

GENETIC SELECTION OF MOUSE LINES SENSITIVE (COLD)
AND RESISTANT (HOT) TO ACUTE ETHANOL HYPOTHERMIA

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Summary

Using the technique of within-family selective breeding, we have generated mouse lines that differ genetically in sensitivity to the acute hypothermia induced by injection of 3 g/kg ethanol (EtOH). After 5 generations of selection, the difference in maximal hypothermic response between COLD and HOT lines was 1.6 °C in the first replicate and 1.2 °C in the second replicate. Estimates of realized heritability were $h^2 = .17$ in each replicate. No differences in EtOH metabolism have developed, so the differences between HOT and COLD mice are presumably in neurosensitivity. These lines of animals should be useful for studying the biological mechanisms underlying neurosensitivity to EtOH. In conjunction with other selectively bred lines, they should improve our understanding of the genetic relationships among EtOH neurosensitivity, tolerance and physical dependence.

Preliminary evidence gathered in our and others' laboratories suggests that the effects of EtOH on body temperature may be considered especially important. These effects are easily quantified (7,17) and are highly strain-specific, which implies that they are genetically-determined (8). The pharmacology (19) and physiology (13) of thermoregulation are reasonably well-studied and provide a good system for the study of conditioned and unconditioned effects of EtOH (22). Finally, effects of EtOH on body temperature may be mediators of other effects of EtOH. For example, EtOH-induced body temperature reduction has been shown to protect animals from the CNS depression of an acute dose of EtOH (1).

Selective breeding is one of the most powerful methodological tools available to the pharmacogeneticist. Selective breeding forces the allelic frequencies of genes affecting the selected phenotype to unity unless opposed by natural selection. That is, within a line, all relevant genes will tend to be forced to the homozygous state, while all non-selected genes will retain their full degree of genetic variability (although many loci begin and remain homozygotic). In a properly executed selection study, differences between selected lines can be attributed almost entirely to the effects of genes influencing the selected response (9,10,16). However, the relatively small number of breeding pairs typically employed in selection experiments will lead to fixation of some genes by chance, a phenomenon known as genetic drift. One

method of estimating the effects of genetic drift is to employ replicate selection lines. Differences between the lines that appear in both genetically independent replicates are more likely to be due to changes in the frequency at relevant loci due to selection rather than to changes in gene frequency due to genetic drift. A great deal of information about the inherited bases of EtOH's effects has come from studies employing LS and SS mouse lines selected for sensitivity to EtOH-induced hypnosis (10) and those employing P and NP rats selected for voluntary consumption of EtOH solutions (18). Since EtOH's effect to lower body temperature in a cool environment is an ubiquitous measure, relevant to sensitivity, tolerance and physical dependence, we reasoned that it would be most useful to have available lines genetically tooled to express maximal and minimal response to this effect.

Methods

Animals and Housing

An initial population of 20 breeding pairs of HS/Ibg mice from the 36th generation was purchased from the Institute for Behavioral Genetics, Boulder CO, U.S.A. These animals were genetically heterogeneous and were originally derived from crossing 8 inbred strains (21). Mice are housed 2-6 to a polypropylene cage (33 x 16 x 13 cm) with sawdust bedding changed twice weekly. A light-dark cycle of 0600 to 1800 light is maintained, and behavioral testing is conducted between hours 3 and 6 of the light period. Colony and testing room temperature is maintained at $25 \pm 1^\circ\text{C}$. Animals have ad libitum access to food and water. All housing and environmental conditions have been approved for adherence to PHS Animal Care Guidelines for our American Association for the Accreditation of Laboratory Animal Care - approved Animal Research Facility.

