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# Mice Genetically Selected for Differences in Open-Field Activity After Ethanol

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CRABBE, J C , E R YOUNG, C M DEUTSCH, B R TAM AND A KOSOBUD *Mice genetically selected for differences in open-field activity after ethanol* PHARMACOL BIOCHEM BEHAV **27**(3) 577–581, 1987 —Starting from a population of genetically heterogeneous mice, selective breeding is being used to develop lines differing in sensitivity to ethanol-induced open-field activity Mice are tested twice for 4 min in an open field The first test is between min 2–6 after injection of saline Twenty-four hr later, a similar test is performed after injection of ethanol (1 5 g/kg) Two independent FAST lines are being selected for ethanol-induced increases in activity, and two independent SLOW lines are being selected for ethanol-induced decreases After four generations of selection, the lines have diverged significantly These lines should be useful for exploring the neuropharmacological basis for the activating and rewarding properties of ethanol

Selective breeding Pharmacogenetics Open-field activity Ethanol stimulation Reward Activity Behavioral genetics

LOCOMOTOR activity offers an attractive system for the study of ethanol (EtOH) sensitivity in rodents Effects of EtOH on activity are easily measured and have clear (although complex) dose-effect characteristics They are highly strain-specific (i e , genetically-determined) [12] The effect of EtOH to stimulate activity has been reasonably well-studied neurochemically, and the neurochemical substrate for ethanol-stimulated activity is generally attributed to catecholaminergic activation [1, 10, 21, 26, 29, 36] This response has been suggested to represent an animal model for the euphoriant and social stimulant effects of alcohol in humans [2,31] An additional interesting feature of this behavioral response to EtOH is that tolerance does not appear to develop with repeated doses [14, 27, 28, 37]

Acute administration of EtOH to rodents leads to complex effects on activity This response is typically referred to as "biphasic," in several senses [31] Most studies report that low to moderate doses of EtOH (roughly speaking, 2 g/kg IP or less) stimulate activity, while higher doses reduce activity [1, 9, 10, 14, 24, 25] (but see [23]) Another sense in which a biphasic response to ethanol occurs may be detected when activity is examined continuously after administration of a single dose of ethanol EtOH may first stimulate and thereafter depress activity [14,33] Other investigators have found that low doses may directly stimulate activity, while higher doses first depress and subsequently stimulate activity in mice tested in groups of three in a closed apparatus [20,29]

In summary, the acute effects of ethanol on activity in rodents are dose-dependent and often biphasic Low doses of ethanol generally are reported to stimulate activity in the period shortly after ethanol administration Higher doses may exert only depressant effects on activity or temporally more complex effects Depending on the species, strain, testing and apparatus conditions employed, exceptions to these generalizations have been reported However, under appropriate conditions, reliable and robust elicitation of the stimulant effect of ethanol shortly after administration is possible in mice

There are many reports of genetic variability in the response of mice to EtOH-stimulated activity Inbred mouse strains differ significantly in initial sensitivity to ethanol challenge [11, 14, 18, 30, 32] We attempted to identify the possible influence of a single gene on the stimulant component of activity and so obtained recombinant inbred strains derived from the cross of C57 and DBA inbred mice These experiments, and those by others [18], suggested that the genetic determination of the response to ethanol activation is probably polygenic [15]

Rat lines have been selectively bred for sensitivity (Most-affected MA) and resistance (Least-affected LA) to alcohol-induced reduction in motor activity in a stabilimeter

