

Response to selection for ethanol-induced locomotor activation: genetic analyses and selection response characterization*

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Abstract. Selectively bred FAST mice are highly susceptible, while SLOW mice are less susceptible, to the locomotor stimulant effects of ethanol. Heritability estimates indicate that approximately 15% of the variance in the FAST lines is of additive genetic origin, while low susceptibility is ostensibly nonheritable. Inbreeding has increased at the rate of 2% per generation, but fertility has been unaffected. Measurement reliability for sensitivity to this ethanol effect was high when measured in both circular ($r=0.6$) and square ($r=0.7$) open-fields. In addition, our results indicate that we have selected for differences in sensitivity to ethanol rather than for differences in habituation to the test environment. The difference in response to ethanol between FAST and SLOW mice extended to tests varying in duration, and to a range of ethanol doses. We conclude that the divergence between FAST and SLOW mice generalizes to related test parameters, and speculate that the genetic architecture underlying the locomotor stimulant response may be simpler than previously proposed.

Key words: Selective breeding – FAST and SLOW mice – Ethanol-stimulated activity – Heritability

Genetic factors influence sensitivity to the stimulant effects of ethanol in mice. Wide variation in locomotor response to ethanol among inbred strains reflects such genetic influences (Crabbe 1986; Randall et al. 1975; Lister 1987; Dudek and Phillips 1990), as does the large difference between mouse lines bred for relative sensitivity to the stimulant effects of ethanol (Crabbe et al.

1987b; Phillips et al. 1989a, b). The importance to alcohol research of variation in this response derives partly from the notion that locomotor stimulation by ethanol and other drugs in mice may model euphoriant or reinforcing drug properties that have been suggested to play an important role in the development of addiction (Ahlenius et al. 1973; McAuliffe and Gordon 1974; Bijerot 1980; Babor et al. 1983; Wise and Bozarth 1987).

Several years ago a program was initiated to produce a genetically-defined animal model composed of selectively bred mouse lines differing specifically in sensitivity to the activating effects of ethanol. FAST (sensitive) and SLOW (insensitive) lines have diverged in response, and differ significantly and consistently in locomotor response to ethanol (Crabbe et al. 1987b; Phillips et al. 1989b). These lines should be useful for examining several issues. Wise and Bozarth (1987) are strong advocates of the notion that one drug property determining addiction potential is ability to induce psychomotor activation. They argue that “the seemingly disparate phenomena of addiction, positive reinforcement, and psychomotor activation are homologous, resulting from activation of a common brain mechanism.” Therefore, if locomotor activation reflects reinforcement, the FAST and SLOW lines will provide an animal model for exploration of ethanol’s euphoric/reinforcing effects. In addition, because selective breeding produces animal lines that differ specifically in allelic frequencies for genes influencing the selection response, but do not systematically differ in frequencies at trait-irrelevant loci, these animals are useful for examining genetic correlations (Phillips et al. 1989b; Crabbe et al. 1990). Thus, the question of whether these lines differ in sensitivity only to low dose stimulation, or also to other ethanol effects, can be addressed, providing information about genetic overlap in the control of sensitivity to various ethanol effects (Phillips et al. 1989b). Lastly, catecholaminergic systems seem to play an important role in the neurochemical mediation of ethanol-induced locomotor stimulation (Ahlenius et al. 1974; Strombom et al. 1977; Friedman et al. 1980; Dudek et al. 1984). Therefore, FAST and

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SLOW mouse lines provide a means for further exploration of the neurobiological basis for locomotor stimulation.

Before using these animals in detailed studies of ethanol's actions, it would first be valuable to estimate the proportion of locomotor response variation under genetic control in such an animal model, and to fully characterize the model. For example, given high response reliability, and establishment of genetic influences, the conjecture that determination of alcohol's acute stimulant potency in human individuals has considerable potential for predicting abuse liability might be strengthened. In this paper, we describe response to 17 generations of selection, present estimates of response heritability, and present results of experiments investigating response reliability, locomotor activity dose-response curves, and habituation of FAST and SLOW mice. Our results indicate that the locomotor response difference to ethanol between FAST and SLOW mice cannot be explained by differences in baseline activity or habituation to novelty, and that within-subject response reliability is high even when designs differing from the selection design are used to measure locomotor activation. We suggest that these lines provide a useful tool for addressing many of the issues raised above.

Experiment 1. Selective breeding for differential susceptibility to ethanol-induced activation

Materials and methods

Animal maintenance. All animals were housed in clear polypropylene cages (28 × 18 × 13 cm) on corn-cob bedding (changed twice weekly) in a filtered Thorens-rack system. Water and rodent block food were freely available, ambient temperature was 21 ± 2° C, and fluorescent lights were on, 12 h daily, beginning at 6 A.M. Mating pairs were housed with offspring until weaning at 21 ± 1 days of age. First litters were weaned, one to six per cage into same-sex litter groups until selection phenotype testing was performed at 5–6.5 weeks of age. After selection of animals for subsequent mating pairs, remaining mice from first litters were not saved for further testing because they represent a population with an abbreviated response distribution. Mating pairs were perpetuated for production of second, third, ... xth litters to be used in additional experiments.

Selection procedures. Two pairs of FAST and SLOW lines and two nonselected randomly bred control (CON) lines were maintained. All lines originated from a heterogeneous stock (HS/Ibg), the product of an 8-way cross of inbred strains chosen for their divergent genetic backgrounds. Originally, 9 out of 18 families from the HS/Ibg foundation population were randomly assigned to serve as the source of progenitors for the first genetic replicate, and the remaining 9 for the second replicate. These 18 families were tested as described below, and a male and a female from each litter were randomly chosen and mated (avoiding brother-sister pairings) to form the non-selected control lines. Mating pairs for the FAST and SLOW lines were similarly established, except that selection for breeding was based on their response to ethanol (see below). Thus, the selection population comprised litters from at least nine mating pairs per line and replication, and in addition, supplemental pairs were mated to guard against fertility problems. The separate sets of mating pairs were maintained as isolated populations for each of the replications, making them genetically independent. For example, FAST-1 mice were never bred with mice from the

FAST-2 population. Selection procedures have been described in greater detail (Crabbe et al. 1987b). Because CON lines were shared with another selective breeding experiment (Crabbe et al. 1987a; Phillips et al. 1990), they were tested for locomotor response to ethanol only every third generation.