Assessment of Sensitivity to Ethanol Hypothermia

The general procedures we employ for hypothermia testing have been published (8). One hour before testing, each mouse is placed in an individual small chamber. These chambers have Plexiglas® walls, floors and lids and have dimensions of 8 x 19 x 8 cm. At $T = 0$ min, a 0.5 mm probe (RET-3) is inserted 2.5 cm into the rectum of the first mouse and baseline temperature is measured with a Sontek Th-8 Digital Thermometer after 5 sec. The mouse is then weighed and injected with EtOH (3 g/kg, 20% v/v in physiological saline, i.p.) and is returned to its individual cage. At $T = 30$ and $T = 60$, (30 and 60 min after injection of EtOH), the test temperature of each mouse is measured. These post-injection intervals yield approximately maximum hypothermic responses after this dose in mice (7, 8). Immediately after the $T = 60$ min temperature assessment, a 20 μl blood sample is taken from the tip of the tail for EtOH concentration determinations.

Blood and Brain Ethanol Concentration (BEC) Determinations

To determine BEC, a method described by Roach and Creaven (23) is used with several modifications. Determinations are made with a Hewlett-Packard Model 5890 gas chromatograph. Flame ionization detector temperature is 230°C , injection port temperature is 175°C , and oven temperature is 154°C . A Porapak Q column is conditioned by heating overnight at 200°C with nitrogen carrier gas flow. Peak areas are determined with an H-P Model 3393A Integrator.

Tail blood samples of 20 μl are transferred to a polyethylene centrifuge tube containing 50 μl of 5 percent zinc sulfate. 50 μl of 0.3 N barium hydroxide and 300 μl of cold distilled water are added. After centrifugation at 8-10,000 g for 5 min, supernatants are stored at -80°C until they are analyzed. For analysis, 2 μl of the supernatant is injected onto the column. Retention time for EtOH is 1.8 - 2.0 min. A standard curve for EtOH (range 1.0-4.0 mg/ml) is determined before measuring. The coefficient of variation among five injections of a sample is less than 4% in this assay.

For brain ethanol concentration determinations, mice were decapitated and brains were rapidly removed, rinsed in ice water, and blotted on filter paper. Preparation follows a previously published procedure (4).

Selection Method

In the S_0 Generation, offspring were produced by 18 of the 20 matings. Nine families were randomly selected for the first replicate of the experiment: the remaining nine families formed the second replicate. All mice in the first replicate were tested for EtOH hypothermia as described. Since most births occur within a narrow range, all mice were tested at the age of 45-49 days, with less than 15% of the mice 1 week younger. Baseline temperatures and test temperatures at 30 and 60 min after ip injection of 3 g/kg EtOH (20% v/v) were determined as described. The maximal reduction from baseline temperature (hypothermia, or HT) was used as the selection criterion. We then selected one female and one male offspring at random from each family. These were mated to form the next generation's control line (CON). Although this assigned some extreme-scoring mice to the CON line, it did not restrict the range of scores available from remaining mice and did not have any apparent impact on selection differentials (see Table II). The highest-scoring male and highest-scoring female were then selected for a Hypothermia Sensitive (COLD) line and the lowest-scoring male and female for a Hypothermia Resistant (HOT) line. The three lines so formed were mated and their offspring (the first selected generation, or S_1) were tested for HT. Each family from that point on was either COLD, HOT or CON and selection was continued each generation on a within-family basis. A rotational mating scheme was employed, as described previously, to reduce inbreeding in the early generations (5). We chose this method over the alternatives of mass selection or combined selection for three reasons: no information about heritability was available; this method is most efficient of space; and because it greatly reduces inbreeding by doubling the effective population size (11). With the remaining 9 families in S_0 , this process was replicated, so we are developing two COLD, two HOT, and two CON lines.

Determination of Response to Selection and Estimates of Heritability

Each generation, both COLD and both HOT lines were tested under identical environmental conditions, so it was possible to compare the selected mice directly. Comparisons between lines were made by two-way ANOVA (line x sex) separately for the two replicates. The difference in HT between COLD and HOT lines indicates the overall realized response to selection (R) achieved. The selection differential (S) can also readily be calculated as the difference between the mean score of the animals from a generation's population that were chosen for matings to produce the next generation, and the mean score of the whole population. These parameters are related by the formula $R = h^2S$ (11), where h^2 is the realized heritability (proportion of total response variance that is due to additive genetic sources). Heritability was estimated by dividing the total realized R by the cumulative S, and by regressing realized R on cumulative S, over five generations (11,14,15).

