Noise avoidance in the C57BL/6J mouse

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Noise was evaluated as an aversive stimulus in the C57BL/6J mouse, using a simple escape/avoidance procedure in which mice could terminate noise by entering and remaining in a designated safe area (corner or side) of a square apparatus. Exposed to pulsed noise of 87.5-90 dB, mice spent 45%-50% of total corner time in the safe corner or approximately 80% of total time in the safe side. Acquisition was significantly faster with moderate intensities of pulsed noise (87.5-90 dB) than it was with high-intensity pulsed noise (100 dB). In comparisons of continuous as opposed to pulsed noise, acquisition was significantly faster with continuous (87.5-dB) noise than it was with pulsed noise, and continuous noise was shown in a choice procedure to be more aversive. Continuous noise caused significant, though not severe, suppression of activity, but pulsed noise caused virtually none. Thus, pulsed noise is a mildly aversive stimulus for C57BL/6J mice and may have promise for the study of stress-induced behaviors in freely moving animals, but continuous noise is clearly more effective for rapid short-term conditioning.

The general intent of this research was to develop noise as an aversive stimulus for behavioral research in the C57BL/6J mouse. Ideally the stimulus would involve only minimal distress and could be used in both acute and chronic studies of freely moving animals. Specifically, we hoped to develop a paradigm suitable for the study of acute ethanol intoxication and also chronic *stress-induced* ethanol consumption in the C57BL/6J mouse. Attempts to develop animal models of stress-induced drinking have generally failed, very often resulting in an overall decrease in ethanol consumption (Dole, 1986). This phenomenon is not surprising in view of the fact that aversive stimuli generally cause inhibition of consummatory behavior (Bolles, 1970). Shock, in particular, is known to cause poststimulus freezing (Fanselow, 1980). Presumably, a mildly aversive noise stimulus would be less likely than shock to elicit behavioral inhibition. If such a stimulus could be shown to be a behavioral *stressor*, in the sense that it would sustain escape behavior, it might have promise for studies of stress-related self-intoxication.

Noise stimulation has been shown to cause physiological stress responses in rats, including changes in plasma hormone levels (Armario, Castellanos, & Balasch, 1985; Armario, Lopez-Calderon, Jolin, & Balasch, 1986) and central neurotransmitter function (Boadle-Biber, Corley, Graves, Phan, & Rosecrans, 1989; Lai & Carino, 1990; Lai, Carino, & Wen, 1989). Early work with rats also suggested that noise would be an effective aversive stimulus, in that animals executed escape behaviors to terminate noise (Bolles & Seelbach, 1964; Campbell, 1955). An

early study (Barnes & Kish, 1957) with C57BL/6 mice also showed that these animals learned to remain on a small platform in order to avoid continuous white noise of 98 dB. However, observations of the animals in the latter research suggested that noise of this intensity tended to elicit bouts of freezing. In more recent research (Leaton & Cranney, 1990), high-intensity (117-dB) white noise has been used as the unconditioned stimulus in Pavlovian fear conditioning; in this case, a conditioned stimulus (light) paired with high-intensity noise was used to induce freezing and to potentiate acoustic startle. Since we specifically wished to avoid the induction of freezing behavior, our research explored a lower range of noise intensity, the highest level being 100 dB.

In the experiments reported here, we used a simple escape/avoidance procedure to define the properties of a noise stimulus that would serve as a mildly aversive stimulus for the C57BL/6J mouse. This strain is of particular interest, because it is genetically predisposed to high voluntary ethanol consumption and has been used widely in ethanol research (Ho, Gentry, Chin, & Dole, 1988). In addition, the C57BL/6J has been studied extensively in hearing research, so both its physiological and its behavioral responses to acoustic stimuli are well-established (Parham & Willott, 1988; Willott & Demuth, 1986). The parameters of noise stimulation used in the present research were based on a substantial literature defining acoustic phenomena in the C57BL/6J mouse (Willott, Kulig, & Satterfield, 1984; Willott, Shnerson, & Urban, 1979).

GENERAL METHOD

Animals

The animals were 160 C57BL/6J mice; equal numbers of males and females were randomly assigned to experimental treatments as detailed below. All animals were third-generation offspring bred from stock obtained from Jackson Laboratories (Bar Harbor, ME).

