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Ethanol-Induced Analgesia in Rats Selectively Bred for Ethanol Sensitivity[1]

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FRIEDMAN, H. J., M. B. BASS AND D. LESTER. *Ethanol-induced analgesia in rats selectively bred for ethanol sensitivity*. PHARMAC. BIOCHEM. BEHAV. 13(6) 773–776, 1980.—Two rat lines selectively bred for ethanol-induced depression of locomotor activity were studied for ethanol-induced analgesia. The effects of ethanol on startle amplitude, extent of overt movements and incidence of audible vocalizations in response to intermittent, noncontingent foot shock. All three responses were dose-dependently depressed by ethanol (0.66 to 2.0 g/kg, IP), and to greater extent in the "most affected" line (MA) than in "least affected" (LA) rats. Ethanol-induced response decrements were reinstated at higher shock intensities, indicating a sensory (i.e., analgesic) rather than a motoric or analgesic basis for these effects. Genes which influence ethanol's motoric effects might, in part, influence sensitivity to its sensory effects.

Ethanol Analgesia Ethanol acute tolerance Startle response Ethanol pharmacogenetics Shock response

MICE and rats are being selectively bred for responsivity to ethanol with the goal of elucidating the brain mechanisms which respond to this drug. Mice have been bred for differences in the duration of ethanol-induced "sleep-time" [9], and rats have been bred for differences in depression of spontaneous locomotor activity following 1.5 g ethanol/kg [13, 14, 19, 20]. Thirteenth generation rats of the "least affected" (LA) line showed about a 40% mean decrease in locomotor activity after ethanol whereas those of the "most affected" (MA) line showed a mean decrease of about 90% [7]. Ethanol-induced activity decrements follow a dose-response relationship in both lines, and a line difference is evident over a range of doses [19]. In addition, the duration of ethanol-induced sleep-time is significantly greater in MA than in LA rats [14]. No major differences in blood [14] or brain [3] ethanol levels or rate of ethanol clearance [7] have been found.

Ethanol has effects on sensory as well as motor systems [17]. For example, a dose of 1.5 g/kg, IP, decreased the amplitude of evoked potentials recorded from the visual cortex of rats [4] and rapid IV infusion of 1.0 g/kg decreased the amplitude of auditory evoked potentials in cats [12]. Ethanol also has analgesic properties; it was reported to increase the heat duration pain threshold in humans [18], the tooth pulp pain threshold in rabbits [11,15], the foot or tail shock intensity threshold and the response to foot shock in rats [1, 5, 6].

The activity measure used as the phenotype for selective breeding is an emitted motor response. Other measures studied, e.g., open field and running wheel activity [14], have also been emitted responses. The effects of ethanol on three elicited responses to suprathreshold foot shock were measured in these experiments; two of the responses were motor responses (startle amplitude and overt movements) and one was non-motor (vocalizations). A dose-response relation was demonstrated and the relation between sensitivity to sensory and motor effects of ethanol was evaluated with LA and MA rats bred for ethanol sensitivity. The possible role of motor impairment in the apparent analgesic action of ethanol was also studied.

METHOD

Animals

Eight male rats from the 15th generation of each line were used. One week prior to testing they were implanted with subcutaneous electrodes while under light CO_2-ether anesthesia [1]. The animals were group housed with water and Purina Lab Chow available ad lib. Vivarium lights were on between 0700 and 1900 hr.

Procedure

Shocks were delivered through the subcutaneous electrode, as previously described [1]. Movement of the animal in response to shock produced a current in a coil mounted on the underside of a stabilimeter platform; a peak reading voltmeter converted the greatest voltage produced within 400 msec of shock onset to a proportional numerical score.

The experimental design was two 4×4 Latin squares so that each rat received each ethanol dose (0.0, 0.67, 1.33 and 2.0 g/kg, IP) in a random order. Ethanol was given as a 10% (w/v) solution in isotonic saline; the zero dose was an isotonic saline injection of the same volume as the highest ethanol dose (20 ml/kg). Twenty min after injection an animal