Measurement of the selection phenotype: locomotor activity (ACT) response to ethanol. Two circular Lehigh Valley open-fields (61 cm, diameter), transected by six pairs of photocell beams and receptors were used for recording horizontal locomotor activity. Photocell beam interruptions were automatically transmitted to a computer. Currently, animals are tested during the light phase (9 A.M. to 3 P.M.) under bright fluorescent lighting conditions. They were ear-punched for identification 7–10 days prior to testing, weighed just before testing, injected IP, with 2.0 g/kg ethanol (20% ; v/v), left in the weighing bin for 2 min, placed into the center of an open-field monitor, and tested for 4 min (minutes 2–6). After a 24-h rest period in the home cage, they were retested as described above, following an IP injection of 0.9% saline. From generations 0–5 the above procedures were utilized except that the order of testing was reversed (saline then ethanol), the ethanol dose was 1.5 g/kg, and testing occurred under dim lighting. Ethanol score minus saline score represented the effect of ethanol on activity (ACT) and is used as the selection phenotype. Animals with the highest ACT scores were selected for the FAST lines and those with the lowest scores (including, in some cases, negative scores) were chosen for the SLOW lines. Changes to the current procedures were prompted by minimal continued divergence between the FAST and SLOW lines, and investigations which indicated greater stimulant response for selection with these alterations. Experiments comparing the different methods of testing are discussed in detail elsewhere (Crabbe et al. 1988), and showed, for example, that the increase in ACT scores under bright versus dim lighting, was not due to increased immobility on saline test days. However, the relative roles of exploration and anxiety under our testing conditions remain to be explored.

Results

Response to selection and estimation of genetic parameters. Mean ACT responses of both replicate FAST, SLOW, and CON lines across 17 generations of selection are presented in Figs. 1A and B. It is apparent that a large response to selection occurred in the first generation in both replicates followed by little further divergence between FAST and SLOW lines through S₆. Another jump in selection response is visible in S₇ in both replicates (especially in replicate 2), corresponding with changes in the selection protocol. Continued response to selection is occurring in both replicates, although replicate 1 lines are diverging less rapidly. CON-1 mean scores have closely resembled those of the SLOW-1 line while CON-2 values have largely been intermediate to the selected line values since S₇.

Positive ACT values in both SLOW lines indicate that we are selecting for less locomotor activation rather than depressed activity. While 55% and 41% of the founding HS/Ibg mice assigned to form replicate 1 and replicate 2 lines, respectively, had negative ACT scores, the magnitude of the depressed response was small. The mean negative scores were -58 and -46 for replicate 1 and 2 HS/Ibg mice, respectively. Percentages of animals with negative scores in the most recently tested generation which included testing of CON mice (S₁₆) were 35%, 51%, and 5% for SLOW-1, CON-1, and FAST-1 mice,

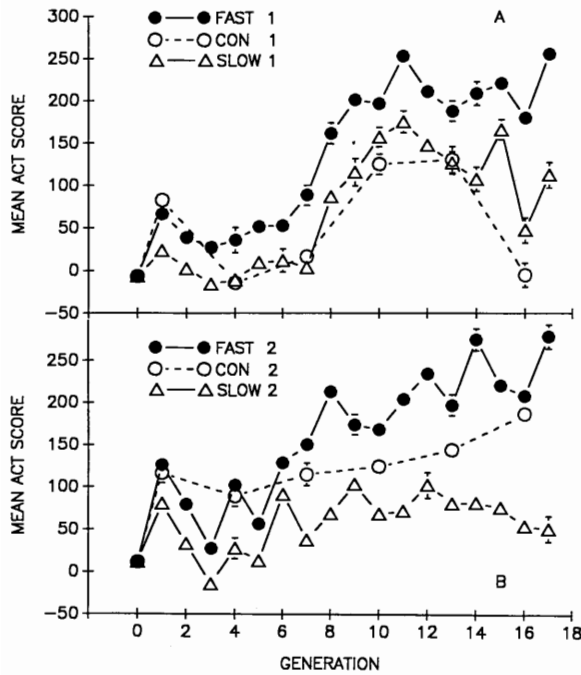


Fig. 1A, B. Response of FAST, SLOW, and CON replicate 1 (*panel A*) and 2 (*panel B*) lines to 17 generations of selection for differential sensitivity to ethanol-induced stimulation. *ACT* represents the difference between a saline activity score and an ethanol activity score collected 24 h earlier. SEM larger than symbol size are shown. Average number of animals tested each generation was 76 for FAST-1, 61 for CON-1, and 69 for SLOW-1; 73 for FAST-2, 62 for CON-2, and 69 for SLOW-2

and 17%, 1%, and 0% for SLOW-2, CON-2, and FAST-2 mice, respectively. There are several published examples of locomotor depression in mice following ethanol treatment (Friedman et al. 1980; Dudek et al. 1984; Crabbe 1986; Dudek and Phillips 1990). We suggest that the relative absence of large depressant responses in our lines is, in part, due to our short test duration, which occurs largely during the ascending phase of the ethanol absorption curve. The general increment in ACT scores of most lines across several generations of the selection suggests the influence of some environmental or methodological factor. We have been unsuccessful at identifying any change(s) that correlates with this progressive increment (e.g., seasonal, new technicians, new test location, etc.).

Changes in experimental design during the course of a selection experiment are not unprecedented (McClearn and Kakhana 1981); however, they make estimation of genetic parameters more complicated and difficult to interpret. The heritability (h^2) of a phenotype is an estimate of the proportion of phenotypic variance presumed to be of additive genetic origin. We have chosen to present h^2 estimates for the entire generation response curves as well as for segments of the curves. Selection differential (*S*) and response to selection (*R*) were calculated from the mean responses of defined populations as follows:

$$S = x'_0 - x_0$$

$$R = x_1 - x_0,$$

where x_0 = the phenotypic value of the parent population, x'_0 = the mean value of only those parents selected to produce offspring for the next generation, and x_1 = the mean value of the offspring of those selected parents. Therefore, *S* provides an estimate of the applied selection pressure and *R* indicates change in the population mean resulting from selection (Falconer 1983).

From Figs. 1 A and B it appears that there is substantial heritability in the direction of ethanol-increased activity in both FAST lines. However, no apparent response to selection for reduced activity in the SLOW lines is evident; in fact, as described above, the locomotor response to ethanol of the SLOW-1 and CON-1 lines has paradoxically been increasing across generations. Heritability estimates for each of the lines determined separately support these characterizations; for the FAST lines $h^2 = 0.14$ and 0.17 , and for the SLOW lines $h^2 = -0.12$ (effectively, 0) and 0.05 , over all 17 generations. Total realized h^2 estimated from the regression of *R* on *S* for the diverging response (i.e., FAST-SLOW) through generation S_{17} was 0.03 for replicate 1 lines and 0.04 for replicate 2 lines (Falconer 1983). These heritabilities are in stark contrast to those calculated after the first generation of selection, which were 0.25 for replicate 1 and 0.36 for replicate 2, supporting the impression of minimal divergence since early in selection. Heritability was also estimated between S_0 and S_5 and between S_6 and S_{17} , since the new protocol was first recruited in the testing of S_6 animals. Values for S_0 - S_5 were $h^2 = 0.06$ and 0.08 for replications 1 and 2, respectively. For generations S_6 - S_{17} , h^2 estimates were 0.06 and 0.07 for the two replications.