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The animals were separated by sex at approximately 1 month of age and were reared in same-sex litter groups, with no more than 4 per cage. The colony was maintained in a sound-attenuated room where the ambient noise level was approximately 60 dB, and a 12:12-h light:dark cycle was adjusted so that behavioral tests could be conducted during the dark phase of the animals' cycle. The light phase began at 1:00 p.m.; testing was conducted between 8:00 a.m. and 12:00 p.m. Experiments were conducted in replications, with all animals tested at approximately the same age, 3 months $\pm$ 6 days.

Apparatus

The apparatus was 32 cm square and 16 cm deep, made of white acrylic plastic, and open at the top; it was located in a sound-attenuated chamber with a window in its lid. To record time in the *safe corner*, each corner of the apparatus was equipped with three sets of infrared photobeams, aimed diagonally across the corner, describing a triangle extending 12 cm on each side wall. To record locomotor activity, two additional sets of photobeams were spaced along the side walls in both planes at 10.5-cm intervals and were aimed directly across the apparatus, to create an intersecting grid. Interruption of one or more of the photobeams by the mouse was recorded on an IBM PC. For each minute of the test period, the computer recorded the number of photobeam interruptions (activity), as well as time spent in each corner of the apparatus.

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 originated from a white noise source consisting of a pseudorandom bit-sequence generator. To provide a one-octave band of noise centered at 10 kHz, the raw noise was filtered with a 7–14 kHz bandpass filter, with -12 dB/octave roll-off outside those frequencies. The frequency for the noise was based on earlier research with C57BL/6J mice, which showed reliable behavioral responding in this frequency range (Willott et al., 1984; Willott et al., 1979).

In the pulsed noise condition, the computer presented 0.2-sec pulses that had a 5-msec rise time and occurred at randomly sequenced intervals of 0.5, 0.9, and 1.6 sec, with the random order restricted so that the same interval was not repeated in succession. Noise was always delivered immediately when an animal exited the safe area.

Intensity of noise was calibrated before each trial with the chamber closed, using a meter modified for remote readout; the shroud of the head on the microphone was removed to decrease directionality of the reading. A spacing template was attached to ensure that the head would be precisely positioned for each reading, with the microphone under the speaker, pointing toward a corner. Like the sound generator, this custom meter was equipped with a 10-kHz filter with knees at 7 and 14 kHz. The custom meter was calibrated against a Brüel & Kjaer meter, equipped with octave filters and using test frequencies common to both meters (8–11 kHz) at a range of intensities from 70 to 105 dB. This procedure ensured that the custom meter provided readings on the same scale as the Brüel & Kjaer meter, relative to the customary sound pressure level of 2 μ Pa.

The method of calibration used in the experimental apparatus, with a meter fixed in position, was not intended to assess the intensity actually experienced by the moving animal, but instead, ensured that the noise stimulus was constant across animals. To provide some idea of intensity variations within the apparatus, measurements were conducted in an identical apparatus equipped with a movable floor that had a sound-measurement hole, such that the microphone could be moved throughout the apparatus. Measurements in this apparatus showed that the intensity of noise delivered from the overhead speaker varied up to 2 dB throughout the apparatus. Sound was always loudest in the center of the apparatus under the speaker, decreasing to a minimum at about 3.5 cm from a side wall, and then increasing to a maximum at about 1 cm from

the wall. This pattern was consistent over the range of intensities used; that is, 100-dB noise fell off to 98 dB, 90-dB noise fell off to 88 dB, and so forth. The ambient noise in the apparatus, also recorded with the chamber closed, was approximately 55 dB and varied less than one dB throughout the apparatus. This noise was present at all times, serving as both a background noise for the pulsed noise and as the *quiet* condition.