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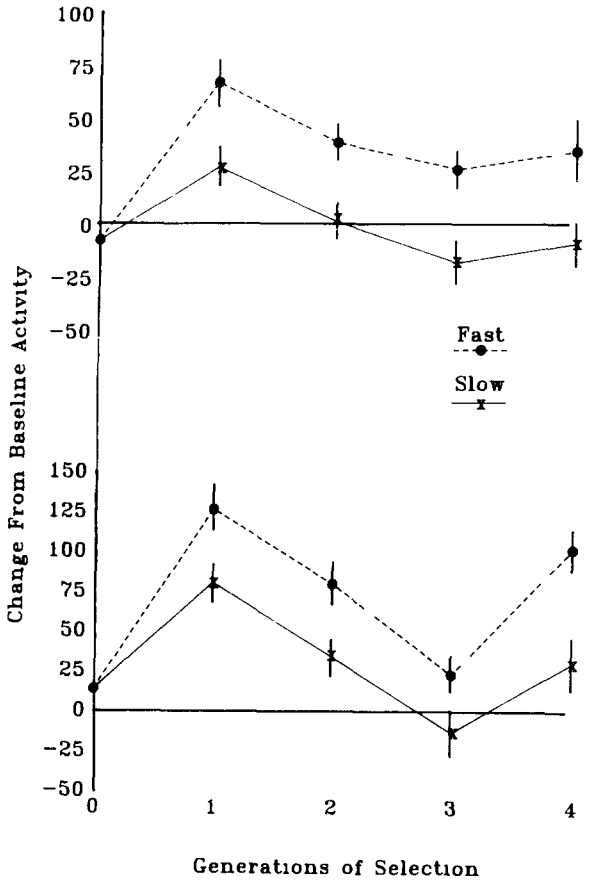


FIG 1 Difference in infrared beam interruptions (EtOH minus saline) during a 4 min test in an open-field apparatus FAST mice are genetically selected for increased activity, and SLOW mice for reduced activity Upper panel first replicate set of lines Lower panel second replicate set of lines

following a 1 5 g/kg IP dose of ethanol Since the thirteenth generation, there has been no overlap between the two lines MA rats are generally more sensitive to ethanol than their LA counterparts Most aspects of sensitivity differences between the lines have been reviewed [6] The MA and LA lines have apparently not been tested for activity after low doses of ethanol, so it is unknown whether they display enhanced activity as described above (Dr E Riley, personal communication)

Selective breeding is perhaps the most powerful methodological tool available to the pharmacogeneticist Within a line, all relevant genes will tend to be forced to the homozygous state, while all non-selected genes will tend to retain much genetic variability (although many loci begin and remain homozygotic) In a properly executed selection study, differences between selection lines can be attributed almost entirely to the effects of genes influencing the selected response This is in contrast to the genetic condition of inbred strains of animals While large strain differences in a phenotype of interest may be fixed in inbred strains, the combination of genes is an accidental process of inbreeding In selected lines, those genes specifically influencing the selected character are fixed to allow future identification of genetically correlated characters A great deal of information about the inherited bases of ethanol's effects has come from studies employing selected mouse and rat lines [17] Several lines of rats and mice have been selectively bred for acute sensitivity to CNS-depressant effects of ethanol, or for phenotypes relevant to physical dependence on ethanol No genetic animal model is currently available for any stimulant effect of ethanol Since ethanol's effect to increase open-field activity is ubiquitous and relevant to sensitivity, tolerance and physical dependence, we felt that it would be very useful to have available lines genetically tooled to express maximal and minimal response to this effect

## METHOD

### *Animals*

Mice from the HS/Ibg genetically heterogeneous stock were purchased from the Institute for Behavioral Genetics (Boulder, CO) These mice served as the foundation stock for the experiment, and all other lines were derived from them by selective breeding All animals are maintained at a colony temperature of 24±1°C and lights are on from 0600 to 1800 hr Mating pairs are housed in Plexiglas cages (28×17×11 5 cm) with stainless steel lids and wood chip bedding Food and water are available ad lib

### *Behavioral Testing*

The general procedures we employ for activity testing have been published [14] Mice are tested in the colony room At time T=0 minutes, the first mouse is weighed to the nearest 0 1 g, injected, and placed in a small individual holding cage At T=2 minutes after injection, each mouse is placed in the middle of one of two Lehigh Valley open fields Testing continues during 4 minutes (minutes 2–6 after injection) The diameter of these round open fields is 61 cm and 7 radially oriented infrared photocells and receptors are distributed equally around the perimeter Activity is electronically recorded as the mouse ambulates and interrupts the photocells While beam interruptions are also sensitive to non-locomotor activity such as grooming, such activity makes up a very small fraction of the total activity displayed by the animals during these short tests, since mice typically ambulate at a high rate in this apparatus Testing is performed under dim light (<5 ft c , or 53 8 lx at the surface of the open fields) Before each mouse is introduced into the field, the field is wiped clean with a slightly damp cloth Immediately upon completion of testing, the mouse is removed from the apparatus