Inbreeding and fertility. Inbreeding, estimated from the effective family size as described by Falconer (1983), has increased at the average rate of just under 2% per generation. It has been possible to maintain this rate of inbreeding because we test approximately 70 mice per line per generation, we maintain over nine families per line on the average, and there is relatively low variability in average contribution per family to the next generation. These conditions in a within-family rotational breeding scheme yield effective breeding population sizes which average just over 27 mice per line per generation. Fertility estimates based on average litter size and percentage of fertile mating pairs across generations indicate no inbreeding depression of reproductive fitness.

Detailed characterization of open-field locomotor responses to ethanol in FAST and SLOW mice

Substantial response to selection has been realized in the activity lines, although divergence between the FAST and SLOW lines has proceeded slowly and in spurts. Given this pattern of results, it is important to examine the selection phenotype carefully to be assured that selection is acting to produce animals differing specifically in ethanol-induced stimulation. Alternatively, we could be selecting for differences in habituation to the novel test-

ing environment, and possibly for differences in baseline activity. These factors were examined in experiment 2.

Reasons for a retarded selection response might be:

1. Exhaustion of all available additive genetic variation in the ACT phenotype early in selection so that no more divergence is possible. Heritability estimates seem to support this conclusion, and some of our results which support the role of a single major gene in the control of this phenotype will be discussed.
2. Presence of a signal to noise problem such that there is not enough genetic variability in the phenotype on which to successfully select. There is a great deal of phenotypic variance in the control lines, which should reflect the variability in the foundation population. However, the proportion of this variance that is attributable to genetic factors is not known. If environmental effects were playing a large part in between-animal variation, reliability of the activity measure might be expected to be low. We investigated this possibility in experiment 3 using the selection experiment design, and in experiment 4 using an altered design.
3. Suboptimal choice of ethanol dose. The ethanol dose currently used in the selection experiment (2 g/kg) has been shown to be stimulating in mice (Crabbe et al. 1982, 1988; Dudek and Phillips 1990). We describe a dose-response study in experiment 5 below, indicating that the doses of ethanol which maximally differentiate FAST from SLOW mice are those which have been used in their selection (1.5 and 2.0 g/kg).
4. Suboptimal choice of test time or duration. For example, Crabbe et al. (1988) found that Swiss Webster mice were equally activated by ethanol 6–10 and 10–14 min after injection, but appeared to be less stimulated 2–6 min after injection. Experiments 4, 5 and 6 examined this factor in greater detail in FAST and SLOW mice.

Experiment 2. Habituation to open field testing

Rationale

It has been suggested that differences may exist between FAST and SLOW mice in habituation or reactivity to the open field testing situation independent of response to ethanol. The activity of one line on day 2 may be more influenced by testing experience on day 1 than that of the other line. Importantly, we could be breeding for this difference rather than for differences in ACT. This experiment was designed to assess the habituation of FAST and SLOW mice to our testing procedures.

Materials and methods

Animals. The animals used in experiments 2–6 were from second or later litters of FAST and SLOW mating pairs. They were separated from dam and sire at 21 \pm 1 days of age and housed on corn-cob bedding in same sex groups of two to five animals per polypropylene cage. In some cases, litters of common age, line and replication were

mixed. Food and water were freely available except during testing, ambient temperature was 21 \pm 2° C, and lights were on between 0600 and 1800 hours. The sex of mice used in different experiments was determined, in part, by availability.

Procedures. In this experiment, 80, S₀, 7–14-week-old, female mice, half FAST and half SLOW, were tested. Two SLOW mice were lost due to bad injections, and short supply permitted testing of only replicate two animals. Mice were randomly assigned to one of two groups. Group ES was composed of 20 FAST and 19 SLOW mice that were locomotor activity tested for 4 min, 2–6 min after ethanol (2 g/kg; 20% v/v; IP) on day 1 and after saline on day 2. Group SS also included 20 FAST and 19 SLOW mice that were identically tested except that the mice received saline on 2 consecutive days, no ethanol. Activity monitors were those described for the selection experiment above.

Results

Mean saline and ethanol activity scores for each line and day are presented in Fig. 2. Data were analyzed with ANOVA grouped on Line and Group with Day as a repeated measure. A significant Line \times Group \times Day interaction was present ($F_{1,74} = 12.3$, $P < 0.001$), therefore, selected simple interaction effects were examined (Keppel 1973). The relevant sources from the three-way interaction were the Line \times Day interactions for each group. Changes that occurred from day 1 to day 2 in group SS occurred similarly in FAST and SLOW mice (no Line by Day interaction), while changes occurring in Group ES were line dependent ($P < 0.001$), reflecting the larger stimulant effect of ethanol in FAST mice.

Habituation to the novel testing environment was further assessed in group SS in a separate ANOVA grouped on Line with Day as a repeated measure. FAST and SLOW mice had similar activity scores, but activity levels on day 2 were significantly lower than on day 1 ($F_{1,37} = 13.1$, $P < 0.001$). This Day effect did not interact with line, indicating similar habituation in FAST and SLOW mice.

The stimulant effect of ethanol was examined in a similar analysis for group ES animals. FAST mice were

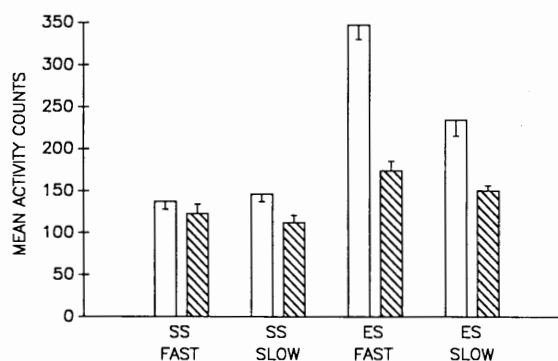


Fig. 2. Mean activity counts on two consecutive days for FAST and SLOW mice tested after saline on both days (group SS), or after ethanol (2 g/kg) on day 1 and saline on day 2 (group ES). Activity was recorded for 4 min beginning 2 min after treatment. SEM are shown. □ Day 1; ▨ day 2

more active than SLOW mice ($F_{1,37} = 23$, $P < 0.001$) and activity was greater on day 1 than on day 2 ($F_{1,37} = 84.3$, $P < 0.001$). Analysis of the significant Line \times Day interaction ($F_{1,37} = 10$, $P < 0.01$) indicated stimulation in both lines that was greater in FAST mice (see Fig. 2). Finally, the ethanol activity test seemed to prevent habituation since the mean saline scores of group ES FAST and SLOW mice were at least as large as those of group SS on day 1.

