# INITIAL SENSITIVITY AND TOLERANCE TO THE ATAXIC EFFECTS OF ETHANOL IN SELECTIVELY BRED HOT AND COLD MICE

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## ABSTRACT

Positive correlations between ethanol (EtOH)-induced hypothermia and ataxia in inbred strains of mice have been demonstrated suggesting that these responses may share common genetic mechanisms. On the basis of these findings and studies of selected lines of rats and mice, the following experiments were performed to determine if mice selected for sensitivity or resistance to EtOH-induced hypothermia would show differences in response to EtOH-induced ataxia. The sensitive line (COLD) was predicted to be more sensitive to EtOH-induced ataxia than the hypothermia-insensitive line (HOT), and to develop tolerance to the ataxic effects of EtOH.

Each replicate line of HOT (HOT1, HOT2) and COLD (COLD1, COLD2) mice was tested for EtOH-induced ataxia. Ataxia was measured by the use of four behavioral tasks: screen test, fixed-speed rotarod, accelerating rotarod and grid test. The screen test and fixed speed rotarod were used to measure initial sensitivity. The accelerating rotarod and grid test were used to measure both sensitivity and tolerance. The grid test also provided a measure of locomotor activity. Overall, the results suggested that HOT and COLD mice were equally sensitive to the ataxic effects of ethanol.

The screen test yielded a dose-dependent decrease in ability to remain on the screen that did not differ between the selected lines. Based on this dose-response effect, 2.5 g/kg EtOH was used as the test dose for the remaining experiments. The onset to ataxia, measured on a fixed-speed rotarod (5 rpm), did not differ in the selected lines; neither latency to fall from the rotarod nor brain ethanol concentration at this time differed between HOT and COLD mice. The third test of ataxia, the grid test, also did not detect any significant differences in sensitivity. HOT and COLD mice had equal ratios of errors to activity counts as measured by the grid test.

The accelerating rotarod was the only test of ataxia which resulted in a differential response in HOT and COLD mice. COLD mice were impaired by the acute ethanol injection as indicated by the decrease in performance compared to their baseline; HOT mice, however, were unaffected. A similar decrease in performance of COLD mice was observed for locomotor activity, measured in the grid test, but the activity of HOT mice was unchanged.

The development of tolerance to ataxia differed between Replicates 1 and 2. The accelerating rotarod indicated that tolerance developed in HOT1 and COLD1 mice but not in HOT2 and COLD2 mice. The grid test revealed no differences in the extent of tolerance development between HOT1 and COLD1 but the magnitude of tolerance in HOT2 exceeded that in COLD2. After multiple exposures to ethanol, COLD mice became tolerant to the locomotor depressant effects of ethanol whereas HOT mice sensitized to the stimulant effect. Blood ethanol concentrations were not different between HOT and COLD mice and therefore cannot explain the difference in sensitivity or tolerance.

In general, the results of all four experiments indicated that HOT and COLD mice were not differentially sensitive to EtOH-induced ataxia or to the development of tolerance to EtOH-induced ataxia. However, since the four tests of ataxia did not yield the same result in terms of differential sensitivity to ataxia in HOT and COLD mice, it is possible that the tasks were not measuring the same behavior. Since the pattern of results from the accelerating rotarod was similar to the locomotor activity in the grid test, results on the accelerating rotarod may have been influenced by sensitivity differences in locomotor activity as well as ataxia. The results suggest that the genetic components underlying sensitivity to ethanol-induced hypothermia are not involved to any significant degree in ethanol-induced ataxia.

# INTRODUCTION

Alcoholism and alcohol abuse are prevalent problems in today's society. They pose social, financial, medical and family problems as well as being major contributors to deaths from accidents (Alcohol and Health, 1993). It is evident that genetics and environment influence the development of alcohol abuse but the extent to which each factor alone affects the outcome is unknown. Results from twin and adoptee studies (Goodwin et al, 1973; Schuckit, Goodwin, and Winokur, 1972) report that children of alcoholics are at a greater risk of abusing alcohol relative to children who are not related to an alcoholic, suggesting that there are genetic factors subserving alcoholism. Environmental influences on the development of alcoholism, such as social and psychological factors, are acknowledged as well (Hesselbrock and Hesselbrock, 1990; Cappell and Greely, 1987). The interaction of genes and environment adds to the complexity of determining inheritance of this syndrome (Cloninger, Sigvardsson, and Bohman, 1988; Cloninger, Bohman, and Sigvardsson, 1981).

The causes of alcoholism are unknown, but the development of physical dependence on, and tolerance to, ethanol probably play a role. Tolerance can develop to many, but not necessarily all, responses to ethanol. In an attempt to understand mechanisms contributing to alcohol abuse, animal models have been developed and utilized to measure various responses to ethanol. Additionally, mice and rats are used to study genetic and environmental factors related to alcohol intoxication.

## *Responses to Ethanol*

Ethanol has a wide range of effects when administered acutely. In general, ethanol is classified as a central nervous system (CNS) depressant, although the severity of a given effect depends on the dose of ethanol administered. Some of the most profound behavioral effects are alterations in

locomotor activity, which encompasses both stimulant and depressant responses, and locomotor incoordination, also referred to as ataxia. Ethanol produces changes in physiological effects as well, most notably a reduction in core body temperature. Each of these is described in more detail below.

Ataxia

An acute ethanol administration produces profound alterations in motor coordination in humans and laboratory animals. These effects are produced in a dose-dependent fashion. Doses of ethanol as low as 2 g ethanol/kg body weight or 0.03 - 0.25 g/100 ml blood result in ataxia (Garriott, 1988). Goldberg (1943) established various methods for determining ethanol intoxication in humans based on motor incoordination, which have proven to be reliable in assessing the degree of intoxication when the results were correlated with blood alcohol content. In mice and rats, ataxia has been easily measured using a variety of methods such as the moving-belt task (Arvola, Sammalisto, and Wallgren, 1958), rotarod (Jones and Roberts, 1967), or the grid test (Belknap, 1975). The rotarod test employs a rod which rotates at a set velocity or acceleration and ataxia is indexed as the latency to fall from the rotating rod. In the grid test apparatus, mice ambulate around an enclosed area atop a grid floor. As a foot slips through the grid, it is counted as a misstep or error; ataxia is quantified as the ratio of missteps to locomotor activity counts. These two tasks are described in more detail under "General Methods". The moving belt test requires the animal to remain on a treadmill to avoid an electric shock that results when the animal falls off the belt. Measurement of ataxia is relatively easy and straightforward using these tasks; typically, the latency to lose coordination or the degree of incoordination as compared to untreated performance is quantified. The importance of investigating the locomotor incoordinating effects of ethanol are best summed up by Deitrich (1990): "...it is

the ataxic, incoordinating effects [of ethanol] that lead to many of the serious consequences such as automobile and industrial accidents, suicide and social and legal problems."

