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Dose-Response Effects of Alcohol Upon Rat Strains Bred for Differences in Reactivity to Alcohol

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WORSHAM, E. D. AND E. X. FREED. *Dose response effects of alcohol upon rat strains bred for differences in reactivity to alcohol.* PHARMAC. BIOCHEM. BEHAV. 7(5) 421–424, 1977. – Two rat strains, designated LA and MA, selectively bred for differential impairment of motor activity following an injection of alcohol, were tested in stabilimeters and compared over a range of ethanol doses. As expected, increasing doses of ethanol produced progressively greater activity decrements in both strains; however, the same dose of ethanol induced a more pronounced decrement in the MA strain than in LA strain at all doses. At the highest alcohol dose (2.25 g/kg), the LA animals were twice as active as were the MA strain at the 1.5 g/kg dose. This strain difference in impairment was evident within 3 min postinjection and remained throughout the 30 min test session. The results are discussed in terms of differential neural and behavioral tolerance to ethanol in the two strains.

Alcohol Activity Dose response Neural tolerance Selective breeding

STRAIN differences in ethanol consumption have been demonstrated repeatedly in mice (e.g. [9,15]). Furthermore, Fuller and Collins [3] have shown that two loci or two independent blocks of closely linked genes control the major portion of variance in ethanol intake and preference among inbred strains of mice selected for characteristics other than alcohol drinking behavior.

In an attempt to obtain more relevant data, Eriksson [1,2] has used selective outbreeding to produce rat strains differing greatly in their voluntary alcohol consumption. Although this work clearly supports a genetic contribution in the self-selection of alcohol, such voluntary selection may not bear much relationship to the consequences of alcohol intake. Recently, Oliverio and Eleftheriou [10] demonstrated linkage for a gene modulating the effects of alcohol upon basal activity levels in mice, a trait different from ethanol consumption but one which may or may not be related to it. Thus, selective breeding on the basis of central nervous system sensitivity to alcohol may be fruitful in the investigation of the physiological or biochemical mechanisms in the brain which respond to alcohol.

One index of CNS responsivity is sleeping time; another is general level of activity. Both represent degrees of impairment of motor coordination produced by alcohol. Selective breeding of mice on the basis of sleeping time in response to a hypnotic dose of alcohol has been reported [4, 5, 8] with a 5-fold difference in sleeping time between the strains in the 14th generation. Since a hypnotic dose of alcohol (4.0 g/kg) produces longer sleeping times in BALB than in C57 mice [12], while subhypnotic doses of alcohol (< = 2.25 g/kg) cause greater motor impairment of C57 than BALB mice [11], we felt that the use of a subhynoptic, but intoxicating, dose of alcohol might be more relevant for an animal model of alcoholism. Elsewhere we [13, 14, 17] have described the selective breeding of two rat strains on the basis of their activity impairment following an intraperitoneal injection of a 1.5 g/kg dose of ethanol. Now at the F14 generation, these strains have been designated as most affected (MA) and least affected (LA) by ethanol. While selective breeding is an end, it is also a means. McClearn [7] has noted that "a successful selective -breeding program gives rise to animal groups derived from the same population who differ widely on a particular trait of interest. These animals constitute prime research material for investigation of correlated characteristics and of mechanism". Given a viable and increasing difference in reactivity to alcohol in succeeding generations of the MA and LA strains, this paper reports one aspect of our continued efforts to investigate possible correlates of the difference – the dose-response relationship.

In early work [13] the dose of 1.5 grams ethanol per kilogram of body weight was selected as the basis for

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[2] Requests for reprints should be mailed to: E.X. Freed, Alcohol Research Laboratory, V. A. Hospital, Lyons, NJ 07939.

selective breeding because the largest decrement between any two of four doses administered occurred when the 1.5 g/kg dose was used. A dose of 0.75 g/kg reduced activity too little, and 2.25 g/kg, 50 percent higher than the dose selected, resulted in only slightly more of a decrement in activity than 1.5 g/kg. Above 2.25 g/kg, the hypnotic doses produced sleep and tended to obliterate measurable differences in motor activity. These differences, however, were only apparent with subhypnotic doses of alcohol. The strains have been equivalent in terms of their baseline levels of activity following saline injection. If significant relationships could be found between lower doses of alcohol and the two strains, then a case might be made for increased sensitivity to alcohol as a result of selective breeding. Furthermore, the differences between the two strains would be documented over a wider range of intoxication.