Experiment 3. Reliability of the selection response

Rationale

The above results suggested that differential habituation is not a major confounding variable in the activity selection program. However, the slightly higher day 2 saline activity scores of ethanol-pretreated mice relative to saline-pretreated animals led us to question measurement and response reliability. State-dependency could account for saline scores in ES animals that resemble those of naive saline-treated mice; ES animals may have reacted to the testing situation as though it had never been experienced because they were in different drug states on days 1 and 2. One might, therefore, predict that animals tested twice with ethanol would exhibit lower scores on the second test day, if part of the activity response to ethanol is determined by reaction to novelty. These issues were investigated by testing the same animals twice using the activity selection procedures. To avoid questions of tolerance/sensitization development, ethanol treatments were separated by 5 days.

Materials and methods

Mice were tested using the current selection protocol, and the equipment described above. Activity testing was in the order ethanol (2 g/kg, 10% w/v, IP) then saline with a 24-h intertest interval. After the second day of testing, animals were placed in clean cages with their original cage-mates and remained undisturbed for 4 days. The ethanol and saline tests were then repeated. Each of these pairs of test scores (ethanol followed by saline) was used to produce an ACT score (ethanol score - saline score) which represented the effect of ethanol on activity. Therefore, two ACT scores were obtained for 25, S_{6-7} , 11-14-week-old, male mice of each line and replicate.

Results

Saline, ethanol and ACT scores were analyzed with repeated measures ANOVA grouped by Line and Replicate, with Day as the repeated measure. There were significant differences between the replicate lines, with replicate 2 animals exhibiting lower baseline activity scores ($F_{1,96} = 6.84$, $P < 0.05$), and larger ACT scores ($F_{1,96} = 3.95$, $P < 0.05$); however, this main effect of replicate did not interact with any other factor. Therefore, data summarized in Fig. 3 are collapsed on replicate.

The lines did not differ in baseline activity. Consistent with experiment 2, habituation to the testing situation

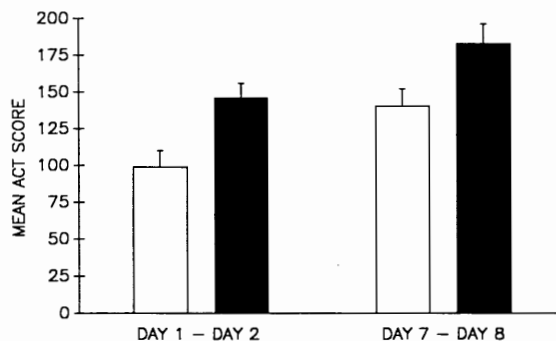


Fig. 3. Mean ACT scores (difference between saline score and ethanol score collected 24 h earlier) for FAST and SLOW mice collected from the same animals in 2 consecutive weeks. Test days are listed in the figure and SEM are shown. □ SLOW; ■ FAST

was indicated by higher saline scores on the first versus the second saline test day (195 versus 148, and 181 versus 146 for SLOW and FAST mice, respectively). The main effect of Day was significant ($F_{1,96} = 47.7$, $P < 0.001$), but did not interact with Line.

On ethanol treatment days, the mean activity of FAST mice was greater than that of SLOW mice ($F_{1,96} = 7.5$, $P < 0.01$). There were no effects of Replicate or Day, or interaction effects. However, when data were analyzed as ACT scores, taking individual differences in baseline activity into account, this pattern of results was slightly altered. As expected, FAST mice were more activated than SLOW mice ($F_{1,96} = 10.4$, $P < 0.002$), and in addition, there was a strong effect of Day on ACT ($F_{1,96} = 22.3$, $P < 0.001$) that was not Line dependent. This effect of Day appeared to be due to sensitization to the stimulant effects of ethanol with repeated testing. However, given the habituation seen on saline treatment days and the similarity of ethanol scores across days within line, this change in ACT is attributed to the reduction in baseline scores rather than to a sensitized response to ethanol.

The correlation between pairs of ACT scores for individual animals was calculated to estimate the reliability of the ACT measure. For all subjects, $r = 0.55$ ($P < 0.001$); for FAST mice $r = 0.55$ ($P < 0.001$); for SLOW $r = 0.49$ ($P < 0.001$). These results suggest that between-subject variation in ACT scores is not simply random; an individual's locomotor response to ethanol is predictable, in part, from previous responses. Importantly, the magnitude of the correlation was similar for the two lines. Correlations between the two ethanol scores indicated that this measure was more reliable (overall $r = 0.69$, FAST $r = 0.71$, SLOW $r = 0.64$; all $ps < 0.001$) than the ACT measure. On the other hand, correlations between saline scores were smaller (overall $r = 0.40$, $P < 0.001$; FAST $r = 0.49$, $P < 0.001$; SLOW $r = 0.33$, NS). In accord with the ANOVA results, this suggests that reactivity to the novel testing situation may play an important role in determining locomotor responses in the non-drugged animal, but may not be so important in the ethanol-treated condition.

Experiment 4. Effects of repeated, long-duration locomotor activity testing

Rationale

Although the activity selection has been successful, maximization of line differences is desirable for the usefulness of this animal model. Differences in biochemical or physiological responses that are genetically correlated with the line difference in ACT would likely be easier to identify if the behavioral difference were larger. Even assuming equal reactivity of FAST and SLOW mice to the testing experience, habituated mice might exhibit an ethanol response less confounded by novelty. In addition, as demonstrated above, ACT scores are likely to be larger in animals that have experienced the testing apparatus in an undrugged state prior to the ethanol/saline test sequence. The length of the activity test was another factor that pilot data indicated might enhance the line difference.

Materials and methods

The activity monitors (Omnitech) used in this experiment were square (40 cm²) with beam interruption sites at 8 points along each side. Ten 12–13-week-old female mice of each line and replicate were tested on 5 consecutive days in the order saline, ethanol, saline, ethanol, saline. The ethanol dose was 2 g/kg (20%, v/v), and animals were tested starting immediately after IP injection for 30 min with data collected in 5-min epochs. Rapid rotational behavior in the home cage was observed in a FAST-1 mouse that elicited extreme activity scores on all test days. The data from this mouse were excluded from further analyses.