Each mouse is tested on two consecutive days at an intertest interval of 24 hr On the first day, basal activity is assessed after administering the mouse a saline injection Immediately after testing on Day One, the mouse is returned to its home cage On Day Two, each mouse is given an injection of ethanol (1 5 g/kg, 20 percent v/v IP)

### *Selective Breeding*

Eighteen families were tested in the foundation population These 18 families were randomly assigned to one of two groups of 9 families, which served as the progenitors for the first and second replicate of the experiment, respectively One male and one female was then chosen from each family at random These mice were mated to form 9 breeding pairs in each replicate, excluding brother-sister matings These lines of mice are the non-selected genetic control lines, and will not be further discussed Use of non-selected controls, and an example of the breeding scheme described, may be

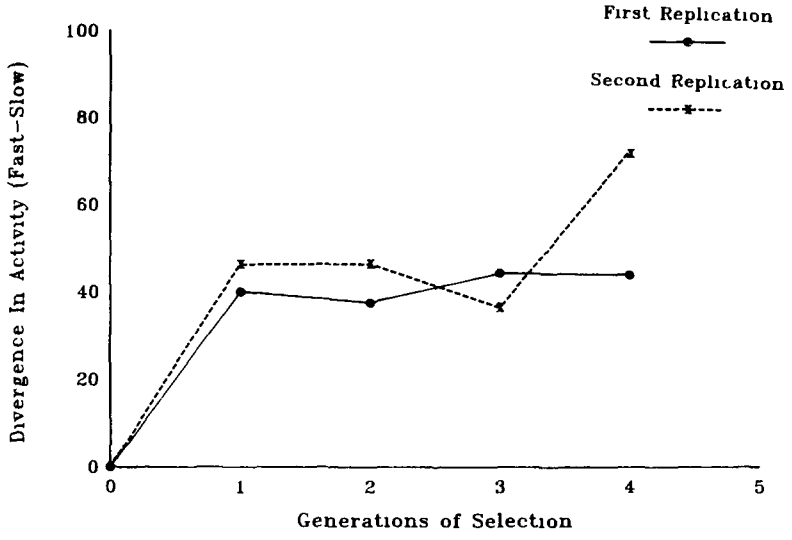


FIG 2 Divergence (FAST—SLOW) in activity across generations of selection in both replicates of the experiments

found in an earlier publication [13] For the remaining mice, the difference between Day 2 (EtOH) and Day 1 (Saline) activity was calculated The male and female from each family showing the largest difference were selected to form a FAST line, and 9 breeding pairs were thus chosen in each replicate The male and female from each family showing the smallest difference (or the greatest reduction in activity) were also chosen, and 9 breeding pairs were thus established in each of the SLOW lines The offspring of each FAST and SLOW line were tested at adulthood as described Each generation, the FAST mice most stimulated by EtOH and the SLOW mice least stimulated (or most depressed) by EtOH were chosen to form the succeeding generation's parents

## RESULTS AND DISCUSSION

Figure 1 shows the change from baseline (saline) activity seen in FAST and SLOW mice from each replication over the first four generations of selective breeding The difference between the FAST and SLOW lines of both sexes was tested by two-way ANOVA (Line × Sex) independently for each replication for the fourth selected generation In the first replication, lines differed significantly, $F(1,116)=6\ 0$, $p=0\ 01$, but neither the sex difference or its interaction with line was significant, $F<1$ The line difference was greater in the second replicate, $F(1,115)=20\ 5$, $p<0\ 0001$, again in the absence of a sex difference or interaction, $F<1$ The gradual increase in the separation of the lines is more easily seen in Fig 2, where the divergence in activity change scores is shown by generation