Locomotor Activity

Although ethanol is usually considered to be a CNS depressant, acute ethanol administration produces dose-dependent and time-dependent biphasic responses. At low doses ethanol is considered to have stimulant properties, while at high doses the depressant effects are observed. Buckalew and Cartwright (1968) demonstrated the dose-dependent biphasic response to ethanol in rats for a variety of behavioral responses including exploratory activity. When locomotor activity was measured in Swiss-Webster mice after an acute dose of ethanol, a rapid onset to the stimulant effects resulted that decreased over time (Read, Cutting, and Furst, 1960). The direction of this biphasic response to ethanol is also dose-dependent (Dudek and Phillips, 1990; Masur and Boerngen, 1980). With a large dose of ethanol, the biphasic response may emerge as a consequence of the temporal factors involved in ethanol absorption. For example, a decrease or increase in activity, which may be the initial observation, is followed in time by the opposite response. For example, an initial decrease in activity was observed after injection of 2 g/kg ethanol in mice and followed by an increase in activity (Matchett and Erickson, 1977). In general, the stimulatory effect of ethanol has been observed in mice and depressant effect has been observed in rats, however, there is a strong influence of dose on these effects.

The apparent differences in species are even more pronounced when responses to chronic ethanol exposure are examined. Masur, de Souza, and Zwicker (1986) showed that chronic injection of ethanol to mice and rats resulted in an enhancement of stimulated locomotor activity in mice, but no

changes in activity in rats. This behavioral sensitization, or increase in ethanol-stimulated activity, has been observed in mice (Phillips, Dickinson, and Burkhart-Kasch, 1994; Cunningham and Noble, 1992; Masur and Boerngen, 1980).

In addition to the species differences and dose- and time-dependent factors that affect the expression of locomotor responding to ethanol, genetic aspects influence locomotor activity. This topic will be discussed in a later section.

Hypothermia

A reduction in core body temperature after acute administration of ethanol illustrates an additional CNS depressant effect of ethanol. Hypothermia is an easily quantifiable physiological response to ethanol in humans and rodents (Kalant and Lê, 1984; Lomax, Bajorek, Chesarek and Chaffee, 1980; Freund , 1973). It is usually measured by taking rectal temperatures or by radio telemetry (Gallaher, Egner, and Swen, 1985). The hypothermic response is dependent on concentration of ethanol (Linakis and Cunningham, 1979) and dose (Ritzmann and Tabakoff, 1976), such that a larger decrease in body temperature results with increasing doses of ethanol. The hypothermic response to ethanol can be influenced by alterations in ambient temperature during exposure to ethanol (Grieve and Littleton, 1979b). Hypothermia is attenuated in mice (Malcolm and Alkana, 1981) and rats (Pohorecky and Rizek, 1981) at ambient temperatures warmer than room temperature and the hypothermic response is increased when mice are exposed to extreme cold temperatures. It has also been suggested that body temperature influences the extent of the expression of sensitivity to the sedative effects of ethanol (Alkana, Finn, Galleisky, Bejanian, Boone, Jones, and Syapin, 1985; Alkana, Boone and Finn, 1985). A decrease in body temperature as a result of an acute ethanol

administration is postulated to lead to a decrease in the temperature of cell membranes, reducing the effect of ethanol on the cell and resulting in decreased sensitivity to the effects of ethanol. Sensitivity to ethanol was determined by measuring latencies to regain righting reflex (sleep-time) and blood and brain ethanol concentrations at wakeup in mice exposed to various ambient temperatures (Alkana et al., 1985). The results indicated that as body temperature increased, the mice became more sensitive to the depressant effects of ethanol (i.e., mice had longer sleep times).

***Tolerance to Ethanol***

Tolerance is a phenomenon known to develop after repeated exposure to a drug. Tolerance is defined as a shift to the right in the dose-response curve; that is, after repeated drug exposure it will take more drug to achieve the same effect, or the same dose will result in a smaller effect than initially observed (Kalant, LeBlanc, and Gibbins, 1971). A change in the response of an organ or tissue as a function of repeated ethanol exposure is described as functional tolerance. Basically, this means that the sensitivity of the tissue has been altered in some way resulting in an attenuation of the initial response to the same amount of drug. A quantitative measurement of intoxication, blood ethanol concentration (BEC), can been used as an indicator of functional tolerance to ethanol (for review, see Kalant, LeBlanc, and Gibbins, 1971). Using this marker, tolerance is characterized by a higher concentration of blood ethanol at a given point (or effect) as compared to the initial response. For example, a mouse that has been made tolerant will recover a particular behavior or physiological function at a higher BEC than when the response was first measured.

Another consequence of repeated drug administration is the development of metabolic tolerance. This refers to a changes in the effective

amount of drug at the target site, including distribution, excretion, and rate metabolism. Monitoring BEC during exposure is a reliable method for determining the development of metabolic tolerance. Since this is not the desired cause of improved performance after a drug exposure, BECs were measured in the present experiments to rule out this effect.