METHOD

Animals

Sixty-two rats from the F12 generation (32 LA and 30 MA) served as the subjects in this experiment. The original parent stock from which selective breeding was initiated consisted of Sprague-Dawley and Long Evans rats from Blue Spruce Farms. The rats were housed in pairs with like-sex littermates and were reared under standard laboratory conditions with a 12-hr light/dark cycle. Food (Purina Lab Chow) and water were available ad lib except where noted.

Apparatus

Six stabilimeter platforms (Lafayette Instrument Company, Model No. A501) with incorporated electronic integrators (Acromag, Inc., Model No. 205 LX–1) were used to measure activity. The stabilimeters were enclosed in sound insulated wooden boxes and external noises were further masked by a white noise generator and ventilating fans. All programming and recording of activity was accomplished by related solid state logic located in an adjacent room.

Procedures

Rats were handled daily one week prior to testing which began when the rats were 65–70 days of age. The animals were tested 4 times at one week intervals using 4 doses (15 ml/kg) of alcohol administered in the following sequence: 0.0, 1.5, 0.75 and 2.25 g/kg of ethanol in isotonic saline.

Following 24 hr of food deprivation, each rat received an IP injection of a 15 ml/kg dose of physiological saline and was immediately placed in the stabilimeter. Activity measures were recorded for each 3-min segment of a 30 min test session. After testing, the rat was removed from the chamber and returned to its home cage. This procedure was repeated with the next 3 alcohol doses at weekly intervals in the order listed above. Activity was always tested at the same time of day.

After testing at the 1.5 g/kg dose, blood alcohol concentrations (BAC) were determined using a method described previously [13]. This procedure gives results which agree with those of simultaneous blood samples and is analogous to the use of man's alveolar air in forensic analysis for BAC. Since the test session was 30 min and since 10 min were allowed for determination of BAC, a total of 40 min elapsed between alcohol injection and BAC determination.

Following completion of testing at the 4 doses described, there was an additional test session with saline to see if the original activity baseline could be recovered after 4 exposures to the testing situation.

RESULTS

The 16–30 min activity scores at each alcohol dose were subjected to a repeated measures analysis with Strain and Sex as between group factors. It was decided to use the 16–30 min data, the procedure used throughout our selective breeding program [13, 14, 17], since activity was found to stabilize in the last 15 minutes of the session. Rats at either extreme of motor impairment, selected for breeding the next generation following testing at the 1.5 g/kg dose of alcohol, were not tested further. This has been the standard procedure for choosing breeders based on their reactivity to alcohol and was necessary to continue our selective breeding efforts. Thus, only the rats not selected for breeding were included in this analysis and the analysis of BAC; these consisted of 20 LA (11 males, 9 females) and 18 MA (11 males, 7 females) animals.

The analysis indicated no effect of Sex or any interactions which included Sex as a factor ($p>0.10$ in all cases). The effect of Strain, however, was found to be highly significant ($F(1,34) = 11.22$, $p<0.005$) as was the effect of Dose ($F(3,102) = 147.63$, $p<0.001$) and Strain x Dose ($F(3,102) = 4.91$, $p<.005$).

Since there was no effect of Sex, the activity scores at each dose were combined for the males and females of each strain and appear in Fig. 1. Although the second saline injection was not included in the analysis, the mean activity score for each strain following this injection is depicted in the figure, and it is evident that both strains essentially recovered their initial saline baseline activity.

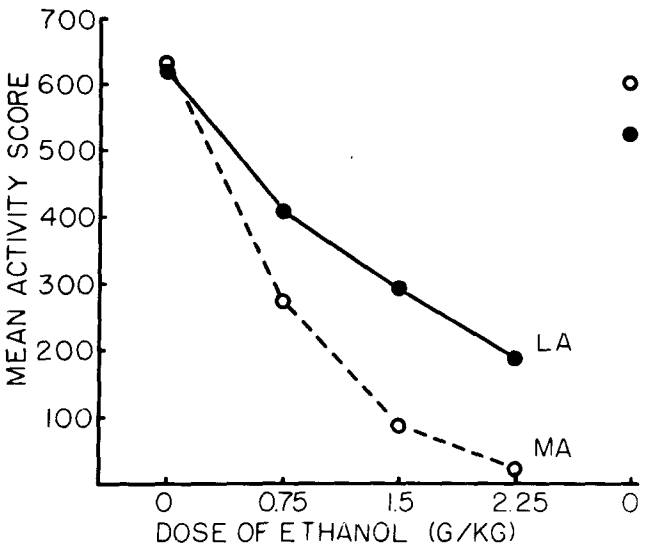

FIG. 1. Mean activity scores in the stabilimeter for the MA (n = 18) and LA (n = 20) strains as a function of ethanol dose.