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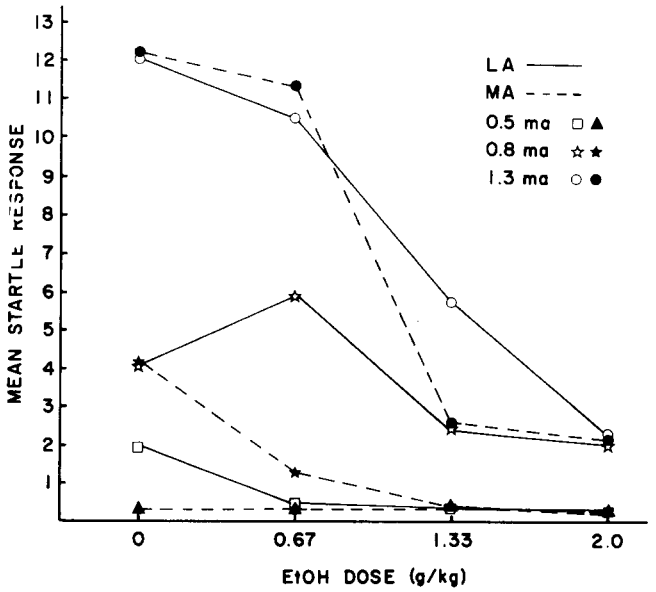

FIG. 1. Effects of ethanol dose and shock intensity on amplitude of the shock-induced startle response in rats selectively bred for sensitivity to ethanol-induced depression of activity.

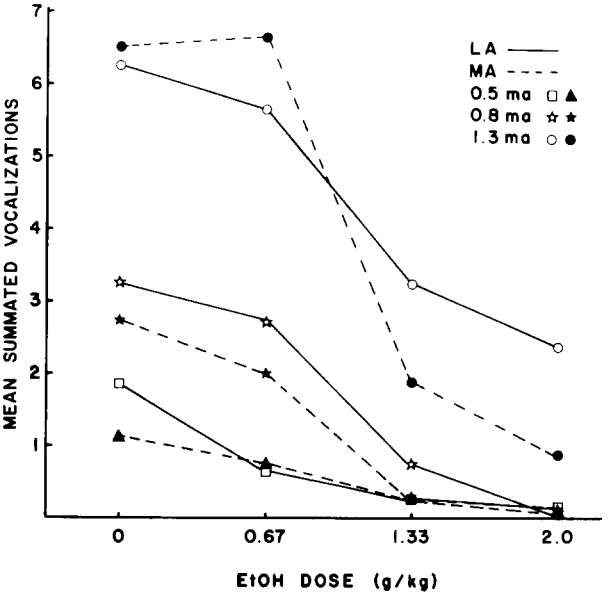

FIG. 2. Effects of ethanol dose and shock intensity on incidence of shock-elicited audible vocalizations in rats selectively bred for sensitivity to ethanol-induced depression of activity. The maximum possible score was 10.

was attached to the shock lead and placed in the test chamber.

Testing consisted of 15 noncontingent 0.5 sec shocks, five at each of three intensities (0.5, 0.8 and 1.3 mA) delivered on a variable time 60 sec schedule (range 15 to 105 sec) in a random order. The shock order was changed each test day; two days separated each successive test day. Three response measures were recorded: (1) amplitude of the startle response displayed by the peak reading voltmeter; (2) presence or absence of an audible vocalization immediately following the shock, independently scored by two observers; and (3) overt movements following the shock, scored independently by both observers and rated as 0 for no discernible response, 1 for a discernible response with, at most, movement of one forepaw, 2 for movement of both forepaws and/or one hindpaw, and 3 for movement of both hindpaws.

One to two weeks later the same LA and MA rats were retested to determine if the response attenuation following ethanol might be attributable to motor impairment and sedation rather than reduced sensitivity to pain. They were given 2.0 g ethanol/kg, IP, 20 minutes prior to testing. Only two shock intensities, 1.3 and 2.5 mA, were used in this experiment. Five shocks at each intensity were delivered in a random order on a variable time 90 second schedule (30 second increments).

The mean of the five responses at each shock level was taken as the startle amplitude score for that intensity. The vocalization score was the sum of the number of vocalizations recorded by each observer for the rat at each shock level for a maximum score of 10. The overt movement score was summed over the five shocks at each shock level and over each observer for a maximum of 30. One observer was blind to shock intensity and the other to the administered dose. Interobserver agreement on vocalizations was found to exceed 95%, and overt movement ratings were highly correlated [1].

RESULTS

Startle amplitudes at each dose and shock intensity are shown in Fig. 1. Analysis of variance indicated a significant ethanol dose effect, $F(3,36)=15.84$, $p<0.0001$. A significant shock×dose×line interaction, $F(6,72)=2.36$, $p<0.04$, indicates that the lines responded differently to ethanol's analgesic action. In addition, there were significant days×shock, $F(6.72)=3.02$, $p<0.02$, days×line, $F(3,36)=4.06$, $p<0.0001$, and days×shock×line, $F(6,72)=6.98$, $p<0.0001$, interactions.