Results

Three repeated measures ANOVAs grouped on line and replicate were performed. The first included only saline data with test Day and Time block as repeated measures. The second analysis was performed similarly on ethanol data. The third included two ACT scores per animal created from the following mathematical operations: ethanol score day 2–saline score day 3, and ethanol score day 4–saline score day 5. Data were grouped as described above, and are presented in Fig. 4. In the analysis of ACT scores, there was no main effect of Replicate, or interaction of Replicate with other variables; therefore, data are presented collapsed on replicate.

There was a significant effect of Day on the activity of saline-treated animals ($F_{2,66} = 46.2$, $P < 0.001$). There were no significant interaction effects, so mean comparisons were performed using the Newman-Keuls test which showed that animals were more active on day 1 than on days 3 and 5 ($P < 0.01$), suggesting habituation to the testing environment; activity was similar on days 3 and 5. There was also a significant effect of Time within session on saline activity levels ($F_{5,165} = 66.8$, $P < 0.001$). This effect was similar across days; however, it interacted with Line ($F_{5,165} = 2.4$, $P < 0.05$). Inspection of the time-course data indicated that this interaction was attribut-

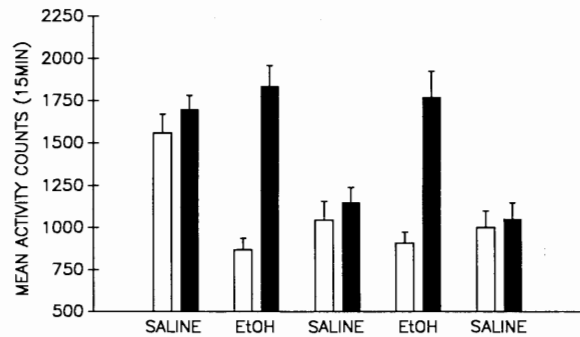


Fig. 4. Mean activity responses on 5 consecutive days for FAST and SLOW mice treated as indicated in the figure. The same mice were repeatedly tested. SEM are shown. *EtOH*, ethanol. □ SLOW; ■ FAST

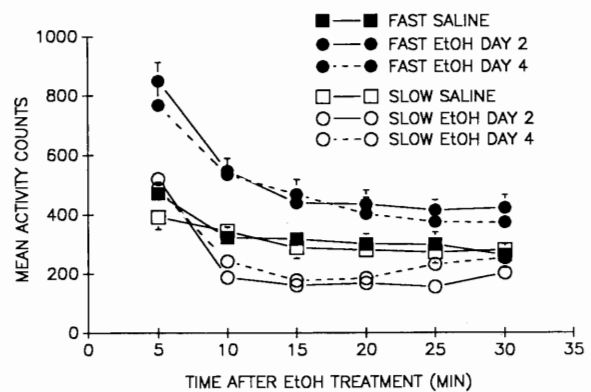


Fig. 5. Locomotor activity time-response curves for FAST and SLOW mice tested on 5 consecutive days. Mice were saline treated on days 1, 3 and 5, and ethanol treated (2 g/kg) on days 2 and 4. Saline values are averages of day 3 and 5 scores. SEM in excess of symbol size are shown. *EtOH*, ethanol.

able to slightly higher activity levels in FAST mice in the first 5 min of testing that then dropped to levels equivalent to those of SLOW mice (see Fig. 5).

FAST mice were much more active on ethanol treatment days than were SLOW mice ($F_{1,34} = 40.3$, $P < 0.001$). There was no difference in activity level between the two ethanol treatment days and there were no significant interaction effects with Day. Activity was strongly dependent on Time within session ($F_{5,170} = 78.4$, $P < 0.001$), and Line interacted with Time ($F_{5,170} = 4.0$, $P < 0.01$). The response patterns of FAST and SLOW mice over time were qualitatively different as can be seen in Fig. 5. The activity of SLOW mice declined in the second 5-min epoch compared to the first, then remained stable. The activity of FAST mice was also reduced in the second 5-min epoch relative to the first, but then continued to decrease, stabilizing after the 15-min time point. At no time were the activity levels of FAST mice as low as those of SLOW animals. In addition, while the activity of ethanol-treated SLOW mice was at or below their saline activity level except during the first 5 min of recording, the activity of FAST mice was stimulated throughout the recording period (see Fig. 5).

Table 1. Correlations among activity scores of FAST and SLOW mice from Experiment 4

	Sal-Day1	EtOH-Day2	Sal-Day3	EtOH-Day4	Sal-Day5	ACT1	ACT2
Sal-Day1	–	0.34	0.42*	0.17	0.35		
EtOH-Day2	0.52*	–	0.10	0.62**	0.01		
Sal-Day3	0.78***	0.31	–	0.28	0.80***		
EtOH-Day4	0.30	0.35	0.38	–	0.45		
Sal-Day5	0.47*	0.16	0.78***	0.52*	–		
ACT1						–	0.73***
ACT2						0.67***	–

All correlations were evaluated with a one-tailed test except those between ethanol and saline scores. Values above the diagonal are correlations for FAST mice; values below the diagonal are correlations for SLOW mice. EtOH, ethanol, SAL, saline. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

As described above, two ACT scores were created per animal; the first saline test day was viewed as an habituation day and not used in the calculation of ACT scores. Data are not presented here as they can be approximated from saline and ethanol data provided in Figs. 4 and 5. Consistent with selective breeding for this character, average ACT values of FAST mice were greater than those of SLOW mice ($F_{1,32} = 29.2$, $P < 0.001$). As for ethanol effects, ACT was time dependent ($F_{5,160} = 16.9$, $P < 0.001$) and the Time effect interacted with Line ($F_{5,160} = 2.4$, $P < 0.05$).

Because we were interested in determining the reliability of activity tests of varying length, correlations were calculated among saline scores, among ethanol scores, and between ACT scores for accumulated time periods. Space restrictions do not permit presentation of all of these correlations. However, correlations between ACT scores were larger for scores accumulated for 15 min than they were for shorter duration tests, and remained relatively stable thereafter. Table 1 presents correlations for the 15 min time point. There were differences between FAST and SLOW mice in the magnitude of these correlations. For example, unlike experiment 3, the activity scores for the 2 ethanol treatment days were not correlated in SLOW mice while they were in FAST mice. This may be attributable to time, since correlations were significant at 5 min for both lines ($r = 0.44$ and 0.54 for SLOW and FAST mice, respectively, $P < 0.05$). This time point is more comparable to the selection testing time, but correlations were still not as large as in experiment 3. Saline scores on days 3 and 5 were highly correlated in both lines, as were the two ACT scores. In contrast to the 15 min correlations, at 5 min, the correlations between the two ACT scores were $r = 0.46$ and 0.62 for SLOW and FAST mice, respectively. This suggests greater reliability of the locomotor stimulant measure for longer duration tests.