The duration and magnitude of tolerance are affected by the test dose, measurement system, and schedule of ethanol administration. The development of chronic tolerance to motor incoordination, hypothermia and inhibition of startle response was measured in Sprague-Dawley rats (Pohorecky, Brick and Carpenter, 1986). In general, at least some tolerance developed after five exposures for all measures, but the rate at which maximal tolerance developed differed according to the response measured. For hypothermia, maximal tolerance developed by the ninth day. Maximal tolerance was not reached until the 17th day for motor incoordination, and no tolerance developed to the ethanol-induced suppression of the startle response. Lê, Khanna, and Kalant (1984) demonstrated the effect of treatment dose of ethanol on the development of tolerance to the motor-impairing and hypothermic effects of ethanol. Groups of rats were given one of five doses of ethanol daily and tested weekly. Maximal tolerance to the motor impairment developed to doses of ethanol < 4 g/kg by the second week; no tolerance developed to the high doses. Tolerance to hypothermia developed to all treatment doses (2, 4, and 6 g/kg) although maximal tolerance was reached at slightly different times. Thus, when measuring tolerance to various effects of ethanol, it is important to keep the factors of dose, schedule and response in mind.

Tolerance can be measured within a single ethanol administration or after multiple, or prolonged, ethanol exposures, giving rise to the further

classification of functional tolerance subtypes. There are three fundamental distinctions made: acute, chronic and rapid tolerance. The defining characteristics for these classifications are vague. Historically, tolerance was thought to develop only as a result of prolonged exposure to ethanol and consequently to develop slowly over the exposure period. However, it is now accepted that tolerance can develop rapidly, even during a single ethanol exposure. Acute tolerance is measured within a single exposure; chronic tolerance develops after repeated, or prolonged, ethanol administration (usually for several days to weeks or longer). Rapid tolerance is measured after a second (challenge) dose; the time between ethanol exposures is usually less than 24 hrs.

Acute tolerance can be defined as the development of tolerance during a single exposure to ethanol (Kalant, LeBlanc, and Gibbins, 1971) and represents the decrease in sensitivity when the response is measured on the descending versus the ascending arm of the ethanol absorption/elimination curve. Mellanby (1919) first described this type of tolerance when he noticed that treadmill performance in dogs was better when measured during the descending arm of the blood ethanol curve than during the ascending arm during the same drug exposure. Thus, BECs at recovery were higher than BECs at the onset of impairment. Another method for determining acute tolerance is to compare BECs at recovery between doses. If animals that are given high doses of ethanol recover at higher BECs than animals given low doses, then acute tolerance is said to have developed (this equals a shift to the right of the dose response curve). Acute tolerance to ataxia was demonstrated by Gallaher, Parsons, and Goldstein (1982). Groups of mice were given increasing doses of ethanol then tested on a static dowel until the recovery of ataxia at which time brain ethanol concentration (BrEC) was determined. The

results showed that mice given the highest doses of ethanol recovered at higher BrEC than mice receiving lower doses. Both of these studies reported recoveries at higher tissue ethanol concentrations with increasing ethanol doses.

Rapid tolerance to the hypothermic and ataxic effects of ethanol has been described by various groups (Grieve and Littleton, 1979a; Crabbe, Rigter, Uijlen, and Strijbos, 1979; Gallaher, Parsons, and Goldstein, 1982). Grieve and Littleton (1979a) used forced ethanol vapor inhalation to render mice tolerant. In this study, mice were initially given an i.p. injection of ethanol and then exposed to ethanol vapors until the behavioral endpoint was reached (loss of righting reflex [LORR] or loss of balance on a rotarod). BECs were measured at this time. The mice were then returned to the chambers at recovery. The development of tolerance was determined by the increase in BEC at each subsequent recovery point. A different, but related, paradigm for measuring rapid tolerance to ataxia was employed by Gallaher, Parsons, and Goldstein (1982). Mice were given an initial injection of ethanol and tested on a rotarod until recovery, at which point a blood sample was taken and a second injection and test were given. This was repeated for five cycles. Mice recovered at increasing BEC values, indicating that rapid tolerance developed. Despite the differences in methods between these two studies, it was clear that tolerance developed in a much shorter time period than previously thought. Crabbe et al. (1979) observed that tolerance developed to the hypothermic response to ethanol was present after a second injection, 24 hours after the initial exposure to ethanol. Tolerance development is not limited to behavioral responses to ethanol. When Purkinje cells of the cerebellum were exposed to ethanol in vitro, depression of firing rate occurred, but was gone when a second dose was

administered approximately 5 min. later (Palmer, Pearson, Donatelli, and Freund, 1994). These authors have described this as rapid acute tolerance.

Chronic tolerance is typically measured after multiple exposures to ethanol that have been given over a prolonged period of time. There are several methods employed for administering ethanol chronically including constant, prolonged exposure to ethanol via forced ethanol vapor inhalation, and through liquid diets or daily repeated injections. The effect of injections and liquid diet may be different from the effect of administering ethanol through forced inhalation. In the latter case, ethanol is always present and the end result is a fairly constant BEC level during the exposure period. Injections or oral consumption may allow the animal to recover between administrations. Since the exposure period to ethanol of all these methods encompasses a longer time than does one or two injections, these methods of intoxication are considered to produce chronic tolerance.

Chronic administration is a useful method for the induction of tolerance and subsequent determination of the acquisition, duration and persistence of tolerance (Lê, Mihic, and Wu, 1992). Chronic tolerance to the sedative effects of ethanol has been investigated by numerous groups using both genetically heterogeneous and selectively bred animals (Grant, Werner, Hoffman, and Tabakoff, 1989; San-Marina, Khanna, and Kalant 1989). Grant et al (1989) exposed rats to ethanol vapors 14 hr/day for 8 days via forced inhalation. BECs were taken daily; an increase in BEC was achieved over time as the concentration of ethanol in the chambers was increased. At the termination of exposure, rats were tested until they regained aerial righting reflex (ARR) and BEC was measured. The results indicated that tolerance developed in some rats; that is, the initial dose of ethanol used to produce loss of ARR was insufficient to produce the same effect after chronic vapor inhalation.