Results and Discussion

Response to Selection

Data were analyzed with separate two-way ANOVAs (Line X Sex) for each generation and replicate. To simplify presentation, sex differences are not reported for all analyses. In no case were there significant interactions of sex with line. In Generation S_5 , the first replicate COLD line had significantly greater HT than the HOT line after injection of EtOH, $F(1,111) = 76.1$, $p < .0001$. This difference was smaller, but also reached significance in the second replicate, $F(1,147) = 53.4$, $p < .0001$. Results over the first 5 generations are shown in Figure 1. Maximum HT increased over generations of

selective breeding in both replicate COLD selected lines. Maximum HT decreased from generation S_3 on in the first replicate HOT line and in generation S_4 and S_5 in the second replicate HOT line. The apparent difference in maximal HT between replicates was evident in Generation S_0 and has been maintained throughout the first 5 generations. This difference is likely due to sampling error. The effect of environmental changes unrelated to the selection is also clearly evident in Figure 1. Even though each generation's data were collected under as nearly identical conditions as possible, generation S_2 generally had more pronounced HT responses than other generations. The reason for this is unknown, but such fluctuations are typical of artificial selection experiments (5,11).

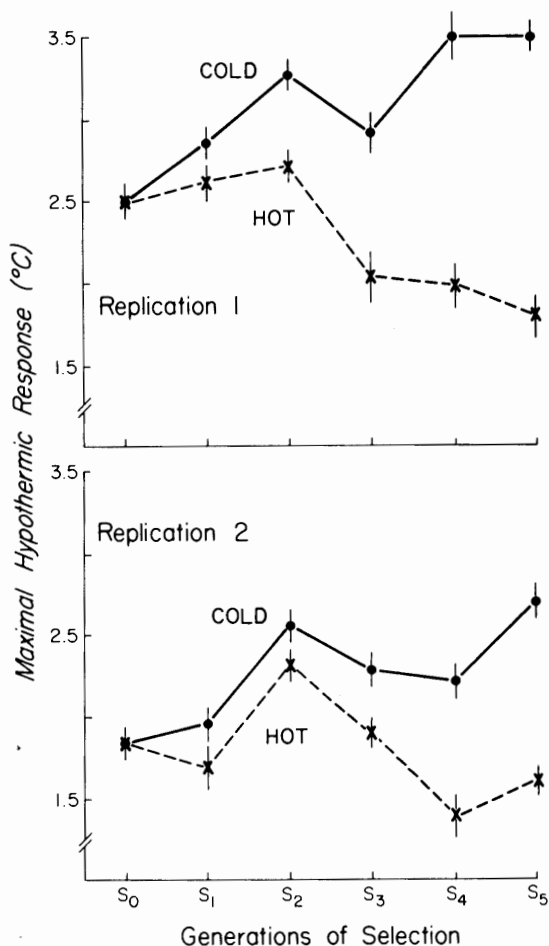


FIG. 1

Maximal change from baseline body temperature (mean \pm SE) is shown each generation for the first (upper panel) and second (lower panel) replicate set of lines. In the S_0 generations, mice had not yet been assigned to be COLD or HOT, so only one data point is shown.

The success of selective breeding in increasing the maximal HT response to EtOH is shown more clearly in Figure 2. The maximal difference between COLD and HOT lines, or the divergence in the selected lines, is plotted as a function of generation of selection. This difference can be seen to be steadily increasing in the first replicate set of lines, and is approximately 1.6°C after 5 generations. Response in the second replicate set of lines is somewhat smaller, approximately 1.2°C after 5 generations.