Experiment 5. Ethanol dose-response locomotor activity curves of FAST and SLOW mice

Rationale

Between-group designs have some advantages over within-subject designs. Most notably, they remove the com-

plication of interpreting previous testing effects on subsequent behavior. The activity of FAST and SLOW mice has previously been assessed only after two doses of ethanol, 1.5 and 2 g/kg. As mentioned above, one possible reason for limited selection response is suboptimal choice of ethanol dose. This study was designed to investigate the dose-specificity of differences in sensitivity to the stimulant effects of ethanol in FAST and SLOW mice using a between-group design.

Materials and methods

FAST and SLOW mice of both replicates were tested in the circular monitors used in the selection experiment, for 30 min, with data collected in 5-min epochs, beginning immediately after injection. Dose groups were administered 0, 1, 1.5, 2, 2.5, or 3 g/kg ethanol. Ethanol dose was adjusted by changing volume of injection of a 20% (v/v) ethanol in saline solution. Nine to 20 male, S_{8-9} , 6–13-week-old mice were tested per line, replicate and dose. The 3 g/kg ethanol groups were tested after completion of other testing in an effort to obtain biphasic dose-response data. At this time, additional mice were tested in the 0 and 2.5 g/kg dose groups for comparability to earlier data. This was the source of the large group size range.

Results

The initial statistical analysis performed was a repeated measures ANOVA grouped on Line, Replicate, and Dose, with Time as the repeated measure. This analysis showed that FAST mice had higher activity levels than SLOW mice ($F_{1,255} = 50.0$, $P < 0.001$), and activity levels of replicate 1 mice were higher than those of replicate 2 ($F_{1,255} = 14.7$, $P < 0.001$); however, this Replicate effect did not interact with any of the other factors. The dose-response curves of FAST and SLOW mice were different (Line \times Dose $F_{5,255} = 4.0$, $P < 0.01$). These curves for mean cumulative 15 min activity scores are presented in Figs. 6 A and B. We present the 15 min curves because 15 min data were most reliable in experiment 4. However, six separate ANOVAs performed on activity data for successively accumulated time epochs revealed significant Line and Line \times Dose effects in every case. Simple main effect analysis of the Line \times Dose interaction was performed within each replicate. Interestingly, FAST-1 and SLOW-1 mice differed only at the two

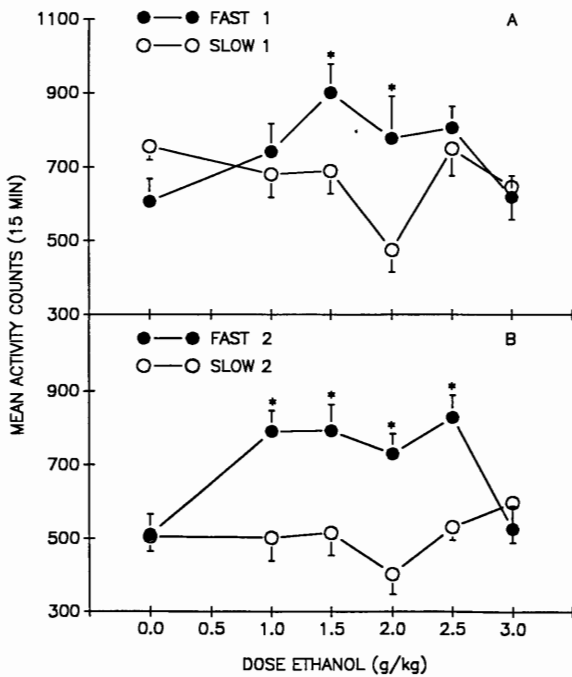


Fig. 6A, B. Locomotor activity ethanol dose-response curves for FAST and SLOW mice of replicate 1 (*panel A*) and replicate 2 (*panel B*). Mice were tested for 15 min beginning immediately after injection. SEM are shown. * $P < 0.05$ for comparison between FAST and SLOW mice of comparable dose group

doses used in their selection. The replicate 2 lines differed across a wider dose range as indicated in Fig. 6B.

The repeated measures ANOVA indicated a significant interaction of Time, Line and Dose ($F_{25,1275} = 2.1$, $P < 0.01$). This 3-way interaction was due to longer lasting stimulation in FAST, relative to SLOW, mice at low doses, and depression of SLOW mice by higher doses at later time points (time-course data not shown).

Experiment 6. Effect of interval after injection on locomotor activity

Rationale

None of the studies described above assessed the importance of interval after ethanol treatment at which testing is initiated to maximization of the locomotor stimulant effect of ethanol. We currently begin testing 2 min after injection for 4 min. In Swiss mice, stimulation appeared to be greater when testing commenced 6 or 10 min after intragastric intubation, possibly due to slower absorption (Crabbe et al. 1988). This experiment examined this time interval factor in FAST and SLOW mice after IP ethanol injection.

Materials and methods

The activity monitors used in this experiment were the same as those used for the activity selection. Experimentally naive, 7–11-week-old,

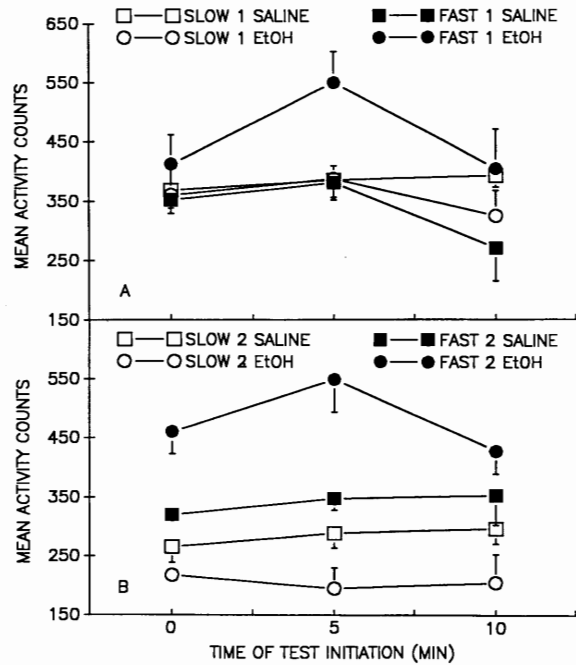


Fig. 7A, B. Mean locomotor activity responses of FAST and SLOW mice of replicate 1 (*panel A*) and replicate 2 (*panel B*) tested for 5 min beginning at time 0, 5, or 10 min after saline or ethanol (2 g/kg) treatment. Each mouse was tested only once, and at only one time point. SEM are shown. *EtOH*, ethanol

S_{14} FAST and SLOW mice of both replicates were tested after IP saline or 2 g/kg ethanol (20% v/v). Three groups of mice ($n = 8$ /line/replicate/group) were tested in each drug condition; one was tested immediately after injection, another 5 min, and another 10 min, after injection. Test duration was 5 min for all mice. Animals of both sexes were included in each group due to inadequate availability of mice of one sex.