These three subtypes of tolerance can be distinguished, to an extent, through the use of neurochemical manipulations. MK-801 blocks rapid and chronic ethanol tolerance. When MK-801 and ethanol were chronically administered to rats for 14 days, no tolerance developed to ethanol-impaired performance on the moving belt task, whereas tolerance developed in the group treated only with ethanol (Wu, Mihic, Lui, Lê, and Kalant, 1993). A similar effect was observed when rats were treated on day 1 with MK-801 and ethanol and tested with ethanol 24 hours later: MK-801 attenuated rapid tolerance to the hypothermic and motor impairment effects of ethanol (Khanna, Wu, Weiner, and Kalant, 1991b). These results suggest that similar mechanisms may be involved in the development of rapid and chronic tolerance. It has been suggested that rapid tolerance can be used as an index of chronic tolerance (Khanna, Kalant, Shah, and Weiner, 1991a). Acute tolerance appears to be governed by different mechanisms. The development of acute tolerance was not affected by the NMDA antagonist, ketamine (Khanna, Kalant, Weiner, Chau, and Shah, 1992). These examples suggest that it may be possible to distinguish among types of tolerance on a neurochemical level.

### *Relationship Between Initial Sensitivity and Tolerance*

Sensitivity has been used to predict the extent of tolerance development (Kalant, LeBlanc, and Gibbins, 1971). According to this prediction, the greater the initial impairment, the greater the amount of tolerance produced. Indeed, hypothermia sensitivity was found to correlate positively with hypothermia tolerance, suggesting that the degree of initial hypothermia sensitivity is associated with the subsequent degree of hypothermia tolerance in mice and rats (Crabbe, Gray, Young, Janowsky, and Rigter, 1981; Khanna, San-Marina, Kalant and Lê, 1989). Grant et al. (1989) showed that rats that were more sensitive to ethanol, as measured by lower BEC at loss of ARR, developed

chronic tolerance whereas rats that were less sensitive did not develop as much tolerance.

***Genetic Animal Models***

Inbred and selectively bred rat and mouse strains have proven to be useful tools for investigating the genetic determinants of ethanol sensitivity. Inbred strains, in which brother-sister matings each generation eventually produce a strain in which all same-sex members are essentially identical twins, can be used to estimate the amount of genetic and environmental influence on a particular behavior. Since all members of an inbred strain are genetically identical, differences among members of a single strain when tested for a particular ethanol response are indicative of environmental influences. An estimate of genetic influences is provided by comparing responses between various inbred strains under identical environmental conditions. The ability to detect differences in responses to an acute ethanol administration between inbred strains of mice or rats suggests there are genetic factors underlying ethanol sensitivity if between-strain differences are significantly greater than within-strain differences.

Testing inbred strains of mice for ethanol-induced locomotor activity led to varying degrees of activity according to strain (Crabbe, Gallaher, Phillips, and Belknap, 1994; Dudek, Phillips, and Hahn, 1991; Randall, Carpenter, Lester, and Friedman, 1975). At a given dose of ethanol, some strains were hyperactive whereas others were hypoactive, relative to baseline locomotor activity. C57BL/6 and DBA/2 inbred strains of mice have been shown to respond differentially to the stimulant effects of ethanol (Tritto and Dudek, 1994; Dudek and Phillips, 1990). DBA/2 were stimulated by ethanol while C57BL/6 were relatively unaffected. A genetic influence on hypothermia sensitivity was provided by studies on inbred mice (Moore and Kakihana, 1978; Crabbe et al.,

1982). Significant strain differences in rectal temperature after 3 g/kg ethanol were observed in several inbred strains of mice. The development of tolerance to hypothermia is also influenced by genotype. Crabbe, Janowsky, Young, Kosobud, Stack, and Rigter (1982) distinguished three effects of repeated ethanol administration in several inbred strains of mice. One group of strains became progressively tolerant, a second group showed no tolerance development and a third also showed no development of tolerance but did reveal increasing baseline temperatures with repeated ethanol exposure.

Selectively bred animals are another model used for investigating the genetics of ethanol sensitivity. The goal of selective breeding is to target a specific phenotype and enrich a population for that character. This is achieved by scoring animals for a particular trait (e.g., a response to ethanol treatment), then mating animals with similar responses to one another. That is, low-responders are mated with other low-responders and high-responders with other high-responders to produce the next generation. As this mating system proceeds, two lines divergent for the response are produced. The amount of inbreeding in selected lines is minimized by avoiding intrafamily matings. By mating nonfamilial animals together, only genes related to the phenotype should be forced to homozygosity while maintaining random assortment of all nonselected genes. This practice reduces the influence trait-irrelevant genes will have on the selected trait through chance fixation. An additional control for environmental effects is provided through replicated lines; that is, the generation of identical phenotypes using the same selection process, so that there are two high-responding lines and two low-responding lines. This procedure has been successful in producing several lines of animals selected for sensitivity to ethanol. These include Long-Sleep (LS) and Short-Sleep (SS) mice, that have been selectively bred for their sensitivity to the hypnotic effects

of ethanol (McClearn and Kakihana, 1981). FAST and SLOW mice have been selected for differential sensitivity to the activational effects of ethanol (Phillips, Burkhart-Kasch, Terdal, and Crabbe, 1991). HOT and COLD mice were selected for resistance or sensitivity to the hypothermic response to ethanol (Crabbe, Kosobud, Tam, Young, and Deutsch, 1987a). A few rat lines have been selected for responses to ethanol such as Alcohol-Tolerant (AT) and -Nontolerant (ANT) lines of rats, selectively bred for their sensitivity (ANT) or resistance (AT) to ethanol-induced motor incoordination (Eriksson, 1990). These selected lines and others are discussed in more detail below.

Use of Genetic Animal Models to Assess Correlated Responses

It is of interest to determine if two responses to ethanol are genetically related. To do this, two responses are measured in genetically defined populations and the degree of association, or correlation, between the responses is determined, yielding information about genetic and environmental influences. The importance of determining such correlations is to gain information regarding the mechanism of action of ethanol. Correlated responses (CR) refer to responses that are under similar genetic influence. Both selected lines and inbred strains can be used to measure CR to ethanol and provide evidence for a genetic association between two phenotypes. To determine CR in selected lines, a response to ethanol, which has not been selected, is measured. Using analysis of variance (ANOVA), differences between the selected lines on the nonselected trait can be determined. In this manner, the absence or presence of a genetic correlation is detected from the results of ANOVA. Significant main effect of selected line, observed in both replicated lines, provides strong evidence for interpreting the responses as CR (Crabbe, Phillips, Kosobud, and Belknap, 1990b). Using selected lines for detecting genetic correlations yields an estimate of genetic as well as

environmental influences since these lines are not specifically inbred. However, if mice are raised and tested under exactly the same environmental conditions, the influence of environmental effects is reduced in the estimation of correlations (Crabbe et al., 1990b). The inclusion of replicated lines acts as a control for spurious environmental effects; if two responses are found to correlate in both pairs of selected lines then one can assume that the correlation is real. If the correlation is found in one replicate only, then more caution needs to be taken when interpreting the data (Crabbe et al., 1990b).