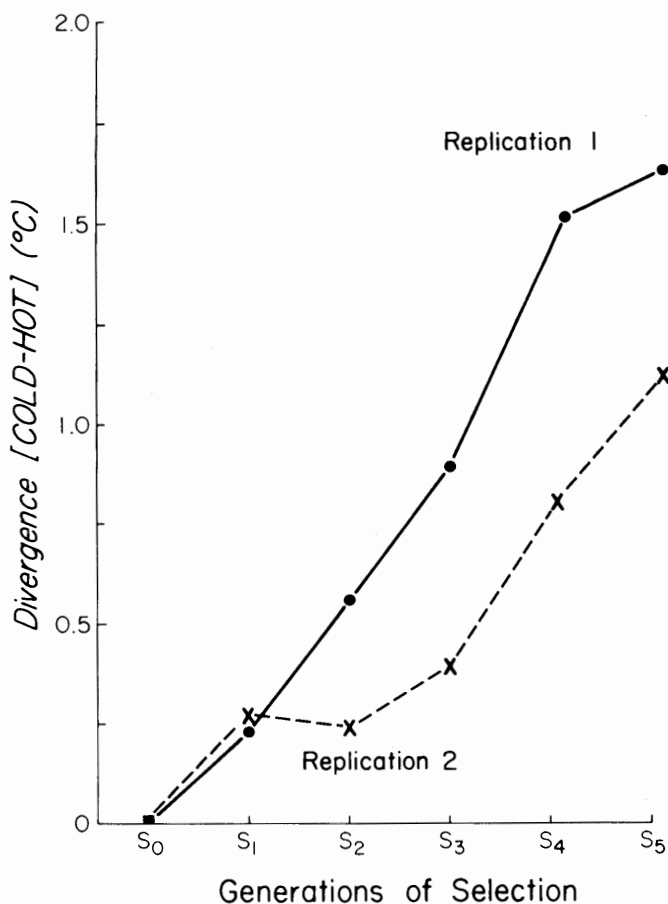


FIG. 2

Divergence in maximal HT response to 3 g/kg EtOH between COLD and HOT selectively bred lines.

Table I gives the mean \pm SE baseline temperature, maximum change in temperature, and blood ethanol concentration for all mice tested through generation S₅. Values for all three lines within a replicate are shown as the same in S₀, since mice had not yet been divided into lines. CON lines were tested in generation 3. COLD and HOT lines did not differ from each other significantly in baseline temperatures for any generation of selection ($p > .10$). Examination of Table I reveals no systematic changes in baseline temperatures during the selection.

Genetic Parameters of Selection

Results are shown in Table II. Number of mice tested per line each generation ranged from 36 to 85 (mean = 73), and actual number of mice per line contributing offspring ranged from 10-18 (mean = 15.4). Since the within-family procedure employed nearly doubles the effective breeding population size (11) and because family size had low variance, the mean effective breeding population size per line per generation ranged from 20-36 (mean = 31.2).

In the COLD lines, selection differential averaged .85°C per generation: that is, mice chosen for mating averaged .85°C larger hypothermic response than the whole population of COLD mice. In the HOT lines, S averaged .65°C per generation: that is, the selected HOT mice had .65°C less hypothermia than the whole population of HOT mice. Combined selection differential was, therefore, approximately 1.5°C per generation for each replicate: that is, the selected HOT and COLD parents differed from each other in maximal HT response by 1.5°C.

Estimates of Heritability

We estimated realized heritability for the total realized response difference between HOT and COLD lines within each replicate. To estimate R we employed the divergence between COLD and HOT lines (See Figure 2), and to estimate S, we summed the selection differentials applied separately to the COLD and HOT lines within a replicate. Total realized heritability after 5 generations estimated for the first replicate was $h^2 = .18$, and for the second replicate, $h^2 = .15$. The current combined estimate of h^2 is therefore .17 in the two replicates. This means that 17% of the variance in EtOH-induced HT in mice is presumed to be of additive genetic origin. A realized heritability of 17% agrees well with other selective breeding studies for pharmacogenetic traits (2, 5, 20). In other response systems, similar heritabilities have led to a large divergence in selection response over generations (2, 5, 20).

Realized heritability was also estimated from the regression of realized response to selection (R) on cumulative selection differential (S) over the first 5 generations of selection (11,14,15). The values obtained for the first replicate were $h^2 = .19$ and for the second replicate, $h^2 = .14$. These regression functions are shown in Figure 3, separately for replications 1 and 2. They agree well with the estimate by the Total Realized Response method. We also estimated realized heritability separately for each line and replicate (data not shown). There were no marked differences in heritability from line to line: estimates ranged from .12 to .21.

