrence of intense noise itself but would show increased contacts during the signal that preceded noise. These results were interpreted as preliminary evidence that C57BL/6J mice show self-administration of EtOH in anticipation of an environmental stressor.

Ethanol Alcohol self-administration Stress Noise C57BL/6J mice

THE intent of the present research was to develop a model of stress-related ethanol (EtOH) self-administration in the C57BL/6J mouse. The critical feature of this model is that self-administration is expected to occur in anticipation of an environmental stressor. Stress or tension reduction has long been assumed to play a role in the etiology of alcohol abuse (3), and this idea has been the basis for a formidable literature showing that EtOH reduces physiological responses to stress (14). Ethanol blunts reactions to stress-induced hormones (14,15), ameliorates stress-induced depletion of the neurotransmitters (7,11), and, perhaps most important, has been linked to the BDZ-GABA receptor systems that mediate anxiety (1,10).

Although pain stimulation has been used successfully in some instances to induce self-administration of EtOH in animals [see review by Pohorecky (14)], efforts to develop animal models of stress-induced EtOH consumption have for the most part been considered unsuccessful (4,14). The explanation may be that aversive stimulation generally causes a suppression of consummatory behavior (2). Shock in particular is known to cause poststimulation freezing (8).

In the present research, we sought to avoid the problem of behavioral suppression in two ways. First, we worked with a fairly moderate stressor, namely, 90-dB pulsed noise. We established in previous research that this noise stimulus is moderately aversive to the C57 mouse but does not elicit significant behavioral suppression (12). Second, we did not attempt to elicit EtOH self-administration during the presentation of the stressor itself. Instead, we designed a procedure to assess EtOH self-administration in anticipation of the stressor. Before they were given access to EtOH, animals were first given 5 weeks of training in a conditioning paradigm in which a low-intensity noise signal, 75-dB pulsed noise, was paired with the more intense 90-dB noise. Control animals received only the low-intensity noise. Self-administration of EtOH was monitored by drink tube contacts. This procedure made it possible to assess signal-induced self-administration of EtOH.

METHOD

Animals

Animals were 64 C57BL/6J mice 2 months old at the beginning of the experiment. Four animals were lost during the course of the experiment as a consequence of apparatus failure. All animals were third-generation offspring bred from stock obtained from Jackson Laboratories (Bar Harbor, ME).

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Animals were separated by sex at approximately 1 month of age and reared in same-sex litter groups, with no more than four animals per cage. They were maintained on a 12 L : 12 D cycle.

Procedure

Housing and data collection. Animals were individually housed in rectangular acrylic cages, 25 × 45 cm and 25 cm deep, with a wire cloth floor and wire cloth lid. Each cage was housed in a separate sound-attenuated chamber equipped with a speaker for delivery of noise stimulation, a fan, and low-watt lighting; male and female mice were housed in separate rooms. Chambers were opened for servicing at the same time each day, 1 h before onset of the light-on period, and animals were otherwise left undisturbed.

Each cage was equipped with a drink monitoring device, based upon methodology developed by Dole et al. (5). This device permitted separate assessment of drinking from water and EtOH tubes during the choice procedure. Drinking tubes were those having closure balls to reduce spillage and evaporation and prevent false positive registration of drink contacts. Drink times on the two tubes were read by computer at 5-min intervals except during the hour when animals were interrupted for servicing.

Noise stimulation. After a 2-week period for adaptation to the chambers, noise stimulation began and continued throughout the course of the experiment. Animals were randomly assigned to noise and control conditions. Animals in the noise condition (Noise) received five 20-min periods of 90-dB pulsed noise, beginning 1 h after onset of the dark (active) period and spaced at 2-h intervals thereafter. Each noise period was preceded by a 5-min signal period of 75-dB pulsed noise. Animals in the control (Ctrl) condition received only the 75-dB pulsed noise. Apart from the difference in intensity, the properties of the signal noise were identical to those of the stressor noise.