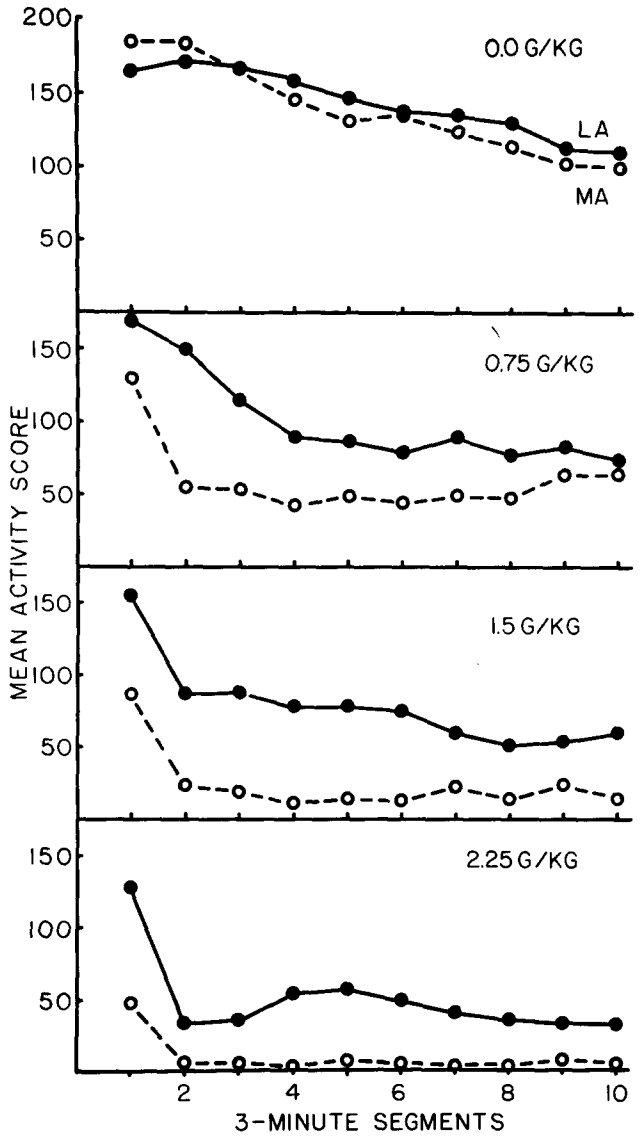

FIG. 2. Mean activity scores in three minute segments for MA (n = 18) and LA (n = 20) rats following ethanol doses of 0.0, 0.75, 1.5 and 2.25 g/kg.

A trend analysis on the linear component of the Strain x Dose interaction yielded $F(1,102) = 8.06$, $p<0.005$, indicating that the dose-response curves for the two strains were different.

In order to evaluate whether the drug effect was peculiar to the last 15 min of the session or occurred throughout the entire test period, the mean activity score for each strain was determined for each three min segment of the 30 min test session. These three min activity scores for each drug dose are presented in Fig. 2. As can be seen, activity became progressively impaired with increasing doses of ethanol. While there were no differences in activity following the saline injection, the strain differences following alcohol administration were evident at all three doses within the first 3-min segment and remained throughout testing.

An unweighted means analysis of the BAC using Sex and Strain as between group factors indicated no effect of Sex or Sex x Strain ($F<1$ in both cases), but the effect of strain was significant ($F(1,33) = 5.0$, $p<0.05$) with the LA animals having a slightly higher mean BAC than the MA strain (0.190% vs 0.174%, respectively). One LA female was not included in the analysis because an equipment malfunction precluded a determination of a BAC in this case.

DISCUSSION

As expected, increasing doses of alcohol produced progressively greater motor impairment in both strains; however, the results clearly demonstrated that the same dose of ethanol induced a more pronounced activity decrement in the MA strain than the LA strain, despite the fact that the LA animals had significantly higher BACs. Thus, the present study confirmed previous findings [13, 14, 17] that this breeding program has successfully separated two strains of rats disparate in their reactivity to a subhypnotic injection of alcohol. Furthermore, these strain differences have now been found to extend over a range of doses between 0.75 and 2.25 g/kg, and they are not specific only to the dose used as the basis for the selective breeding procedures. These differences also were obtained following several injections of alcohol as well as the single acute injection used previously [13]. The evidence is that the strain differences are robust and reliable.