Results

Figures 7 A and B show the results at each time point after injection for replicate 1 and 2 mice, respectively. In a 5-way ANOVA assessing activity (Line \times Replicate \times Sex \times Dose \times Time), the main effects of Line, Replicate, and Dose were significant ($F_{s_{1,144}} = 15.0$, 7.6, and 4.6, respectively, $P_s < 0.05$). However, there was no significant effect of Time or Sex. The only significant interaction effect was Line \times Dose ($F_{1,144} = 10.5$, $P < 0.01$). This supports the visual interpretation that FAST mice were activated by ethanol while SLOW mice were slightly depressed (replicate 2) or unaffected (replicate 1). The activity of FAST and SLOW mice after saline treatment was similar at each time after injection with the exception of that of FAST-1 mice which showed lower activity when testing began 10 min after injection. The response of SLOW mice after ethanol treatment was similar across time. However, relative to saline-treated mice, ethanol-treated FAST-2 mice appeared most stimulated at the two earlier test initiation times, and FAST-1 mice when tested 5 min after ethanol.

Discussion

Selective breeding for sensitivity to the stimulant effects of ethanol has been successful in producing replicate FAST and SLOW mouse lines that consistently differ in stimulant response under a variety of conditions. A large response to selection was seen in offspring of the first selected generation in both replicates. This is not an uncommon result in artificial selection experiments for pharmacological responses (e.g. Eriksson and Rusi 1981; McClearn and Kakihana 1981). However, the virtual absence of further significant changes with additional selection in one replicate suggests the major effect of a single gene determining susceptibility to ethanol-induced activation. We have no further direct evidence to support this contention; however, it is interesting that we found a large difference in stimulant response between one replicate pair of HOT and COLD mice, selected for sensitivity to ethanol hypothermia (Crabbe et al. 1989). The originating population for the hypothermia lines was the same as that for the FAST and SLOW lines, and it is tempting to speculate that the major "activating gene" was randomly fixed in the highly-stimulated HOT-2 mice. Gora-Maslak et al. (1990) have used quantitative trait loci analysis of recombinant inbred strain data to identify a number of genes correlated with sensitivity to the locomotor stimulant effects of ethanol. Genes correlated with sensitivity were clustered on chromosomes 2 and 4. Although a major gene effect was not detected, this could be because the particular gene was not present in either the C57BL/6J or DBA/2J genomes that served as parental strains for the recombinant inbreds. Changes in our activity selection testing parameters have led to further response. This could be due to the recruitment of additional genes that affect the stimulant response under the new conditions. Breeding experiments will likely be needed to define the genetic architecture underlying this trait. For example, the FAST and SLOW lines could be used in a Mendelian analysis of ethanol-induced locomotor stimulation to estimate the number of genes influencing stimulant sensitivity. Such an analysis was performed with the Long-Sleep and Short-Sleep mice, bred for sensitivity to ethanol's sedative effects (Dudek and Abbott 1984). FAST and SLOW mice could also be crossed with extreme scoring inbred strains, or crossed among themselves, using both genetic replications, in diallel analyses. Clearly, these biometrical genetic approaches would be better performed after response to selection is complete, and inbred FAST and SLOW strains have been produced.

Heritability estimates indicate that sensitivity to the stimulant effects of ethanol is partly genetically determined, and that response to selection has ostensibly been limited to FAST mice. Heritability estimates were substantial after the first generation of selection, and although these estimates for the first 17 selection generations of the two FAST lines seem small (0.14 and 0.17), they are only slightly smaller than those for other highly successful selection programs (McClearn and Kakihana 1981; Phillips et al. 1990). The continuing response of replicate 2 lines to selection is more apparent than that

of replicate 1 lines. It is possible that the limits of selection have been attained. This in no way undermines the usefulness of these mouse lines. Studies in which we have detected correlated responses to selection serve to provide information about commonalities of drug action, and about convergence of genetic mediation of various ethanol effects (Phillips et al. 1989a,b). For example, FAST and SLOW mice did not differ in hypothermic response to ethanol, or in loss of righting reflex duration after ethanol administration, but they did differ in sensitivity to the ataxic effects of ethanol (FAST more sensitive). They also differed in blood ethanol concentration at regaining of the righting reflex, suggesting a neurosensitivity difference between the lines (Phillips et al. 1989b). However, a recent replication of this experiment showed no difference between the lines (Phillips et al. 1989a). Thus, sedative sensitivity does not seem to be genetically correlated with stimulant sensitivity in these lines, but a correlation between ethanol's stimulant and ataxic effects is apparent.

The reliability of the locomotor activity test suggests that the activity responses to ethanol of these mice are consistent during repeated tests. However, reliability varied somewhat with testing procedure, possibly due to incomplete genetic overlap in mediation of the responses measured using different tests. In general, we believe that the current test time and duration are appropriate for detection of ethanol's stimulant effects, and, therefore, for continued selection. In fact, there is some indication from experiment 6 that testing immediately after ethanol administration may be inadequate for detecting stimulation if the test is short. This problem may be obviated by using tests of longer duration should such designs be favored. One strength of the activity lines is that they differ in response to ethanol under a variety of conditions. We have shown that both within-group and between-group designs can be used to detect the line difference in ethanol sensitivity, that the line difference is detectable in square as well as circular activity monitors, and that tests of different duration can be used.

The ethanol doses (1.5 and 2.0 g/kg) used in the selection of FAST and SLOW mice seem to be those that best differentiate the lines. This may, in fact, be a result of selection. However, in non-selected Swiss Webster mice, these doses were clearly stimulating, with 2.0 g/kg having a larger effect than 1.5 g/kg (Crabbe et al. 1988). Therefore, we feel confident that the slow divergence of FAST and SLOW mice is not due to choice of a suboptimal ethanol dose.