The properties of the noise stimulation and calibration procedures have been described in detail previously (12). Briefly, computer-controlled noise was delivered through a Piezo tweeter suspended 37 cm above the floor of the living cage. Raw white noise was filtered with a 7- to 14-kHz bandpass filter to provide a one-octave band of noise centered at 10 kHz; the frequency of the noise was based upon earlier research studying acoustic phenomena in C57BL/6J mice (16). Noise was delivered in 0.2-s pulses having a 5-ms rise time and occurring at randomly sequenced intervals of 0.5, 0.9, and 1.6 s, with the random order restricted so that the same interval did not repeat in succession. The intensity of noise was calibrated using a meter modified for remote readout and also equipped with a 10-kHz filter; this custom meter was calibrated against a B&K meter. Measurements made throughout the living cage showed that noise was loudest (90 dB) directly below the speaker and fell off a maximum of 2.5 dB at points most distant from the speaker. Background noise was approximately 55 dB. The method of calibration used in this experiment was not intended to assess the intensity actually experienced by the moving animal at any given time; it was intended to ensure that noise stimulation was constant across animals.

Ethanol choice. After 5 weeks of noise conditioning, animals were tested for EtOH self-administration; the noise program was continued as before, and EtOH self-administration was assessed using a two-bottle choice procedure. To ensure that all animals would be exposed to EtOH before choice data were assessed, we implemented the following procedures. On the first day of EtOH administration, animals were exposed to a 5% solution of EtOH, with EtOH present in both drinking tubes during the last 6 h of the dark cycle. On the second day, they were exposed to a 10% solution of EtOH in both drinking tubes for the same period. On the third day, the choice procedure was instituted, with a 10% EtOH solution continuously available in one drinking tube and water available in the other. The choice procedure was continued for 3 days. The position of the EtOH and water tubes were alternated daily; tubes were refilled and weighed to provide an additional estimate of overall intake. As noted above, drinking time was read for both tubes at 5-min intervals.

Noise avoidance. Animals from the second replication of this experiment (n = 31) were subsequently tested for avoidance of the 75-dB noise stimulus to confirm the effectiveness of the conditioning procedure. After the 3 days of EtOH choice procedure, EtOH was removed and animals were continued in the noise program for an additional 2 weeks. They were then tested for noise avoidance following a procedure described previously in full detail (12). Briefly, the animal was placed in the center of an apparatus 32 cm square and 16 cm deep, made of white acrylic plastic, open at the top. The apparatus was situated in a sound-attenuated chamber, and 75-dB noise identical to that described above was delivered through a Piezo tweeter suspended 37 cm above the floor. After a 2-min habituation period, 75-dB noise was delivered, and the animal could terminate the noise by remaining in the randomly designated safe half of the apparatus. Photobeams were positioned at 2-cm intervals along the side walls describing a grid of 256 intersections. Interruptions of the beams were read by a PC that controlled noise delivery and recorded time on the safe side per minute of noise exposure.

RESULTS

EtOH Self-Administration

Computer-read data for EtOH and water consumption were averaged over the 3 days separately by period (signal, noise, and quiet) during the first half of animals' dark or active period. Data from the second half of the dark period were not analyzed because animals had achieved levels of intoxication that could be expected to interfere with sensory processes.

Figure 1 depicts average time of water tube contacts (left panel) and average time of EtOH tube contacts (right panel) for the three time periods. As noted above, animals in the Noise condition (black bars) were exposed to both the 75-dB signal (SIG) and the 90-dB noise (NOISE) but animals in the Ctrl condition (hatched bars) were exposed only to the 75-dB signal. Comparisons of tube contacts for quiet (QUIET) periods preceding and following Noise periods showed them to be virtually identical; thus, the data were averaged over these periods.