By measuring responses to ethanol in several inbred strains, it is possible to estimate the extent of genetic correlation between pairs of traits. Thus, in a manner similar to that used for selected lines, two or more traits are measured in inbred strains and the strain means for each trait are correlated. An advantage to using inbred strains is that the same mouse does not have to be tested for both responses. Assuming sources of environmental variance have been kept constant while testing, a significant correlation suggests that the responses are genetically related. This relationship can be positive, suggesting that common genetic factors are producing a increase in the magnitude of each response, or negative, suggesting that the genetic factors act ot produce an increased effecot of one response but a decreased effect of the second response. A nonsignificant correlation indicates that the responses are genetically unrelated, assuming the appropriate number of strains has been used in order to detect a significant result.

Using inbred strains of mice, Crabbe and colleagues (1982, 1983) estimated genetic correlations for a variety of responses related to sensitivity and tolerance to ethanol, including locomotor activity, hypothermia, LORR, and ataxia. A positive correlation between ataxia sensitivity and hypothermia sensitivity was found (Crabbe, 1983): baseline temperature was correlated with

both the hypothermic response and the ataxic response to ethanol, suggesting that there are common genetic factors regulating ethanol-induced hypothermia and ataxia. A positive correlation between the initial hypothermic response and tolerance to hypothermia was found using inbred strains (Crabbe et al., 1982), a finding consistent with reported phenotypic correlations (Crabbe et al., 1981). This suggests that there are common genes influencing the expression of both these traits. On the other hand, open-field activity did not correlate with hypothermia, ataxia or loss of righting reflex. These suggestions of positive correlations in inbred strains led to the studies performed in this thesis.

Studies of correlated responses to selection are briefly described below, focusing on the ataxia, hypothermic, and activational responses to ethanol. Information is divided into sections according to the selected trait. These studies provide additional information to the results of experiments conducted with inbred lines. Because HOT and COLD mice were chosen as the model for hypothermia sensitivity, a review of studies of HOT and COLD mice are presented in a separate section.

Ataxia

Successful selection for an ataxia phenotype in rats and mice signifies that there are heritable components predisposing the animal to ethanol-induced incoordination. The Alcohol-Tolerant (AT) and -Nontolerant (ANT) lines of rats have been selectively bred for their sensitivity (ANT) or resistance (AT) to ethanol-induced motor incoordination using the tilting-plane test, and secondarily, the rotarod test (Eriksson, 1990). The same results from selection with the tilt-plane were observed using the accelerating rotarod, i.e., ANT rats were more sensitive than AT rats. The tilt-plane test involves placing a rat on a slightly roughened platform that can then be tilted forcing the rat to slide off. The change in angle at time of fall between baseline and drug treatment indicates

intoxication. No significant differences in acute tolerance to motor impairment, measured on the tilt-plane, were detected (Eriksson, Deitrich, Rusi, Clay, and Petersen, 1982). When AT and ANT rats were tested for ethanol-induced hypothermia or LORR no differences in initial sensitivity were apparent (Eriksson and Sarviharju, 1984; Lê and Kiianmaa, 1989), but these lines differed in tolerance development to LORR (Lê and Kiianmaa, 1989). AT and ANT rats did not differ in sensitivity to hypothermia induced by < 4 g/kg ethanol (Eriksson and Sarviharju, 1984).

LORR may be considered to be an extreme manifestation of ataxia. Long-Sleep (LS) and Short-Sleep (SS) mice, selectively-bred for their sensitivity to the hypnotic effects of ethanol, have been tested for their sensitivity to a wide range of ethanol responses. SS mice are more sensitive to the locomotor stimulant effects of low-doses of ethanol (Sanders, 1976). There is equivocal evidence regarding ataxia as measured by fixed-speed rotarod: in one study no differences were found (Sanders, 1976), whereas in another study LS were more sensitive to the ataxic effects (Stinchcomb, Bowers and Wehner, 1989). LS were found to be more sensitive than SS mice to the ataxic effects of ethanol as measured by the grid test (Dudek and Phillips, 1983, 1990). The effect of genotype on the hypothermic response has been demonstrated in LS and SS mice (Moore and Kakihana, 1978). LS mice were more sensitive to the hypothermic effects of 2 g/kg ethanol than SS mice, although some degree of hypothermia resulted in both lines of mice.

Locomotor Activity

The role of genetics in the expression of ethanol-induced locomotor activity has been investigated in selected lines of mice and inbred strains. FAST and SLOW lines of mice have been selectively bred for stimulated or nonstimulated response to 1.5 g/kg ethanol (Crabbe, Young, Deutsch, Tam, and

Kosobud, 1987b). The dose of ethanol used for selection was later changed to 2 g/kg (Phillips, Burkhart-Kasch, Terdal, and Crabbe, 1991). FAST and SLOW mice have been tested for ataxia and hypothermia. The results for ataxia were inconsistent (Crabbe, Feller, and Phillips, 1990a). In general, FAST mice appeared to be more sensitive than SLOW mice to the motor impairment effects of ethanol, suggesting the existence of a genetic correlation. No line differences were observed for ethanol-induced hypothermia (Phillips, Limm, and Crabbe, 1989). LS and SS mice differed in ethanol-induced locomotor activity in a dose-dependent manner; SS mice are more activated than LS, especially at doses >1.5 g/kg ethanol (Dudek and Phillips, 1990; Dudek, Abbott, and Phillips, 1984). A line of rats has been selected for reduction in locomotor activity as measured on a stabilimeter test. Most Affected (MA) and Least-Affected (LA) rats showed significant divergence when selected with 1.5 g/kg ethanol. Both lines of rats have been tested for sensitivity to ethanol-induced hypothermia. MA rats were more sensitive to this effect than LA rats (Mayer, Khanna, Kim, and Kalant, 1983).