EXPERIMENT 1

Since one objective of the present research was to determine a stimulus that would be suitable for chronic administration, we explored discontinuous or pulsed noise composed of short (.2-sec) randomly scheduled noise bursts. The properties of this stimulus were based on earlier work in acoustic startle with the C57BL/6 mouse (Willott et al., 1984; Willott et al., 1979). However, the stimulus was not adopted for its capacity to elicit startle. The parameters of noise stimulation required for acoustic startle are, of course, well established for both the rat (Davis, 1980) and the C57BL/6 mouse (Willott et al., 1984). However, it has also been determined that acoustic startle shows rapid habituation when the stimulus is repeated on a short-interval schedule (Davis, 1970; Willott & Demuth, 1986; Wilson & Groves, 1973). The purpose of using pulsed or discontinuous noise was to establish a stimulus that would be less likely than continuous noise to induce sensory adaptation and acoustic damage when used chronically. In the early work showing that noise induced escape behavior, continuous high-intensity white noise had been used (Bolles & Seelbach, 1964; Campbell, 1955; Montgomery & Monkman, 1955); thus, it remained to be determined whether pulsed noise of moderate intensity would be an effective aversive stimulus.

Method

Experiment 1 was designed to confirm the effective intensity for noise stimulation, with mice tested at 100, 90, 87.5, and 75 dB. These specific intensities were chosen to bracket the conditions that we planned to use in experiments involving chronic noise. In the sound-attenuated chambers used for chronic research, noise calibrated to 90 dB at the center of the chamber ranges from a high of 90 dB at the center of the animal's cage to a low of 87.5 dB in the more distant areas of the cage. Thus, the 87.5-dB condition was included to ensure that the lowest intensity experienced in this program would be effective. On the basis of preliminary work, we did not expect a difference between animals tested at 90 and at 87.5 dB. The 75-dB condition was included because this noise stimulus is used as a signal for louder noise in our chronic research.

Design. Sixty mice were randomly assigned to five groups, with 6 males and 6 females in each group: a control group that did not receive noise stimulation apart from ambient chamber noise (55 dB), and four noise groups that received pulsed noise at 100, 90, 87.5, or 75 dB. All animals, including the controls, were randomly assigned a designated safe corner; animals in the noise groups could terminate the noise by entering the safe (quiet) corner. Sequence of corner assignment was constant across conditions and was rotated within conditions.

Procedure. Animals were removed from their home cages approximately 30 min before testing and placed in individual holding containers. At the beginning of the noise conditioning session the mouse was removed from the container and placed in the center of the apparatus directly below the speaker. For animals in noise

conditions, the noise was started immediately and the mouse could terminate the noise by entering its randomly designated corner. The session continued for 7 min, with data recorded by the computer.

The noise stimulus was controlled by a trained observer who interrupted the computer-generated noise when any part of the animal's body, apart from its tail, was inside the corner. In order to be "blind" to the intensity being administered, the observer wore headphones that conveyed noise bursts synchronized with those given to the animal, but constant in intensity.

Results

Escape/avoidance. Escape/avoidance was evaluated as the absolute amount of time spent in the designated safe corner and as the percent of total corner time spent in the safe corner (%CT). As elaborated below, %CT was used to preclude the possibility that *safe time* could be accumulated simply as an artifact of behavioral suppression. The data from the 1st minute of the session were excluded from analysis, since some animals had not encountered the safe corner during this minute. Figure 1 depicts %CT per minute as a function of noise intensity, averaged across Minutes 2-4 of the session (left side) and Minutes 5-7 (right side). These data were analyzed by analysis of variance, with follow-up comparisons using Duncan's multiple range test with $p = .05$. As inspection of the figure suggests, the analysis of these data revealed a significant noise $\times$ time interaction [$F(4,50) = 3.74, p < .01$], in which the 87.5-dB and 90-dB groups showed significantly increased %CT in Minutes 2-4 as well as Minutes 5-7, but the 100-dB group did not differ from the controls until Minutes 5-7. The 75-dB group did not differ from the controls in either time period. The analysis of these data also showed a main effect of noise intensity, in which the 87.5-, 90-, and 100-dB groups all differed significantly from the controls, and in addition, the 87.5-dB group had significantly higher %CT than did both the 90-dB and the 100-dB groups [$F(4,50) = 9.68, p < .01$].

Finally, females showed higher %CT ($M = 36.6\%$) than did males ($M = 29.6\%$) [$F(1,50) = 8.73, p < .01$].