Inbreeding

Using data from Table II, we estimated the cumulative change in the inbreeding coefficient (ΔF), computed according to Falconer (11). After 5 generations of selection, approximately 6-7% of segregating loci in each line have been fixed homozygous by chance factors unrelated to the selection process. This is near the expected rate of 1.5% per generation and agrees with our experience with selection for another EtOH trait (5). Thus, we are successfully minimizing trait-irrelevant inbreeding in these lines.

TABLE I

Mean \pm SE Temperatures and Blood Ethanol Concentrations for All Tested MiceGenerations
of Selection

		BASELINE TEMPERATURE (°C)					
		HOT ₁	COLD ₁	CON ₁	HOT ₂	COLD ₂	CON ₂
FEMALE							
S ₀		38.02 \pm .08	38.02 \pm .13	38.02 \pm .08	37.87 \pm .09	37.87 \pm .09	37.87 \pm .09
S ₁		38.50 \pm .09	38.50 \pm .08	----	38.00 \pm .13	38.22 \pm .08	----
S ₂		38.31 \pm .09	38.18 \pm .13	----	38.14 \pm .10	38.19 \pm .08	----
S ₃		37.84 \pm .16	38.15 \pm .09	38.16 \pm .09	37.97 \pm .06	37.94 \pm .08	37.76 \pm .15
S ₄		37.80 \pm .11	38.10 \pm .10	----	37.69 \pm .07	37.91 \pm .07	----
S ₅		38.16 \pm .08	38.25 \pm .10	----	37.83 \pm .08	37.96 \pm .08	----
MALE							
S ₀		37.52 \pm .09	37.52 \pm .09	37.52 \pm .09	37.42 \pm .09	37.42 \pm .09	37.42 \pm .09
S ₁		38.01 \pm .09	38.13 \pm .12	----	37.46 \pm .14	37.51 \pm .10	----
S ₂		37.67 \pm .09	37.96 \pm .12	----	37.74 \pm .10	37.92 \pm .09	----
S ₃		37.69 \pm .12	37.47 \pm .12	37.59 \pm .08	37.45 \pm .14	37.68 \pm .11	37.16 \pm .15
S ₄		37.48 \pm .09	37.54 \pm .11	----	37.14 \pm .08	37.49 \pm .07	----
S ₅		37.53 \pm .10	38.10 \pm .10	----	37.40 \pm .09	37.53 \pm .08	----
		MAXIMUM CHANGE IN TEMPERATURE (°C)					
		HOT ₁	COLD ₁	CON ₁	HOT ₂	COLD ₂	CON ₂
FEMALE							
S ₀		2.69 \pm .00	2.69 \pm .00	2.69 \pm .00	1.88 \pm .14	1.88 \pm .14	1.88 \pm .14
S ₁		2.87 \pm .16	3.17 \pm .16	----	1.82 \pm .17	2.21 \pm .13	----
S ₂		3.01 \pm .16	3.50 \pm .26	----	2.26 \pm .16	2.57 \pm .16	----
S ₃		2.08 \pm .28	3.05 \pm .23	2.54 \pm .16	1.97 \pm .13	2.44 \pm .13	1.25 \pm .30
S ₄		2.19 \pm .21	3.75 \pm .21	----	1.58 \pm .20	2.50 \pm .16	----
S ₅		2.28 \pm .20	3.71 \pm .21	----	1.75 \pm .13	2.92 \pm .13	----
MALE							
S ₀		2.37 \pm .00	2.37 \pm .00	2.37 \pm .00	1.81 \pm .13	1.81 \pm .13	1.81 \pm .13
S ₁		2.40 \pm .14	2.50 \pm .16	----	1.57 \pm .21	1.73 \pm .14	----
S ₂		2.46 \pm .16	3.11 \pm .17	----	2.39 \pm .17	2.56 \pm .13	----
S ₃		1.98 \pm .18	2.80 \pm .20	2.40 \pm .16	1.81 \pm .17	2.10 \pm .18	0.92 \pm .24
S ₄		1.83 \pm .17	3.18 \pm .20	----	1.23 \pm .20	1.94 \pm .13	----
S ₅		1.61 \pm .15	3.34 \pm .17	----	1.46 \pm .20	2.43 \pm .14	----
		BLOOD ETHANOL CONCENTRATION (MG/ML)					
		HOT ₁	COLD ₁	CON ₁	HOT ₂	COLD ₂	CON ₂
FEMALE							
S ₀		3.14 \pm .06	3.14 \pm .06	3.14 \pm .06	2.92 \pm .06	2.92 \pm .06	2.92 \pm .06
S ₁		3.20 \pm .06	3.27 \pm .07	----	3.22 \pm .08	3.11 \pm .08	----
S ₂		3.23 \pm .07	3.30 \pm .07	----	3.11 \pm .09	3.11 \pm .08	----
S ₃		3.14 \pm .05	3.18 \pm .06	3.03 \pm .09	3.11 \pm .09	3.12 \pm .05	2.80 \pm .10
S ₄		3.35 \pm .09	3.61 \pm .15	----	2.82 \pm .08	2.93 \pm .08	----
S ₅		2.92 \pm .08	3.07 \pm .05	----	2.96 \pm .05	2.87 \pm .06	----
MALE							
S ₀		2.97 \pm .07	2.97 \pm .07	2.97 \pm .07	2.95 \pm .05	2.95 \pm .05	2.95 \pm .05
S ₁		3.12 \pm .07	3.11 \pm .06	----	3.15 \pm .05	3.05 \pm .05	----
S ₂		3.22 \pm .08	3.14 \pm .05	----	3.13 \pm .06	3.13 \pm .07	----
S ₃		3.15 \pm .05	3.17 \pm .06	3.04 \pm .06	3.09 \pm .05	3.07 \pm .08	2.97 \pm .06
S ₄		3.32 \pm .05	3.55 \pm .17	----	2.79 \pm .06	2.86 \pm .05	----
S ₅		2.89 \pm .06	2.86 \pm .07	----	2.64 \pm .08	2.81 \pm .05	----