In conclusion, FAST and SLOW mice should provide a good tool for mechanistic examinations of ethanol's stimulant effects. For example, we plan to begin using neurotransmitter receptor specific drugs, beginning with dopaminergic agents, to investigate the roles of specific transmitters in determining sensitivity to ethanol's stimulant effects. This approach, using mice of other genotypes, has provided evidence for the involvement of dopamine in this response (e.g., Strombom et al. 1977; Friedman et al. 1980; Dudek et al. 1984), encouraging further investigation. Whether or not FAST and SLOW mice will provide a good animal model for

ethanol's euphoric effects remains to be determined. One way in which we plan to examine this is by measuring intravenous ethanol self-administration in these animals. If propensity to self-administer ethanol correlates with susceptibility to stimulation, this will lend some support to the hypothesis that ethanol stimulation reflects ethanol reinforcement.

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References

- Ahlenius S, Carlsson A, Engel J, Svensson TH, Sodersten P (1973) Antagonism by alpha-methyltyrosine of the ethanol-induced stimulation and euphoria in man. *Clin Pharmacol Ther* 14:586-591
- Ahlenius S, Brown R, Engel J, Svensson TH, Waldeck B (1974) Antagonism by nialamide of the ethanol-induced locomotor stimulation in mice. *J Neural Transm* 35:175-178
- Babor TF, Berglas S, Mendelson JH, Ellingboe J, Miller K (1983) Alcohol, affect, and the disinhibition of verbal behavior. *Psychopharmacology* 80:53-60
- Bijerot N (1980) Addiction to pleasure: a biological and social-psychological theory of addiction. In: Lettieri DJ, Sayers M, Pearson HW (eds) *Theories on drug abuse: selected contemporary perspectives*. National Institute on Drug Abuse, Rockville, pp 246-255
- Crabbe JC (1986) Genetic differences in locomotor activation in mice. *Pharmacol Biochem Behav* 25:289-292
- Crabbe JC, Johnson NA, Gray DK, Kosobud A, Young ER (1982) 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
- Crabbe JC, Kosobud A, Tam BR, Young ER, Deutsch CM (1987a) Genetic selection of mouse lines sensitive (COLD) and resistant (HOT) to acute ethanol hypothermia. *Alcohol Drug Res* 7:163-174
- Crabbe JC, Young ER, Deutsch CM, Tam BR, Kosobud A (1987b) Mice genetically selected for differences in open-field activity after ethanol. *Pharmacol Biochem Behav* 27:577-581
- Crabbe JC, Deutsch CM, Tam BR, Young ER (1988) Environmental variables differentially affect ethanol-stimulated activity in selectively bred mouse lines. *Psychopharmacology* 95:103-108
- Crabbe JC, Kosobud A, Feller DJ, Phillips TJ (1989) Use of selectively bred mouse lines to study genetically correlated traits related to alcohol. In: Kuriyama K, Takada A, Ishii H (eds) *Biomedical and social aspects of alcohol and alcoholism*. *Excerpta Medica*, Elsevier, Amsterdam, pp 427-430
- Crabbe JC, Phillips TJ, Kosobud A, Belknap JK (1990) Estimation of genetic correlation: interpretation of experiments using selectively bred and inbred animals. *Alcoholism: Clin Exp Res* 14:141-151
- Dudek BC, Abbott ME (1984) A biometrical genetic analysis of ethanol response in selectively bred Long-Sleep and Short-Sleep mice. *Behav Genet* 14:1-19
- Dudek BC, Phillips TJ (1990) Distinctions among sedative, disinhibitory, and ataxic properties of ethanol in inbred and selectively bred mice. *Psychopharmacology* 101:93-99
- Dudek BC, Abbot ME, Garg A, Phillips TJ (1984) Apomorphine effects on behavioral response to ethanol in mice selectively bred for differential sensitivity to ethanol. *Pharmacol Biochem Behav* 20:91-94
- Eriksson K, Rusi M (1981) Finnish selection studies on alcohol-related behaviors: general outline. In: McClearn GE, Deitrich RA, Erwin VG (eds) *Development of animal models as pharmacogenetic tools*. US Government Printing Office, Washington, pp 87-117
- Falconer DS (1983) *Introduction to quantitative genetics*, 2nd edn. Longman, New York
- Friedman HJ, Carpenter JA, Lester D, Randall CL (1980) Effect of alpha-methyl-*p*-tyrosine on dose-dependent mouse strain differences in locomotor activity after ethanol. *J Stud Alcohol* 41:1-7
- Gora-Maslak G, McClearn GE, Crabbe JC, Phillips TJ, Belknap JK, Plomin R (1991) Use of recombinant inbred strains to identify quantitative trait loci in psychopharmacology. *Psychopharmacology* (in press)
- Keppel G (1973) *Design and analysis: a researcher's handbook*. Prentice-Hall, Englewood Cliffs, New Jersey
- Lister RG (1987) The effects of ethanol on exploration in DBA/2 and C57BL/6 mice. *Alcohol* 4:17-19
- McAuliffe WE, Gordon RA (1974) A test of Lindesmith's theory of addiction: the frequency of euphoria among long-term addicts. *Am J Sociol* 79:795-840
- McClearn GE, Kakihana R (1981) Selective breeding for ethanol sensitivity: Short-Sleep and Long-Sleep mice. In: McClearn GE, Deitrich RA, Erwin VG (eds) *Development of animal models as pharmacogenetic tools*. US Government Printing Office, Washington, pp 147-159
- Phillips TJ, Feller DJ, Crabbe JC (1989a) Selected mouse lines, alcohol and behavior. *Experientia* 45:805-827
- Phillips TJ, Limm M, Crabbe JC (1989b) Correlated behavioral responses as potential predictors of sensitivity to ethanol's rewarding effects and addiction liability. In: Kiiianmaa K, Tabakoff B, Saito T (eds) *Genetic aspects of alcoholism*. The Finnish Foundation for Alcohol Studies, Helsinki, pp 197-206
- Phillips TJ, Terdal ES, Crabbe JC (1990) Response to selection for sensitivity to ethanol hypothermia: genetic analyses. *Behav Genet* 20:473-480
- Randall CL, Carpenter JA, Lester D, Friedman HJ (1975) Ethanol-induced mouse strain differences in locomotor activity. *Pharmacol Biochem Behav* 3:533-535
- Strombom U, Svensson T, Carlsson A (1977) Antagonism of ethanol's central stimulation in mice by small doses of catecholamine-receptor agonists. *Psychopharmacology* 51:293-299
- Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. *Psychol Rev* 94:469-492