Figure 1. Mean percentage of total corner time in the safe (quiet) corner (%CT), $\pm$ SE, as a function of intensity of pulsed noise, with data averaged across Minutes 2-4, left side, and Minutes 5-7, right side. The asterisk indicates significant difference from controls ($p < .05$).

Table 1
Time per Minute in Designated Safe Corner
as a Function of Noise Intensity

Intensity	Mean Designated Corner Time (Seconds/Minute) $\pm$ SE	
	Minutes 2-4	Minutes 5-7
Controls	8.26 $\pm$ 0.50	6.34 $\pm$ 0.71
75 dB	10.65 $\pm$ 0.66	7.16 $\pm$ 0.83
87.5 dB	17.06 $\pm$ 2.60	21.26 $\pm$ 2.82
90 dB	14.19 $\pm$ 2.15	13.79 $\pm$ 2.23
100 dB	11.07 $\pm$ 1.61	18.16 $\pm$ 2.9

However, sex did not interact with noise [$F(4,50) = 1.47$], time [$F(1,50) = 1.90$], or noise and time ($F < 1$).

The decision to express escape/avoidance data as %CT was based on analyses of real time in the safe corner—that is, designated corner time (DCT)—and also on analyses of average corner time (ACT)—that is, total time per minute spent in any of the four corners divided by four. The data for designated corner time are summarized in Table 1. As inspection of the data suggests, analysis of DCT produced a pattern of results identical to those for %CT, described above. However, another feature of these data—namely, the relatively low DCT levels—prompted us to perform additional analyses. Although the 87.5-dB group showed approximately three times as much DCT as did the control group by Minutes 5-7, the actual time spent in the designated corner was approximately 20 sec/min, or just one third of the total time. This raised the possibility that increased time in the safe corner was an artifact of an overall increase in corner time for noise-exposed animals. To assess this possibility, we analyzed average corner time. For the control animals, the ACT per minute was 8.06 sec, and for 75-dB animals, it was 8.31 sec. The three higher intensity noise groups showed slightly higher ACTs; the ACT was 9.90, 9.68, and 10.07 sec for the 87.5-, 90-, and 100-dB groups, respectively. Although noise did cause this small but reliable increase in ACT [$F(4,50) = 4.07, p < .01$], the pattern of results was quite different from that for DCT and %CT. In pairwise comparisons, there were no significant differences among the three highest intensity noise groups, nor did any single group differ from the controls; furthermore, there was no interaction between noise and time. There was a main effect of time in which ACT decreased slightly from Minutes 2-4 (mean ACT = 9.44 sec) to Minutes 5-7 (mean ACT = 8.96 sec) [$F(1,50) = 4.37, p < .05$]. The measure of %CT was adopted in order to compensate for this nonspecific increase in CT.

Activity. Activity data—mean number of photobeam interruptions—were also analyzed by analysis of variance. Since time in the safe corner would presumably be associated with a reduction in activity, it might be expected that the data for activity would be the mirror image of that for escape/avoidance. However, this was not the case. In contrast to the %CT data, there was no significant interaction between noise condition and time. Overall activity decreased from a mean of 46.3 interruptions in Minutes 2-4 to a mean of 39.5 in Minutes 5-7 [$F(1,50)$

= 26.3, $p < .01$). The 87.5-, 90-, and 100-dB groups all showed significantly reduced activity ($M = 37.6, 41.5$, and 39.8) relative to controls ($M = 50.4$) [$F(4,50) = 3.53$, $p < .05$], but did not differ from each other. Activity was not significantly reduced for the 75-dB group ($M = 45.11$). There were no significant effects of sex either as a main effect or by way of interaction.

EXPERIMENT 2

The purpose of Experiment 2 was to explore the relative effectiveness of continuous as opposed to pulsed noise. As discussed previously, continuous noise would presumably be unsuitable for experiments involving long-term administration. However, we hypothesized that continuous noise would be superior for short-term noise-escape experiments, since it would make the safe area more easily discriminable and would likely induce less variability. Thus, it might have promise as a paradigm for the study of factors affecting acute drug response.