TABLE II

Genetic Data for HOT and COLD Selected Lines and Nonselected Control Lines

Generations
of Selection

	Total Tested	Number of Families	S	N	$X(\sigma^2)$	N_e	Cumulative ΔF
<u>HOT₁</u>							
S ₀	79	9	.71	16	1.78(.17)	29.49	---
S ₁	74	9	.77	18	2.00(.00)	36.00	.0139
S ₂	83	6	1.01	12	2.00(.00)	24.00	.0347
S ₃	44	7	.56	18	2.57(.24)	32.14	.0503
S ₄	67	8	.98	16	2.00(.00)	32.00	.0688
<u>COLD₁</u>							
S ₀	79	9	.77	18	2.00(.00)	36.00	---
S ₁	67	10	.67	18	1.80(.18)	33.03	.0139
S ₂	77	10	1.18	18	1.80(.16)	33.33	.0280
S ₃	81	9	1.09	18	2.00(.00)	36.00	.0440
S ₄	76	8	1.14	14	1.75(.21)	25.34	.0621
<u>CON₁</u>							
S ₀	79	9	--	16	1.60(.26)	28.32	---
S ₁	--	9	--	16	1.78(.17)	29.49	.0177
S ₂	--	8	--	16	2.00(.00)	32.00	.0347
S ₃	72	9	--	18	2.00(.00)	36.00	.0503
S ₄	--	8	--	16	2.00(.00)	32.00	.0650
<u>HOT₂</u>							
S ₀	79	9	.49	16	1.78(.17)	29.49	---
S ₁	60	8	.47	16	2.00(.00)	32.00	.0170
S ₂	80	7	.96	14	2.00(.00)	28.00	.0326
S ₃	70	9	.80	18	2.00(.00)	32.43	.0505
S ₄	81	7	.91	14	2.00(.00)	28.00	.0684
<u>COLD₂</u>							
S ₀	79	9	.56	18	2.00(.00)	36.00	---
S ₁	81	7	.57	14	2.00(.00)	28.00	.0139
S ₂	85	8	.69	16	2.00(.00)	32.00	.0318
S ₃	74	8	.78	16	2.00(.00)	32.00	.0474
S ₄	81	9	.87	18	2.00(.00)	36.00	.0630
<u>CON₂</u>							
S ₀	79	9	--	18	2.00(.00)	36.00	---
S ₁	--	8	--	16	2.00(.00)	32.00	.0139
S ₂	--	6	--	12	2.00(.00)	24.00	.0295
S ₃	36	6	--	18	3.00(.00)	36.00	.0503
S ₄	--	5	--	10	2.00(.00)	20.00	.0677