The data summarized in Fig. 1 were evaluated by analysis of variance (ANOVA). It is clear from the figure that the onset of the 75-dB signal resulted in an increase in average tube contacts relative to the other periods, $F(2, 116) = 16.45$, $p < 0.05$. However, the most interesting feature of these results is the fact that only animals in the Noise condition showed increased EtOH contacts during the SIGNAL period, as reflected in a significant interaction between noise condition, time period, and drug choice, $F(2, 116) = 3.41$, $p < 0.05$. Separate analysis of the EtOH data confirmed the inter-

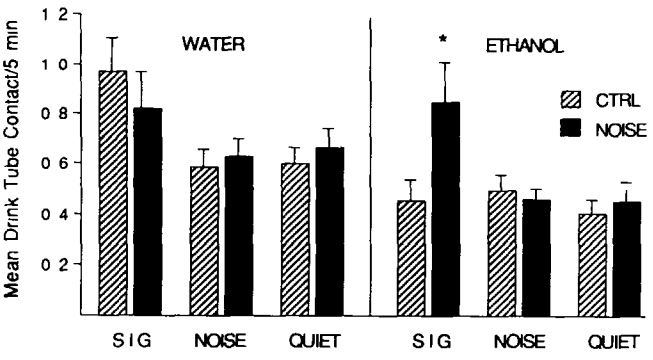

FIG. 1. Mean tube contacts per 5-min period (±SEM) for water (left) and ethanol (EtOH) (right). Noise animals (black bars) received 75-dB pulsed noise during the signal period (SIG) and 90-dB pulsed noise during the noise period (NOISE). Control animals (hatched bars) received only the 75-dB signal. All animals showed increased water contacts during SIG, but only Noise animals showed significantly increased EtOH contacts during SIG. *Differs from Ctrl ($p < 0.01$).

action between noise condition and time period $F(2, 116) = 4.46$, $p < 0.05$, with simple effects showing that Noise animals showed significantly more EtOH tube contact than Ctrl animals during the SIGNAL period, $F(1, 116) = 9.45$, $p < 0.05$, but did not differ from Ctrl during any other period. In the analysis of the water data, there were no differences between the Noise and Ctrl animals, and, consistent with the overall analysis, both groups showed increased water drinking during the SIGNAL period $F(2, 116) = 7.19$, $p < 0.05$.

ANOVA of these data also showed a significant interaction between gender and drug in which female mice consumed relatively more EtOH than male mice $F(1, 58) = 12.46$, $p < 0.05$. For female mice, 48.73% of total tube intake was EtOH and for male mice 36.9% was EtOH. The data in Fig. 1 are not broken down by gender because gender did not interact with either of the factors of interest—noise condition or period. Neither was the main effect of gender significant.

Finally, it is evident from the figure that overall water intake was higher than EtOH intake, $F(1, 58) = 6.41$, $p < 0.05$; expressed in percent, EtOH intake accounted for approximately 42.6% of the total daily intake. This pattern was confirmed in the assessments of intake by tube weight. Animals averaged 1.9 g EtOH per day and 2.32 g water. The pattern in gender was also confirmed; for females, 49.3% of total fluid intake was EtOH and for males 39.8% was EtOH. The correlation between computer-assessed + intake and tube weight-assessed intake was $r = 0.86$.

Inspection of tube contacts across 24 h showed that tube contacts were highest and showed the least variance during the first 6 h of the dark period, the period for which data are presented; contacts then dropped off sharply during the seventh and eighth hours and were sporadic during the remainder of the dark phase. There were almost no tube contacts during the light phase. The pattern was the same for water and EtOH tube contacts, and for male and female mice, although the absolute levels differed as discussed above.

Noise Avoidance

Noise avoidance was assessed as percent time spent on the safe or quiet side of the apparatus during the 10 min of exposure to 75-dB noise. The data were evaluated by t-tests comparing % safe time to the expected value of 50%. Ctrl animals did not differ significantly from chance in percent time on the safe side ($M = 53.08\%$), $t(14) = 1.03$. In contrast, Noise animals showed a small but highly reliable increase in time on the safe side ($M = 56.81\%$) relative to chance, $t(15) = 3.33$, $p < 0.01$.