A second purpose of Experiment 2 was to provide further information on the relative effectiveness of the 87.5- and 90-dB pulsed noise conditions. Although the results of Experiment 1 suggested that acquisition of escape/avoidance was more rapid for 87.5- than for 90-dB noise, this effect was unexpected, and we had some doubt about its reliability. In order to reduce variability and allow a better comparison of these stimulus conditions, we altered the procedure to provide a 2-min period of exposure to the apparatus before introduction of the noise stimulus. It has been shown that preexposure to the apparatus reduces the degree of freezing in response to acoustic stimulation (Borszcz, Cranney, & Leaton, 1989; Leaton & Cranney, 1990).

Method

Sixty mice were randomly assigned to five groups, with 6 males and 6 females in each group: a control group; and four noise groups that received 90-dB pulsed noise, 90-dB continuous noise, 87.5-dB pulsed noise, and 87.5-dB continuous noise, respectively. The procedure was the same as in Experiment 1, except that a 2-min preexposure period was given prior to the start of the noise.

Figure 2. Upper panel: mean percentage of total corner time in the safe (quiet) corner (%CT), $\pm$ SE. Lower panel: mean number of activity photobeam interruptions, $\pm$ SE. Noise conditions are pulsed (P) versus continuous (C) and 87.5 versus 90 dB; data are averaged across Minutes 2–4 of noise exposure, left side, and Minutes 5–7, right side. The asterisk indicates significant difference from controls ($p < .05$).

Results

Escape/Avoidance. The upper panel of Figure 2 depicts %CT as a function of noise treatment, averaged across Minutes 2-4 of noise exposure (left side), and Minutes 5-7 of noise exposure (right side). Inspection of the figure shows a significant interaction between noise and time [$F(4,50) = 3.64, p < .05$], in which only the animals exposed to 87.5-dB continuous noise showed significantly increased %CT relative to controls in Minutes 2-4, whereas all of the noise groups differed from controls during Minutes 5-7. The main effects of noise [$F(4,50) = 2.63, p < .05$] and time [$F(1,50) = 12.30, p < .01$] were also significant.

The main effect of sex was not significant in these data; nor did sex interact significantly with time or noise.

As in Experiment 1, we also analyzed the data for DCT and ACT. The data for DCT are summarized in Table 2. As inspection of the data suggests, analysis of DCT produced a pattern of results identical to those for %CT, described above. In contrast, the analysis of ACT produced a very different pattern of results. As in Experiment 1, there was some tendency for noise to cause a general increase in ACT, but the main effect of noise was not significant [$F(4,50) = 2.34, p = .07$]. Examination of the data, however, suggested that ACT was increased for continuous noise groups, but not for pulsed. A factorial analysis of the four noise groups supported this assertion, showing that ACT per minute was significantly higher for continuous noise ($M = 10.17$ sec) than for pulsed noise ($M = 8.70$ sec) [$F(1,44) = 5.25, p < .05$]. There was no interaction with decibel level or with time. This pattern of results for ACT is the approximate mirror image of the activity data described below.

Activity. The lower panel of Figure 1 depicts activity data as a function of noise treatment. As the figure shows, the pattern of results for activity was very different from the pattern for %CT. There was a significant main effect of noise [$F(4,50) = 5.94, p < .01$], reflecting reduced activity for the continuous noise groups only. The effects of noise on activity were virtually identical during the two time blocks. Thus, in contrast with the escape/avoidance results, there was no significant main effect of time and no significant interaction between time and noise.

The main effect of sex was not significant in the activity data; nor did sex interact significantly with time or noise.

Table 2
Time per Minute in Designated Safe Corner
as a Function of Noise Condition

Noise Condition	Mean Designated Corner Time (Seconds/Minute) $\pm$ SE	
	Minutes 2-4	Minutes 5-7
Controls	9.65 $\pm$ 1.57	6.91 $\pm$ 1.20
87.5 dB pulsed	11.50 $\pm$ 2.30	15.46 $\pm$ 2.45
87.5 dB continuous	15.75 $\pm$ 2.55	20.59 $\pm$ 3.90
90 dB pulsed	11.57 $\pm$ 1.16	12.66 $\pm$ 1.85
90 dB continuous	11.42 $\pm$ 2.31	18.53 $\pm$ 3.13

EXPERIMENT 3

The results of Experiment 2 supported our expectation that acquisition of escape/avoidance would be more rapid for continuous noise. However, the %CT of 87.5-dB pulsed noise animals in Experiment 2 was low relative to that in Experiment 1. This left some question about the relative effectiveness of pulsed and continuous noise. We explored this further in Experiment 3, using a slightly different procedure.