Impairment of motor coordination has been used extensively to assess the effects of alcohol, but the causal mechanism underlying this impairment may not be unitary. In a previous paper [14] investigating BACs across a number of generations, it was suggested that the motor impairment shown by these strains probably reflected differences in sensitivity to alcohol unrelated to the blood alcohol levels attained after injection. Although it is conceivable that the two strains absorb alcohol differentially during the 30 min test period and also differ in the rate of alcohol metabolism in such a way as to result in virtually identical BACs 40 min after injection, we do not feel that this is too likely for several reasons. First, the difference in motor impairment is evident within the first 3 min postinjection and remains fairly stable throughout the 30 min test period. While BACs during the 30 min following alcohol injection have not been monitored continuously, unpublished data from the F14 generation (n = 32) on the BACs of these strains 3, 20 and 90 min following an alcohol injection (1.5 g/kg) yielded no strain difference in BAC at any of these time periods. Furthermore, the rate of alcohol metabolism determined in fasted F5 rats given 1.5 g/kg of ethanol was not different in these two strains [6], lending further support to the view that the differences between the strains are effected at a central level and do not depend on a difference in the disposition of alcohol. Both the rate of alcohol metabolism and the BACs following the other two doses of alcohol remain to be investigated.

Although there appears to be an inherent difference in the neural response to alcohol between these two strains, their mean activity levels following a saline injection were virtually identical. Taken in conjunction with earlier results [14] demonstrating no differences in open field behavior between these two strains following a saline injection tested either in a light or dark condition, this would seem to argue against emotionality differences in these strains as a factor accounting for all of the differential reactions following alcohol administration. In addition, it does not seem likely

that differences in habituation to the testing situation can account for these strain differences since an additional saline injection following the end of testing resulted in an almost complete recovery of baseline activity by both strains.

In addition to the large activity differences following the same dose of alcohol, the activity of the strains appeared qualitatively different. Upon removal from the stabilimeter, the MA animals showed obvious signs of intoxication such as motor disorientation and ataxia, even following the lowest alcohol dose, while the LA animals appeared ataxic only following the highest alcohol dose. As seen in Fig. 1, following the 0.75 g/kg alcohol dose, the MA animals had an activity decrement comparable to the LA rats following twice that dose. At the highest alcohol dose (2.25 g/kg), the LA animals were still twice as active as were the MA strain following a 1.5 g/kg dose. In fact, that same dose (2.25 g/kg) which was often hypnotic (defined as no measurable activity for 9 of the last 15 test minutes) for the MA animals was subhypnotic in all LA animals.

It is evident from the three minute activity scores presented in Fig. 2 that the differences in activity impairment following alcohol were present even during the first three min segment and remained throughout the entire testing period. In fact, the strain differences appeared to be slightly greater during the initial stages of intoxication; therefore, the use of the 16–30 min portion of the session is probably conservative. However, we prefer to use the 16–30 min activity data as this allows time for any initial novelty effects of the chamber, as well as any emotional effects from the injection procedure, to dissipate. Furthermore, we have found that activity tends to stabilize after 10–15 min exposure to the chamber.

The strain differences seem to be greatest following the 1.5 g/kg alcohol dose; the highest dose virtually eliminated activity in the MA strain, making it impossible for the strain differences to increase and making it necessary to change the behavioral measure to sleeping time to compare these strains at alcohol doses higher than 2.25 g/kg. A previous study on earlier generations [14] investigated sleeping times in these strains following a 2.5 g/kg or 3.0 g/kg alcohol dose and found that the MA strain had sleeping times 2–3 times longer than the LA strain in the eighth and ninth generation, although there were no strain differences in prior generations. It would be interesting to compare these strains on an extremely low dose of ethanol since Strange, Schneider and Goldbort [16] and others have reported that low doses increase activity in certain mouse strains. Possibly, as Strange *et al.* [16] have reported with mice, a higher sensitivity to the effects of alcohol would be manifested as a significant increase in activity in the MA animals while the LA animals would be relatively unaffected.

In summary, the results of this investigation demonstrated a greater tolerance to alcohol in the LA strain than the MA strain over a range of subhypnotic alcohol doses. Selective breeding based on behavioral tolerance has not received much attention and we can only speculate on the factors that might contribute to the inherent genetic difference in neural sensitivity to alcohol in these strains. At this time there is no reason to believe that it is specific to ethanol, and one way of gaining insight into the possible mechanisms which may account for this difference might be the investigation of other depressant drugs. Certainly these strains offer a unique opportunity for the investigation of physiological and biochemical mechanisms underlying individual differences in behavior in response to alcohol.

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