We also analyzed activity scores after saline in the two replications after four selected generations Since animals are being chosen for mating on the basis of the difference between EtOH and saline scores, we did not expect to see a systematic change in the saline scores between the lines In all analyses, the number of mice within a replication, line, and sex combination ranged from 22–36 In the first replication, the main effects of line and sex were not significant, $F<1$ The Line × Sex interaction, however, was significant, $F(1,116)=6\ 0$, $p<0\ 05$ This reflected the fact that male FAST mice were more active than female FAST mice (249±13 counts versus 226±8), while female SLOW mice were more active than male SLOW mice (245±11 counts versus 216±11, mean±SEM) In the second replication, no significant differences were found, $F<1\ 5$ in all cases Mean activity of all mice in the second replication after saline was 192±4 counts

We conclude that the result of selective breeding has been to generate mouse lines that differ significantly in their response to EtOH-induced open-field activity The effect of environmental factors unrelated to selection can be seen in Fig 1 In the first selected generation, for example, mice responded to EtOH on the average with relatively more stimulation than in other generations This was generally true across genotype, and therefore is due to some unexplained difference (e g , seasonal) in the environment affecting all genotypes The rather small divergence of lines after only a few selected generations is typical of experiments which employ within-family selection [13, 16, 19] Experience with selection for other phenotypes [13,16] has shown that continuation of this process for several more generations can lead to dramatic separation of the lines as more and more gene combinations favoring increased or decreased activation by EtOH are recruited into the lines

One of the more interesting features of ethanol-stimulated locomotor activity is the question of tolerance development to this effect The existing data are consistent in reporting that tolerance does not seem to develop to the effects of low doses of ethanol to stimulate activity in mice [14, 27, 28, 37] This suggests that ethanol-stimulated activity is mediated by a neuronal substrate distinct from that or those underlying the development of tolerance to depressant effects of higher ethanol doses Since tolerance develops to the effects of caffeine and two enkephalin analogues to stimulate locomotor activity in rats [7,22], this failure to develop tolerance after chronic ethanol treatment cannot be intrinsic to the motor response system itself, but must reside in the effects of ethanol on the response system

Although the depressant effects of ethanol are considered to be its principal defining features, stimulant effects analo-

gous to the low-dose stimulation of activity have been reported in man [2,5] Pohorecky [31] has reviewed the evidence strongly indicating that ethanol's effects in man are biphasic with respect to dose Lower doses elicit euphoric responses, increase talkativeness, and exert similar "social stimulant" actions, while higher doses can have opposite emotional and behavioral effects She reviews some studies implicating catecholaminergic effects of ethanol in the stimulant properties of the drug in man [31] One group [2] administered alpha-methyl-para-tyrosine to volunteers and reported that this CA inhibitor blocked elation, talkativeness, happiness and alertness and shortened the duration of euphoria as scored by observers blinded to condition Assuming that free choice consumption of ethanol by rats may serve as an index of ethanol's reinforcing properties, Amit and his coworkers [3, 4, 8, 34] showed that blockade of central noradrenergic synthesis, or serotonergic synthesis, reduced ethanol intake Rats develop gradually higher rates of self-infusion of ethanol at low, but not high doses [35] A lively controversy surrounds the suggestion that alcohol's reinforcing effects are due to specific stimulation of a noradrenergic brain reward system [3]

In summary, there is a variety of evidence generally consistent with the notion that low-dose effects of ethanol are rewarding in humans, these effects are accompanied by catecholaminergic activation, and that the stimulant effect on activity in rodents seen soon after administration of low doses of ethanol may serve as an animal model of the effects in humans While this hypothesis is anything but firmly substantiated, it suggests that the activating effect of ethanol in rodents may have some relevance for the eventual understanding of compulsive self-administration of alcohol by humans, particularly given the failure to detect tolerance development to this effect It is our hope that the FAST and SLOW lines will provide a convenient genetic animal model for discovering the neurochemical substrate of EtOH-induced activation