The Scheffe test for post-hoc comparisons (alpha=0.05) was used to determine whether particular means differed significantly. There was no line difference in startle amplitude at the zero dose at any shock intensity. At the lowest shock level (0.5 mA) no effect of dose or line was evident. At 0.8 mA, MA rats showed significantly lower startle amplitude than LA rats after 0.67 g/kg. LA rats showed no significant decrease in startle amplitude from 0 to 2.0 g/kg, whereas MA rats showed a decrease that was maximal at 1.33 g/kg, and nearly so at 0.67 g/kg. The lines were also differentially affected by ethanol at 1.3 mA, but the differences became evident at higher doses. There was a further reduction in startle amplitude of LA rats from 1.33 to 2.0 g/kg, but maximal analgesia was again produced by 1.33 g/kg in MA rats.

On the vocalization measure (Fig. 2), there were significant effects of dose, $F(3,36)=11.38$, $p<0.0001$, and shock intensity, $F(2,72)=3.27$, $p<0.05$. Significant interactions of shock×dose×line, $F(6,72)=3.30$, $p<0.01$, and shock×day×line, $F(6,72)=4.93$, $p<0.001$, were also found. There was no line difference after saline treatment. At 0.5 mA shock, vocalization in LA rats was lower after 1.33 g/kg than after saline, but there was no such attenuation in MA rats, possibly due to their slightly lower response incidence under saline. The lines were affected similarly by ethanol at 0.8 mA shock; vocalizations declined with increasing dose. The lines did, however, differ in response to ethanol at 1.3 mA shock.

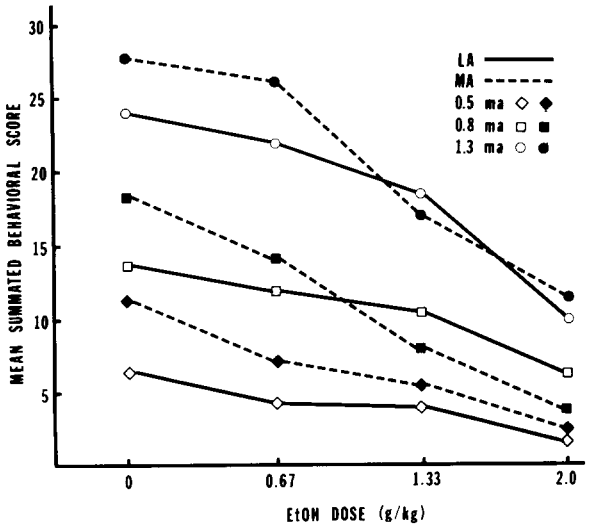

FIG. 3. Effects of ethanol dose and shock intensity on overt movements elicited by shock in rats selectively bred for ethanol-induced depression of activity. The maximum possible score was 30.

Linear trends for dose were evident for each line, and the shock×line×dose linear interaction was significant, $F(1,72)=5.01$, $p<0.03$, indicating that incidence of vocalization in MA rats was more sensitive to depression by ethanol than was that of LA rats.

Overt movement scores are presented in Fig. 3. There were main effects of dose, $F(3,36)=28.2$, $p<0.0001$, days, $F(3,36)=6.28$, $p<0.002$, and shock intensity, $F(2,72)=4.41$, $p<0.02$. Significant interactions were: shock×dose, $F(6,72)=3.61$, $p<0.005$, shock×dose×line, shock×days, shock×days ×line (all $p<0.0001$). MA rats were more reactive than LA rats at all shock intensities after saline injection. A line difference in response to ethanol was evident at 0.5 and 0.8 mA shock. MA rats were significantly affected by the low dose of 0.67 g/kg at both intensities; LA rats showed no decrement in response until a dose of 1.33 g/kg at 0.8 mA and until 2.0 g/kg at 0.5 mA.

Responses to the two shock intensities on the second test sequence are shown in Table 1. Paired t tests indicate that the responses in both lines to 2.5 mA shock were significantly greater than those to 1.3 mA shock on all three measures. Furthermore, responses to 2.5 mA were significantly greater than the responses to 1.3 mA after 2.0 g/kg in the first sequence. On the other hand, there were no differences in response to 1.3 mA between the two sequences. The reinstatement of responsivity to shock by sufficient intensity indicates that ethanol's effect on reactivity is not attributable to motor impairment. The results suggest an influence on sensory responsiveness.