### *HOT and COLD Selected Lines*

Response to selection

Starting from a genetically heterogeneous stock of mice which was derived from crossing eight inbred strains (McClearn, Wilson, and Meredith, 1970), HOT and COLD lines of mice have been selectively bred for their different responses to the hypothermic effect of an acute 3 g/kg dose of ethanol (Phillips, Terdal, and Crabbe, 1990; Crabbe et al., 1987a). Mice with the greatest change from baseline temperature were mated to produce the sensitive line (COLD); mice with the smallest change were mated to produce

the less-sensitive line (HOT). Replicates of each line were also produced so that there are four lines: HOT1, HOT2, COLD1, COLD2. In addition, two control lines are maintained that are randomly bred. Figure 1 shows the response to selection up to generation 36. As of that generation, COLD mice showed a mean decrease of ~8°C body temperature, whereas HOT mice only lost ~1.0°C from baseline temperature. Not only do these lines differ in acute sensitivity, but they also differ in the development of tolerance to hypothermia. COLD mice develop tolerance to the hypothermic effect of ethanol but HOT mice do not, even when the two lines have been equated for initial response (i.e., having the same degree of change in body temperature) (Crabbe, Feller, and Dorow, 1989) . The only condition thus far detected under which HOT mice develop tolerance is when they are exposed to ethanol at extreme ambient temperature (4°C) (Limm and Crabbe, 1992).

Response to other ethanol-induced behaviors

HOT and COLD mice have been tested for severity of ethanol withdrawal and locomotor activity using standard laboratory protocols. When ethanol-stimulated locomotor activity was assessed for 4 min (2-6 minutes after injection in HOT and COLD mice, the results indicated that a difference was evident in Replicate 2 but not in Replicate 1. HOT2 mice were more sensitive (i.e., more activated) than COLD2 mice but there was no difference between these lines in Replicate1 (Crabbe, Kosobud, Feller, and Phillips, 1988). A similar replicate difference appeared for withdrawal severity. After 72 hr in ethanol inhalation chambers, withdrawal scores, calculated as area under the withdrawal curve over 24 hrs, for HOT1 mice were significantly greater than COLD1 mice but no differences were evident between HOT2 and COLD2 (Crabbe et al., 1988). It

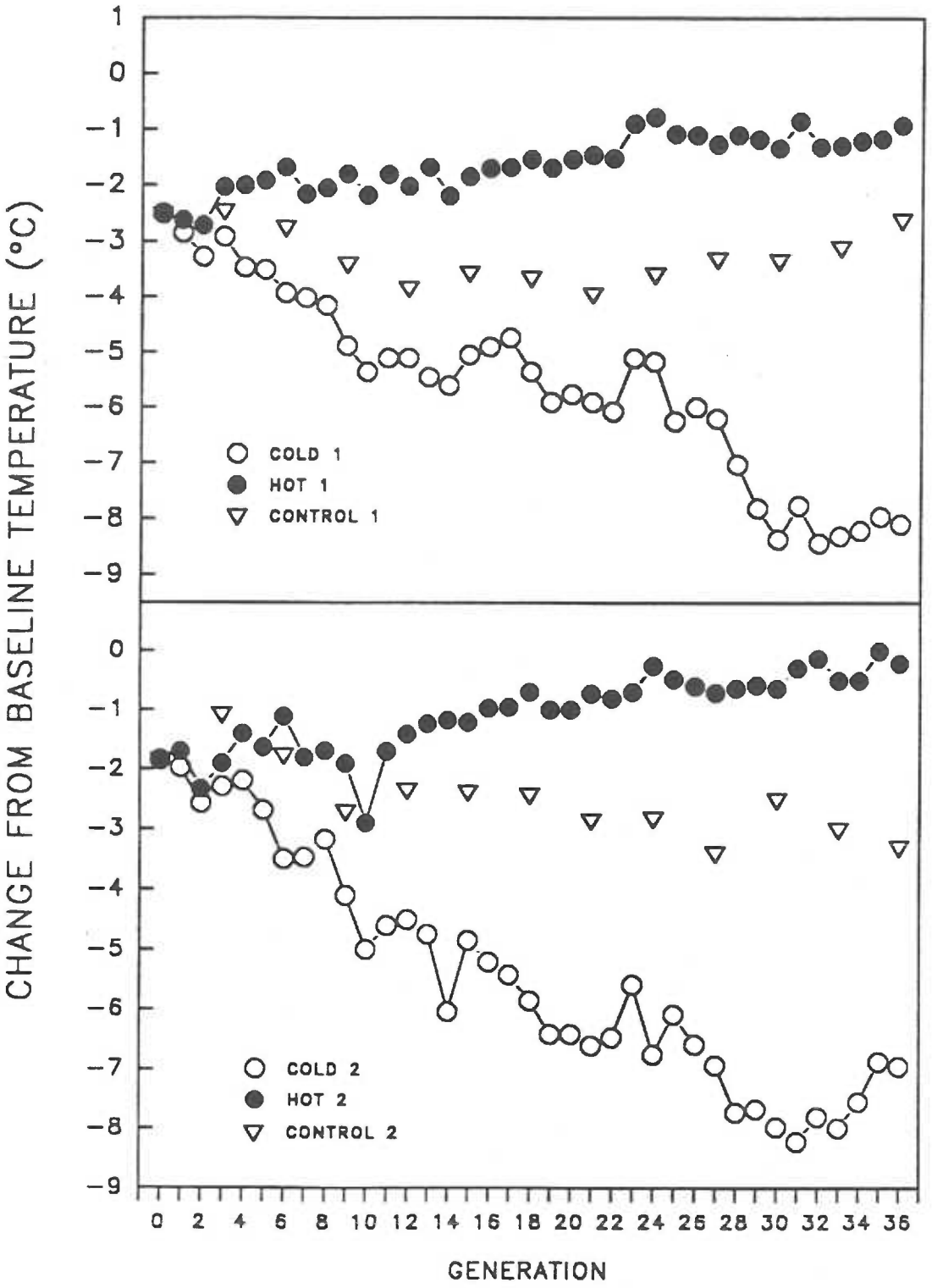


Figure 1. Response to selection. Temperatures represent the largest (COLD) or smallest (HOT) change from baseline temperature (°C) either 30 or 60 minutes following injection of 3 g/kg EtOH. CONTROL lines are not selected and are tested every third generation. The mean for each generation is shown; SE are smaller than symbol.

appears that there is a negative genetic correlation between withdrawal severity and hypothermic sensitivity as well as between ethanol-stimulated activity and hypothermia.