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## REFERENCES

1 Ahlenius, S , R Brown, J Engel, T H Svensson and B Waldeck Antagonism by nialamide of the ethanol-induced locomotor stimulation in mice *J Neural Transm* **35** 175–178, 1974
2 Ahlenius, S , A Carlsson, J Engel, T H Svensson and P Soderstern Antagonism of alpha methyltyrosine of the ethanol-induced stimulation and euphoria in man *Clin Pharmacol Ther* **14** 586–591, 1973
3 Amit, Z and Z W Brown Actions of drugs of abuse of brain reward systems A reconsideration with specific attention to alcohol *Pharmacol Biochem Behav* **17** 233–238, 1982
4 Amit, Z , Z W Brown, D E Levitan and S O Ogren Noradrenergic mediation of the positive reinforcing properties of ethanol I Suppression of ethanol consumption in laboratory rats following dopamine beta-hydroxylase inhibition *Arch Int Pharmacodyn Ther* **230** 65–75, 1977
5 Babor, T F , S Berglas, J H Mendelson, J Ellingboe and K Miller Alcohol, affect, and the disinhibition of verbal behavior *Psychopharmacology (Berlin)* **80** 53–60, 1983
6 Bass, M B and D Lester Selective breeding for ethanol sensitivity least affected and most affected rats In *Development of Animal Models as Pharmacogenetic Tools*, edited by G E McClearn, R A Deitrich and V G Erwin Washington USDHHS-NIAAA Research Monograph, No 6, 1981, pp 193–202
7 Brady, L S and S G Holtzman Effects of intraventricular morphine and enkephalins on locomotor activity in nondependent, morphine-dependent and postdependent rats *J Pharmacol Exp Ther* **218**· 613–620, 1981
8 Brown, Z W , Z Amit, D E Levitan, S O Ogren and E A Sutherland Noradrenergic mediation of the positive reinforcing properties of ethanol II Extinction of ethanol-drinking behavior in laboratory rats by inhibition of dopamine-beta-hydroxylase Implications for treatment procedures in human alcoholics *Arch Int Pharmacodyn Ther* **230** 76–82, 1977
9 Buckalew, L and G Cartwright General and differential behavioral effects of five ethanol dosages on the albino rat *Psychol Rep* **23** 1151–1154, 1968
10 Carlsson, A , J Engel and T H Svensson Inhibition of ethanol-induced excitation in mice and rats by α-methyl-p-tyrosine *Psychopharmacologia* **26**· 307–312, 1972
11 Crabbe, J C Sensitivity to ethanol in inbred mice genotypic correlations among several behavioral responses *Behav Neurosci* **97** 280–289, 1983
12 Crabbe, J C Genetic differences in locomotor activation in mice *Pharmacol Biochem Behav* **25** 289–292, 1986
13 Crabbe, J C , A Kosobud, E R Young, B R Tam and J D McSwigan Bidirectional selection for susceptibility to ethanol withdrawal seizures in *Mus musculus* *Behav Genet* **15** 521–536, 1985
14 Crabbe, J C , N Johnson, D Gray, A Kosobud and E R Young Biphasic effects of ethanol on open-field activity sensitivity and tolerance in C57BL/6N and DBA/2N mice *J Comp Physiol Psychol* **96** 440–451, 1982
15 Crabbe, J C , A Kosobud, E R Young and J Janowsky Polygenic and single-gene determination of response to ethanol in BXD/Ty recombinant inbred mouse strains *Neurobehav Toxicol Teratol* **5** 181–187, 1983
16 DeFries, J C , M C Gervais and E A Thomas Response to 30 generations of selection for open-field activity in laboratory mice *Behav Genet* **8** 3–14, 1978
17 Deitrich, R A and K P Spuhler Genetics of alcoholism and alcohol actions In *Research Advances in Alcohol and Drug Problems, Vol 8*, edited by J Smart, H C Cappell, I Glaser, Y Israel, H Kalant and E Sellers New York Plenum Publishing Corp , 1984, pp 47–98
18 Dudek, B C , M E Abbott and T J Phillips Stimulant and depressant properties of sedative-hypnotics in mice selectively bred for differential sensitivity to ethanol *Psychopharmacology (Berlin)* **82** 46–51, 1984
19 Falconer, D S *Introduction to Quantitative Genetics, Second Edition* London Longman Group Ltd , 1983
20 Frye, G D and G R Breese An evaluation of the locomotor stimulating action of ethanol in rats and mice *Psychopharmacology (Berlin)* **75** 372–379, 1981
21 Frye, G D , G R Breese, R B Mailman, R A Vogel, M G Ondrusek and R A Mueller An evaluation of selectivity of fenmetozole (D11-524) reversal of ethanol-induced changes in central nervous system function *Psychopharmacology (Berlin)* **69** 149–155, 1980
22 Holtzman, S G Complete, reversible, drug-specific tolerance to stimulation of locomotor activity by caffeine *Life Sci* **33** 779–787, 1983
23 Holtzman, S G and F H Schneider Comparison of acetaldehyde and ethanol Depression of motor activity in mice *Life Sci* **14**: 1243–1250, 1974