DISCUSSION

The primary finding of the present research was that C57BL/6J mice showed significantly increased EtOH self-administration during a period of 75-dB pulsed noise, which preceded the onset of more intense 90-dB pulsed noise; this effect can be seen in the SIGNAL period of Fig. 1. This finding suggests evidence of stress-related drinking or, more specifically, drinking in anticipation of a stressor. It is unlikely that the increased EtOH intake was attributable solely to the alerting or activating properties of the signal. The 75-dB noise did indeed cause a general activation, as reflected by the fact that both Noise and Ctrl groups showed increased water tube contacts during this period. However, only the Noise group showed increased EtOH tube contacts during the signal period.

Another important finding was that the subsequent test for noise avoidance confirmed the effectiveness of the 75-dB signal for Noise animals. When animals could avoid this noise simply by remaining in the randomly designated half of a square apparatus, Ctrl animals did not differ from chance in the amount of time spent on the quiet side. In contrast, Noise animals showed a low but highly reliable level of noise avoidance, suggesting that the period of conditioning had invested the 75-dB noise with aversive properties or prevented animals from adapting to any initial aversive properties. In either case, the avoidance data support the view that the 75-dB pulsed noise was at this point mildly aversive. The present research did not address the question of whether the 75-dB signal was a conditioned stimulus in the classical sense. Instead, we addressed the limited but important problem of developing an animal model of drinking in anticipation of a stressor. For this reason, we wished to maximize the similarity between the signal and the noise stressor. It was our intent to create a situation that would take full advantage of stimulus generalization to create a period of mild anxiety.

Another aspect of the present results that deserves comment is the fact that EtOH intake for Noise and Ctrl animals did not differ during the delivery of the more intense noise stimulus, the period designated "NOISE" in Fig. 1. The lack of difference during this period does not represent a problem for the present work. In fact, one of the important premises of this approach is that it is unrealistic to expect increased EtOH intake during the actual presentation of an environmental stressor (2).

We chose the C57BL/6J mouse for this research because this animal is known for its genetic predisposition to consume EtOH (9). Dole et al. (6) suggested that the genetic role would be better described as "permissive" and also that "efforts to model human drinking behavior in rodents should start with genetically permissive strains." Applied to the present model, the predisposition of the C57 to consume EtOH represents a distinct advantage in that it provides a baseline level of drinking that can then be influenced by environmental factors. The signal-induced drinking that occurred in the present results cannot be attributed solely to genetic factors but may represent an interaction between genetic and environmental factors, such as conditioned anxiety.

Another finding of interest in the present research was the higher percent overall EtOH consumption by female mice. Recent work has shown that, relative to body weight, female C57BL mice consume more EtOH than males but show lower levels of blood acetaldehyde, implying a more rapid metabolism of alcohol (13). We did not find gender interactions with noise condition or signal-related drinking. However, assessment of blood alcohol levels and the role of gender in these levels will be an important consideration for future research looking at longer periods of signal-induced drinking.

In summary, the present study offers preliminary evidence that C57BL/6J mice show increased EtOH self-administration in the presence of a low-intensity noise that signals the onset of more intense noise. The present paradigm has several important advantages. First, animals are not removed to a novel environment that would be expected to inhibit consummatory behavior but instead are exposed to the stressor in their living environment. Second, the stressor itself is sufficiently mild that it does not elicit behavioral suppression. Third, the paradigm does not involve pain stimulation and therefore avoids the issue of EtOH-induced analgesia. Although the present paradigm does not satisfy the criteria for an animal model of alcoholism, we suggest that it provides a useful model for the study of stress-related drinking or, more specifically, EtOH self-administration in anticipation of a stressor.

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