In the procedure in Experiments 1 and 2, animals spent reliably more time in the safe corner, but they did not spend anything approaching 100% of the time in that area. This procedure has the advantage of preventing both ceiling and floor effects, so that manipulations might be expected to increase or decrease the time in the safe area. On the other hand, the fact that the animal must learn to remain in a relatively small area to avoid the noise means that acquisition of the task must compete with the animal's natural exploratory tendencies. Altogether, the task may represent a fairly difficult learning/memory problem for the animal. We therefore modified the procedure to simplify the demands placed on the animal. In Experiment 3, animals could passively avoid the noise stimulus simply by remaining in one designated half of the apparatus. In this experiment, we compared 87.5-dB pulsed and continuous noise. We expected that animals in the continuous condition would acquire the avoidance behavior more rapidly and would show more reliable avoidance.

Method

Sixteen mice were randomly assigned to two conditions, with 4 males and 4 females in each condition: 87.5-dB pulsed noise, or 87.5-dB continuous noise. For each animal, half of the apparatus was designated as the safe area. Groups were assigned in equal proportions to the two sides of the apparatus. The procedure of Experiment 3 involved two further changes: (1) the length of the avoidance period was increased to 20 min, and the animal was given a 2-min period of preexposure to the apparatus, followed by 20 min of noise avoidance; and (2) the software controlling the experiment was revised so that the computer could interrupt the noise when the animal was on the safe side, thus eliminating the need for an observer to control the noise.

Results

The data from this experiment are summarized in Figure 3, which presents average percent time on the safe side over 2-min blocks of noise exposure. The dotted line indicates the expected value of 50% time on the designated side. As inspection of the figure suggests, both groups achieved significant avoidance relative to the expected value of 50% by the 4th minute of noise exposure and continued to differ from chance at all subsequent points. Both groups showed a dramatic increase in time on the safe side over the 20-min session of noise exposure; this was reflected in a significant main effect of time [$F(9,126) = 6.44, p < .01$].

These data also confirmed the prediction that continuous noise would sustain more rapid acquisition of avoid-

Figure 3. Mean percentage of total time on the safe (quiet) side, $\pm SE$, as a function of noise condition: 87.5-dB pulsed (P) and 87.5 dB continuous (C); data are averaged across blocks of 2 min of noise exposure. Line designates expected value of 50% time on a side.

ance. As the figure shows, the continuous noise group showed a sharp increase in percent time on the safe side from the first 2-min block of noise exposure to the second [$t(7) = 3.76, p < .05$], but the pulsed group did not. Thus, the continuous group was spending close to 80% of the time on the quiet side by the 4th minute of noise exposure, whereas the pulsed group was still spending just over 60%. A planned comparison between the two groups on this block of time showed that the difference was significant [$t(14) = 2.59, p < .05$]. As for the degree of avoidance by the two groups, Figure 3 shows that overall percent time on the safe side was somewhat higher for the continuous group, but this effect was not significant in the analysis of variance of these data [$F(1,126) = 3.30, p = .09$]. A more important feature of these data, however, may be the degree of variance. Inspection of the figure shows that for the last 10 min of noise exposure, the variance was extremely low for the continuous noise group. Thus, the results of Experiment 3 also suggest that escape/avoidance behavior may be more reliable in the presence of continuous noise.

EXPERIMENT 4

Together, the results of Experiments 2 and 3 suggest that continuous noise is more effective than pulsed noise, in the sense that it supports earlier and more reliable acquisition of escape/avoidance. One interpretation of this finding is that the continuous noise stimulus is more aversive to the animals. Experiment 4 was designed to provide a direct test of this hypothesis. Thus, the animal was forced to choose between pulsed and continuous 87.5-dB noise. When the animal entered one side of the apparatus, pulsed noise was delivered, and when it entered the other side, continuous noise was delivered.