24 Jarbe, T U C and G C Ohlin Interactions between alcohol and other drugs on open-field and temperature measurements in gerbils *Arch Int Pharmacodyn Ther* **227**: 106–117, 1977
25 LeBlanc, A E , R J Gibbins and H Kalant Generalization of behaviorally augmented tolerance to ethanol, and its relation to physical dependence *Psychopharmacologia* **44**: 241–246, 1975
26 Liljequist, S , U Berggren and J Engel The effect of catecholamine receptor antagonists on ethanol-induced locomotor stimulation *J Neural Transm* **50** 57–67, 1981
27 Masur, J and R Boerngen The excitatory component of ethanol in mice A chronic study *Pharmacol Biochem Behav* **13.** 777–780, 1980
28 Masur, J , M L Oliveira de Souza and A P Zwicker The excitatory effect of ethanol Absence in rats, no tolerance and increased sensitivity in mice *Pharmacol Biochem Behav* **24** 1225–1228, 1986
29 Matchett, J and C Erickson Alteration of ethanol-induced changes in locomotor activity by adrenergic blockers in mice *Psychopharmacologia* **52** 201–206, 1977
30 McClearn, G E , J R Wilson and W Meredith The use of isogenic and heterogenic mouse stocks in behavioral research In *Contributions to Behavior-Genetic Analysis The Mouse as a Prototype,* edited by G Lindsey and D Thiessen New York Appleton-Century-Crofts, 1970, pp 3–22
31 Pohorecky, L A Biphasic action of ethanol *Biobehav Rev* **1** 231–240, 1977
32 Randall, C L , J A Carpenter, D Lester and H J Friedman Ethanol-induced mouse strain differences in locomotor activity *Pharmacol Biochem Behav* **3**: 533–535, 1975
33 Read, G W , W Cutting and A Furst Comparison of excited phases after sedatives and tranquilizers *Psychopharmacologia* **1.** 246–350, 1960
34 Rockman, G E , Z Amit, G Carr, Z W Brown and S O Ogren Attenuation of ethanol intake by 5-hydroxytryptamine uptake blockade in laboratory rats I Involvement of brain 5-hydroxytryptamine in the mediation of the positive reinforcing properties of ethanol *Arch Int Pharmacodyn Ther* **241** 245–259, 1979
35 Sinden, J and J LeMagnen Parameters of low-dose ethanol intravenous self-administration in the rat *Pharmacol Biochem Behav* **16:** 181–183, 1982
36 Strombom, U H and B Leidman Role of dopaminergic neurotransmission in locomotor stimulation by dexamphetamine and ethanol *Psychopharmacology (Berlin)* **78** 271–276, 1982
37 Tabakoff, B and K Kiianmaa Does tolerance develop to the activating, as well as the depressant, effects of ethanol? *Pharmacol Biochem Behav* **17**: 1073–1076, 1982