Shown for each generation are the total number of mice tested, the number of families and number of mice (N) producing testable offspring in the next generation, the selection differential (S), the mean and variance (S^2) of reproductive family size, the estimated effective breeding population size [$N_e = 4N/(2 + \sigma_k^2)$], and the cumulative estimated change in the inbreeding coefficient, ΔF . These values are estimated according to Falconer (11).

Role of EtOH Metabolism

One way in which the COLD and HOT lines could be differing in response to EtOH is pharmacokinetically. If COLD lines were simply to achieve higher BEC after acute injection than HOT lines, they would be expected to differ in HT response on the grounds of dose rather than because of a presumed difference in CNS sensitivity to EtOH. Table I shows that after 5 generations of selection, this is not the case. Data were analyzed by separate two-way ANOVAs (Line X Sex) within each replicate and generation. In both replicates, same-sex COLD and HOT lines did not differ significantly in BEC at 60 minutes after injection in any generation. BEC at 30 and 60 minutes after

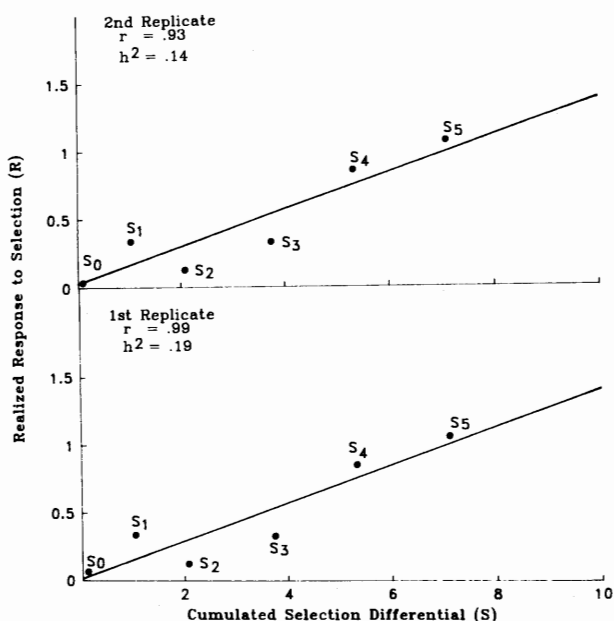


Fig. 3

Estimates of realized heritability (h^2) for regression of realized response to selection (R) on cumulated selection differential (S) for the data in Tables I and II. First replicate, lower panel; second replicate, upper panel.

injection are known to be highly genetically correlated (8), and BEC is known to have equilibrated with brain EtOH concentrations by 60 minutes after ip injection (12). We assume, therefore, that the BEC at 60 minutes adequately estimates brain EtOH concentration at the time of maximal HT, although more detailed experiments would be necessary to demonstrate this unequivocally.

The temporal pattern of HT response is similar in the COLD and HOT lines (data not shown). While it is certainly conceivable that as selection progresses one line will begin to attain maximal HT (or regain normal body temperature after injection) more quickly than the other, we have not seen this happen so far. The percentage of animals in the four selected lines attaining maximal HT at 30 (versus 60) minutes post-injection ranged from 54% to 89%, but no apparent line-specific pattern of changes has developed during the course of selection (data not shown). Collapsing across lines, the proportion of animals tested attaining maximal scores at 30 minutes varied between 63% (S_2) and 87% (S_0). The difference between generations was significant [$\chi^2(5) = 55.4, p < .001$]. Since the lines did not differ systematically, this suggests to us that differences across generations in the time of maximal HT is due to environmental variability, thus resembling the differences in average maximal HT across generations previously discussed.