When the sedative effects of ethanol, as measured by LORR and regain of righting reflex, were conducted with HOT and COLD mice (Finn, Bejanian, Jones, McGivern, Syapin, Crabbe, and Alkana, 1990), a significant line difference was found in BrEC at regain of righting when mice were tested at 22°C ambient temperature. HOT mice recovered at higher BrEC than COLD mice suggesting that COLD mice were more sensitive to ethanol than HOT mice. The duration of LORR was not significantly different between the selected lines.

Rapid tolerance to the ataxic effects of ethanol in HOT and COLD mice has been assessed using the rotarod test (Gallaher, unpublished). On day 1, one group of mice was tested to recovery from ataxia after 2 g/kg ethanol, at which time a blood sample was taken and the mouse was given a second ethanol injection. This procedure continued for a total of five injections with five recoveries. A rapid tolerance curve was generated mapping BEC at recovery over time. The time course for the development of rapid tolerance was not different between the lines. No differences in initial recovery, as indicated by BEC levels at time of first recovery, were observed either. Twenty-four hours later the tolerant mice and drug-naive mice were tested for brain ethanol levels at the time of onset of ataxia. There were no differences in BrEC at onset of ataxia between the lines or treatment groups.

In the HOT and COLD lines, genes involved in producing ethanol-induced hypothermia have been selected for in the COLD line and genes conferring resistance to hypothermia were selected in the HOT line. These lines are useful for investigating the influence of genetic factors involved in

ethanol-induced hypothermia on other ethanol-induced behaviors. Since the inbred strain studies have suggested a correlation between ataxia and hypothermia, ataxia, as well as other potentially correlated responses to ethanol, have also been examined in the HOT and COLD lines using various methods.

## RATIONALE AND SPECIFIC AIMS

Previous studies using inbred mouse strains indicate that there are positive genetic correlations between initial sensitivity and tolerance to hypothermia (Crabbe et al., 1981, 1982), initial sensitivity to ataxia and initial sensitivity to hypothermia (Crabbe, 1983; Crabbe et al., 1982) and ataxia tolerance and hypothermia tolerance (Crabbe et al., 1982). Selectively-bred mice can be used to understand the genetics of the behavioral and physiological effects of ethanol. In the following experiments using HOT and COLD selected lines, differences in ataxia sensitivity were assessed to see if any of the genes influencing hypothermia sensitivity were also involved in sensitivity to ethanol-induced ataxia. Tolerance was tested to determine if 1) HOT and COLD mice can develop tolerance to the ataxic effects , and 2) if tolerance developed, whether it developed differently for these lines. It has been shown that tolerance to one response to a drug does not necessarily generalize to another response to that same drug (Pohorecky, Brick, and Carpenter, 1986; Kalant, LeBlanc, and Gibbins, 1971). It was predicted that there would be a difference in sensitivity and tolerance to the ataxic effects of ethanol between the HOT and COLD selected lines.

A variety of tests were available to measure ataxia (e.g., grid test, rotarod, tilt-plane). These tests are designed differently so that they may be measuring other effects of the drug as well, which could complicate the interpretation of the

results. Each of the behavioral tests chosen for study may measure ataxia as well as other biologically-relevant effects of ethanol. For example, there may be contributory effects of muscle weakness and locomotor activity. It is possible that tolerance may develop to various responses at different rates, if at all. By using multiple tests of ataxia, there is a better chance of revealing differences between the HOT and COLD lines of mice than if only one of the tests were used.

The specific aims of the study were two-fold: One was to assess differences in initial sensitivity to the ataxic effects of ethanol between HOT and COLD mice. It was predicted that COLD mice would be more sensitive to the ataxic effects of ethanol. The second goal was to determine if HOT and COLD mice differed in the development of tolerance to ataxia. Based on their tolerance to hypothermia, COLD mice were predicted to develop more tolerance than HOT mice.

## GENERAL METHODS

### *Animals and Animal Husbandry*

HOT1, HOT2, COLD1 and COLD2 mice were used in all experiments. These mice were derived from an initial breeding population of genetically heterogeneous mice (HS/Ibg) obtained from the Institute for Behavioral Genetics, Boulder, CO. HOT and COLD mice were maintained at the Animal Research Facility, VAMC, Portland, OR. Mice were housed 4-5 in a polypropylene cage with corncob bedding that was changed twice a week. The colony room was kept on a 12:12 light-dark cycle with lights on at 6 am. Daily room temperature ranged between 20° - 21° C. Mice received ad libitum food (standard rodent chow, Purina) and water. Two to seven days prior to the start of experiments, mice were moved into a second colony room where they

remained for the duration of the experiment. Female mice were used in experiment 1 due to the unavailability of sufficient numbers of male HOT and COLD mice at the time the experiment was conducted. In all other experiments, male mice were used since they had been used in earlier pilot studies.

### *Drugs*

Ethyl alcohol (Pharmaco ethyl alcohol, 200 proof) was diluted to a working solution of 20% (v:v) with 0.9% saline. Solutions were made fresh daily. All ethanol doses were injected intraperitoneally (i.p.) according to body weight in a volume of 0.2 - 0.6 mL. Untreated mice were given an equivalent volume of 0.9% saline.

### *Apparatus*

Four methods for assessing ataxia were used: screen test, fixed-speed and accelerating rotarods, and grid test. Initial sensitivity was measured with all four apparatus, while tolerance was determined by the accelerating rotarod and grid test only.