Method

Twelve mice, 6 males and 6 females, were studied in the choice paradigm. Assignment of side for pulsed as opposed to continuous noise was random, with the restriction that the two conditions were evenly represented in the two sides of the apparatus. The procedure for this experiment was the same as for Experiment 3, except that pulsed 87.5-dB noise was experienced on one side of the apparatus and continuous 87.5-dB noise was experienced on the other.

Results and Discussion

The results of this experiment are summarized in Figure 4, which shows percent time on the side of pulsed noise averaged over 4-min blocks. As the figure shows, the animals developed a significant preference for pulsed

Figure 4. Mean percentage of total time on the side of pulsed noise, $\pm SE$, for animals given a choice between pulsed and continuous noise at 87.5 dB. Data are averaged across blocks of 4 min of noise exposure. The asterisk indicates significant difference from the expected value of 50% time on a side ($p < .05$).

as opposed to continuous noise. On the last three blocks of the session, the animals spent significantly more time on the pulsed side, relative to the expected value of 50% [$t(11) = 3.40, 2.37, \text{ and } 3.02$, with $p < .05$, for Minutes 9–12, 13–16, and 17–20, respectively]. In effect, the animals treated the pulsed side as a safe area and showed avoidance of the continuous side, suggesting that continuous noise is more aversive than pulsed. This finding is consistent with the previous results, which showed that animals learn to avoid continuous noise more rapidly.

GENERAL DISCUSSION

The results of the present research show that noise of moderate intensity is an effective aversive stimulus for the C57BL/6J mouse. In Experiment 1, mice acquired escape/avoidance more rapidly at moderate intensities (87.5–90 dB) than at high intensity (100 dB). Our observation of 100-dB animals suggests that noise of this intensity elicits behaviors that might be expected to interfere with escape/avoidance learning. In the 100-dB condition, mice were sometimes seen to crouch in unsafe areas for seconds at a time. This observation accords with early work in which 98-dB white noise induced freezing in C57BL/6 mice (Barnes & Kish, 1957). It also accords with recent work in which high-intensity (117-dB) acoustic startle stimulation was used as an unconditioned fear stimulus (Leaton & Cranney, 1990); in this work, the high-intensity noise was able to invest a conditioned stimulus with the capacity to elicit freezing and potentiate acoustic startle in rats.

Although the present results provide evidence that acquisition of escape/avoidance is more rapid with noise of moderate intensity than with noise of high intensity, the specific intensities that are most effective will undoubtedly vary, depending on characteristics of the experimental and housing conditions. As detailed in the procedure, animals in the present research were reared in a sound-attenuated animal colony. Animals reared in a standard colony or shipped by air freight might be expected to show a different range of effective intensity.

With intensity held constant, animals show more rapid acquisition of avoidance when the noise stimulus is continuous rather than pulsed (Figures 2 and 3), and when given a choice between the two, they show avoidance of continuous noise (Figure 4). Taken as a whole, the results of these experiments establish that acquisition of noise avoidance will be more rapid with a moderate-intensity continuous noise stimulus.

In discussing the relative effectiveness of different intensities and of pulsed as opposed to continuous noise, we have interpreted the present results as showing that C57BL/6J mice learn a specific behavior in order to terminate or avoid noise stimulation. Several factors argue against the possibility that a general behavioral suppression or defensive response (Bolles, 1970) could account for this pattern of results. First, and perhaps most important, is the fact that escape/avoidance was assessed in terms of %CT in Experiments 1 and 2. With time in the

designated safe corner expressed as a percent of total CT, elevated safe time could not be accumulated simply as an artifact of an increase in ACT. Second, the activity measure—interruption of intersecting photobeams—provided at least a crude measure of locomotor activity. Since passive avoidance of the noise would be incompatible with moving about the apparatus, it could be expected that the pattern of results for activity would be the mirror image of those for escape/avoidance. However, this was not the case for either Experiment 1 or Experiment 2. As discussed above, the analysis of escape/avoidance data in both experiments revealed an interaction between noise condition and time, in which acquisition was more rapid for some noise conditions. In contrast, the analysis of activity data in both experiments revealed no interaction between noise condition and time.