DISCUSSION

Startle amplitude, vocalization and overt movements were reliable indicators of pain, and ethanol produced analgesia on all the measures, decreasing the response to shock as a function of dose. The attenuation of response to shock appeared to be a reduction in sensitivity to pain rather than sedation or motor impairment since the response was restored when the shock level (and the degree of pain) was increased.

Some distinctions between the three measures were evident. The motoric responses were more greatly influenced by repeated testing than were vocalizations. Effects of days and days×shock interactions on overt movements and on startle amplitude were significant. Although shock intensities in these studies were suprathreshold, present observations appear to be in accordance with results of studies of shock thresholds: movement, but not vocalization thresholds, were elevated by previous experience with shock [8].

Line differences in response to ethanol were found with all three measures. The MA line was more sensitive: at low doses, analgesia was more pronounced, and maximal analgesia was attained at lower doses than in the LA line. These results are consistent with line×dose interactions observed with depression of locomotor activity [19], the effect for which these animals were selectively bred.

TABLE 1

RESPONSES TO FOOTSHOCK WITH ETHANOL IN RATS BRED FOR ETHANOL SENSITIVITY

Measure	Line	First sequence 1.3 mA	t[a]	Second sequence 1.3 mA	t[b]	Second sequence 2.5 mA	t[c]
Startle							
Amplitude	LA	2.19 ± 1.68	1.63	6.69 ± 2.20	2.85*	11.89 ± 1.33	5.15†
	MA	2.04 ± 1.73	0.94	0.39 ± 0.14	4.04†	8.48 ± 2.02	3.11*
Vocalization	LA	2.38 ± 1.13	0.28	2.63 ± 1.43	2.70*	5.75 ± 1.49	2.79*
	MA	0.88 ± 0.52	0.74	1.63 ± 0.96	3.78†	5.13 ± 1.27	3.93†
Overt movements	LA	9.75 ± 1.92	1.13	11.50 ± 2.15	7.55†	19.38 ± 1.69	4.86†
	MA	11.25 ± 3.14	1.01	7.60 ± 2.02	4.95†	18.75 ± 1.97	2.79*

Values are means ± SE.

[a]Student's t-test for paired data ($df=7$) comparing responses to 1.3 mA in first and second test sequences.

[b]Student's t-test for paired data ($df=7$) comparing responses to 1.3 and 2.5 mA in second test sequence.

[c]Student's t-test for paired data ($df=7$) comparing responses to 1.3 mA in first sequence and 2.5 mA in second sequence.

*$p<0.05$.

†$p<0.01$.

The MA and LA lines were similar in their baseline (zero dose) responses on startle amplitude and vocalization, but differed on overt movements. Line differences in the absence of ethanol have been noted previously: LA rats are more active in the running wheel [14] and swim more slowly than MA rats [2]. The hypothesis was advanced that the degree of response-produced feedback could be a source of these line differences; MA rats may have a more sensitive neural apparatus and more intense feedback from running wheel activity or swimming might be more aversive [14]. In the present experiments, however, it is unlikely that there is any appreciable difference in the degree of feedback associated with startle amplitude and overt movements, two indices of essentially the same response.

In some cases initial differences in responses of rat strains can have implications for evaluating drug effects. In measurement of the threshold for the flinch-jump response to foot shock, Fisher rats were not only more reactive than Sprague-Dawley rats, but also displayed less relative morphine analgesia [16]. However, differential sensitivity to ethanol by the MA and LA rat lines was not attributable to differences in initial performance with impairment of swimming [2] or with analgesia in this study. Ethanol produced a differential effect in the lines with the MA rats exhibiting greater analgesia although they were initially more reactive on the overt movement measure.

Differences between the lines bred for ethanol sensitivity, both with and without ethanol, may help to elucidate ethanol's mechanism of action and the inter-relationships between its various effects. Line differences in ethanol analgesia reported here are in the same direction as the depression of locomotor activity for which the lines have been selectively bred. This might suggest common mediation of these effects, but the line difference in depression of activity is more pronounced with virtually no overlap between the lines. Both effects are likely to be polygenic, the selection process acting on populations of genes. Since analgesic effects involve sensory processes, different populations of genes may be involved. Present results suggest partial, but perhaps appreciable overlap of the populations of genes mediating these effects. Descendents of hybrids obtained from crossing the lines can be used to further explore this possibility.

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