We also tested additional offspring from Generation S_5 . Mice from each of the four selected lines were injected with 2, 3, or 4 g/kg EtOH (20% v/v, ip) and blood samples were drawn from a random sample of each group for analysis at 30, 60, 120, and 180 minutes after injection. No more than three samples were drawn from an individual mouse. Results from the three doses tested were very similar. The analysis of the results after 3 g/kg, given in Table III, demonstrated that Widmark's β , the apparent linear decline in BEC over time after injection estimated from linear regression on data points 60 minutes and later after injection, did not differ among lines. Furthermore, analysis of the data by three-way ANOVA (Line X Replicate X Time) revealed that the lines did not differ significantly at any time point. Finally, to see whether differences in distribution and/or absorption of EtOH could lead to higher brain concentrations of EtOH, we sacrificed some mice at 30 and some at 60 minutes after injection of 3 g/kg EtOH and determined brain EtOH

Table III

Ethanol Metabolism in HOT and COLD Mouse Lines
from Generation S_5 after 3 g/kg EtOH Dose

Line	Replicate	BEC (mg/ml)				Widmark's β (mg/ml/hr)
		Time after Injection (min)				
		30	60	120	180	
COLD	1	3.40 \pm .03	3.09 \pm .14	2.57 \pm .12	1.37 \pm .24	.846
	2	3.24 \pm .22	3.20 \pm .12	2.51 \pm .24	1.74 \pm .10	.728
HOT	1	3.20 \pm .11	3.05 \pm .15	2.29 \pm .23	1.52 \pm .06	.763
	2	3.26 \pm .20	3.11 \pm .21	2.31 \pm .21	1.52 \pm .26	.793

Number of mice = 6-11/group. BEC did not differ significantly except as a function of time [$F(3,11) = 78.9, p < .0001$]. For all other factors and interactions, $F \leq 1.4$ (ns). All values were determined after ip injection of 3 g/kg EtOH 20% v/v, and are mean \pm SE.

concentrations. The effect of time was significant [$F(1,8) = 7.6, p < .05$], and mean \pm SE brain EtOH concentration at 30 min was higher ($3.61 \pm .08$ mg/g) than at 60 min ($3.04 \pm .20$ mg/g). Selected lines did not differ significantly in brain EtOH at either time, and the interactions were not significant ($p > .10$).

Results from the non-selected control lines, which were tested again in the third generation, are shown in Tables I and II. The control line for the first replicate is intermediate between its COLD and HOT line in HT responsiveness, while for the second replicate control line, HT was less than for its HOT line. It is too early in the selection process to be able to say whether or not this is due to asymmetry in response to selection, sampling error, or restriction of variability due to the smaller response to selection in the second replicate. CON lines will be tested regularly each third generation, so it should be possible to answer this question in the future. CON lines have BEC values that do not differ significantly from those of their corresponding HOT and COLD lines (See Table I).

We are concurrently conducting a similar selection for EtOH-induced activation in an open field. Lines selected for high (FAST) and low (SLOW) levels of EtOH-induced activity have diverged after four generations of selection to differ significantly (unpublished). It will be of interest to determine whether lines selected for genetic susceptibility to one effect of EtOH also differ in sensitivity to other effects. Based on experiments estimating genetic correlations between responses to ethanol in inbred mouse strains, we would predict that FAST and SLOW mice would not differ systematically in HT response to EtOH, and that COLD and HOT mice would not differ in EtOH-induced activity (3). It is more difficult to predict whether COLD and HOT mice should differ in susceptibility to EtOH physical dependence. Studies with inbred strains estimated a reasonable negative genetic correlation between HT and withdrawal severity (6). However, lines selected for differences in withdrawal severity (5) do not differ markedly in acute HT sensitivity to EtOH (4). The existence of a battery of lines genetically selected for sensitivity to a variety of effects of EtOH should allow definitive assessment of the genetic relationships among EtOH neurosensitivity, tolerance, and dependence.

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