In Experiment 1, there was a very slight, but reliable, decrease in beam crossings for the three highest intensity noise groups, but the groups did not differ from each other. In Experiment 2, in which animals were given a 2-min preexposure to the apparatus, beam crossings for the pulsed groups were virtually identical to those of the no-noise controls. The continuous groups did show a significant decrease in beam crossings, but, as noted above, and as clearly illustrated in Figure 2, the effects of noise condition on activity were virtually identical in the two blocks of time, but the effects of noise condition on %CT were very different. Finally, in both Experiments 1 and 2, the analyses of overall CT or ACT, which we would take to be an index of general behavioral suppression, produced a pattern of results that was the mirror image of the pattern for activity. In both experiments, there were very small but reliable increases in ACT that mirrored the decreases in beam crossings. Given the consistency of these two measures, it seems possible to draw some tentative conclusions regarding behavioral suppression. We suggest that continuous moderate-intensity noise does produce a measurable degree of behavioral suppression. In terms of absolute numbers, however, this effect is very small. Apparently, it neither impedes nor greatly facilitates escape/avoidance behavior.

As indicated in the introduction, one purpose of the present research was to develop noise as a stimulus that would be suitable for experiments involving acute ethanol intoxication in the C57BL/6J mouse. On the basis of the present results, we adopted 87.5-dB continuous noise in experiments with the safe-corner paradigm in studies of acute ethanol intoxication. In work recently completed, we used this paradigm to study the effects of prior exercise training on ethanol intoxication. We found that 5 weeks of voluntary wheel running reduced the effects of acute ethanol intoxication on escape/avoidance behavior in C57BL/6J mice (Mollenauer, Bryson, Speck, & Chamberlin, 1991).

Another objective of the present research was to develop noise as a stimulus that would be suitable for the chronic study of ethanol intoxication in C57BL/6J mice. Although the continuous noise stimulus would seem to be most effective in the noise avoidance paradigm, its

desirability for chronic stimulation seems questionable. It has been shown in the mouse, for example, that 30 sec of continuous exposure to white noise at 95 or 110 dB results not only in significant loss of auditory sensitivity, but also in alterations in normal coding processes (Willott & Lu, 1982). Use of discontinuous or pulsed noise would not be expected to eliminate these sensory effects, but it might be expected to ameliorate them somewhat. In fact, pulsed noise delivered for several hours a day for periods of 6 weeks has been shown to produce continuing alterations in serotonergic regulation (Boadle-Biber et al., 1989). This finding supports the idea that pulsed noise is appropriate for experiments involving long-term exposure. From the perspective of developing a model of stress-induced ethanol intoxication, a very important feature of the present results, discussed above, is the fact that animals in the pulsed noise conditions showed little or no reduction in activity. In these conditions, animals continued to move freely about the apparatus, accumulating numbers of photobeam interruptions that did not differ from controls. Even during an extended session of 20 min in the safe-side procedure, animals exposed to 87.5-dB pulsed noise showed an asymptote of less than 80% of time/minute spent on the safe side (Figure 3). In other words, they tended to confine their activity primarily to the quiet side, but regularly made circuits of the entire apparatus. These results indicate that pulsed noise of moderate intensity is clearly aversive, but that it does not cause severe inhibition of exploratory behaviors. Thus, pulsed noise of moderate intensity shows promise as an aversive stimulus that allows fairly normal levels of behavior.

In summary, the present findings provide behavioral evidence that noise of moderate intensity (87.5–90 dB) is a mildly aversive stimulus for the C57BL/6J mouse. At the intensities employed here, it does not produce pain or cause severe behavioral inhibition. Thus, it can be administered to freely moving animals in a novel environment or in the home cage. In addition, the safe-corner paradigm used in the present research has a number of advantages. Animals show reliable avoidance of noise fairly early in a 7-min test period, and they continue to improve across the period, providing continuous, as opposed to discrete, data. Even in the safe-side procedure, avoidance of moderate-intensity noise shows an asymptote of less than 100%; thus, the paradigm is capable of detecting either facilitation or impairment of avoidance.

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