#### Screen Test

The screen test has been used to measure muscle weakness produced by a drug. It was originally developed as an alternate test to the rotarod to determine the degree of ataxia (Coughenour, McLean, and Parker, 1977). The apparatus consists of a wire mesh screen (1 $cm^2$; 15.5 cm X 16.5 cm) placed in the center of 33.5 $cm^2$ framed, wire surface. The area between the screen and the frame is covered with silver duct tape, creating a slick surface to prevent the mice from climbing up to, and over, the edge of the frame. The screen is elevated 25 inches above sawdust bedding. The screen apparatus can be rotated so that the subject is perpendicular to ground ("vertical") or parallel to, and facing, the ground ("horizontal") with the subject hanging upside-down.

Rotarod

The rotarod, originally designed to test neuromotor performance, measures balance maintenance (Dunham and Miya, 1957; Jones and Roberts, 1967). The apparatus is a rotating cylinder (PVC piping), 3-in. in diameter, divided into six compartments separated by white acrylic disks extending 3-5/8 in. beyond the walking surface (Flair Plastics, Portland, OR). The surface of the cylinder is covered with sandpaper (320 grade) to reduce slipping. The rotarod is suspended 18 in. above sawdust bedding onto which the mouse falls. The rod is attached to a motor that can be set to rotate the rod at a constant speed or a gradual acceleration. The rotarod requires the mouse to remain on the cylinder as it rotates. The apparatus used in these experiments can be run at a constant velocity ("fixed-speed rotarod") or at a constant acceleration ("accelerating rotarod"). In Experiment 2, a fixed-speed of 5 rpm was chosen. This speed has been used in other experiments when HOT and COLD mice were being tested. Experiment 4 used the accelerating rotarod. The rotarod was adjusted to accelerate 20 rpm per minute (or an increase of 1 rpm every 3 seconds).

Grid Test

The grid test was originally described by Belknap (1975). The apparatus as modified by Dudek and Phillips (1983) consists of a 6-in.$^2$ clear Plexiglas box with a 0.5-in. wire grid bottom suspended above a metal plate by placement of a 1 cm-thick Plexiglas frame between the grid and plate. An electrical signal is recorded by a computer when a foot of the mouse comes in contact with the metal plate while contact is maintained with the grid. The grid apparatus is placed in an Omnitech activity monitor which allows locomotor activity to be recorded simultaneously. Locomotor activity is detected when photocell beams

are broken. There are two photocells per side of the apparatus. Each time the mouse slips through the grid and contacts the floor a misstep is recorded. The ratio of these measures (missteps/activity count) is indicative of the degree of impairment (the higher the ratio the greater the impairment). The ratio score is used instead of the number of errors since ratio corrects for individual differences in activity.

The development of ethanol tolerance using the grid test with repeated, scheduled injections has been shown in preliminary experiments by Phillips (unpublished) using inbred and recombinant inbred strains (BXD strains) of mice. The same schedule of injection and testing was used in this experiment. The grid test measures both ataxia (motor incoordination) and locomotor activity (horizontal activity).

***Blood and Brain Ethanol Concentration Determination***

Ethanol concentration was estimated from retro-orbital sinus (ROS) blood or whole brain (BrEC). All blood or tissue was taken at the termination of an experiment. For retro-orbital blood sampling, mice were gently restrained with their heads immobilized. A pipette was inserted into the retro-orbital sinus and 20-$\mu$l of blood removed. Blood samples were added to 50-$\mu$l zinc sulfate ($ZnSO_4$) and kept on ice until further processed. 50-$\mu$l of $Ba(OH)_2$ was added to each sample, agitated and centrifuged at 12,000 x g. When BrEC were determined, mice were cervically dislocated immediately after testing. Whole brain, including brainstem, was removed and immediately frozen on dry ice. Processing continued as described above and in Crabbe et al. (1987a). Supernatant was removed, put into a fresh vial and ethanol concentration measured by gas chromatography as previously described (Crabbe et al., 1987a). Standards of known ethanol concentration were prerun to calibrate the assay.

***Data Analysis***

Data for all experiments were analyzed using analysis of variance (ANOVA). The independent variables of LINE (HOT or COLD) and REPLICATE (1 or 2) were always initially included. A third independent variable, TREATMENT, was used in Experiments 1, 3 and 4. In all cases, TREATMENT represented an experimental manipulation that differed between two or more groups of mice. More specifically, in Experiment 1, TREATMENT refers to the dose of ethanol given to each group of mice; in Experiment 3 and 4, TREATMENT refers to the grouping of mice given chronic saline (CS) or 2.5 g/kg chronic ethanol (CE). When a three-factor interaction reached statistical significance, each replicate was analyzed by independent two-factor ANOVAs to reduce the complexity of multiple factor interactions (> 2), which are difficult to interpret. Significant two-factor interactions were further analyzed by simple main effects. Post-hoc multiple comparisons were analyzed by Newman-Keuls. For all analyses, results were considered significant when $p < .05$. More specific details of each analysis are described below.

In Experiment 1, LATENCY data were analyzed by a three-factor ANOVA (LINE, REPLICATE, and TREATMENT). Experiment 2 data were subjected to analysis by a two-factor ANOVA (LINE and REPLICATE).

In Experiments 3 and 4, the effects of an acute ethanol exposure or the effects of repeated ethanol treatment were analyzed. In experiment 3, initial sensitivity was determined by three-factor ANOVA (LINE, REPLICATE, and TREATMENT) on Day 3. Dependent variables measured in this experiment were RATIO, ERRORS, and ACTIVITY. The effects of repeated ethanol were analyzed by a three-factor, between-groups ANOVA (LINE, REPLICATE, and TREATMENT) for Day 11. This analysis allows for a direct comparison of the

treatment groups and phenotypes to assess tolerance or sensitization and controls for the effects of repeated handling and testing between the treatment groups. However, that analysis does not adequately describe the events taking place in the CE treatment group each test day. Therefore, to address the question of whether or not tolerance (or sensitization) developed at any point during treatment, a repeated-measures ANOVA was included for data from each LINE (i.e., HOT1-CE, HOT2-CE, COLD1-CE, COLD2-CE) in the CE group only. The repeated measure was designated as DAYS. It should be noted that the purpose of this analysis was not to compare selected lines, but rather to detect significant changes in response within each line.

RPM was analyzed in Experiment 4 in the same ANOVA designs as described above (Expt. 3). Data were analyzed as change scores, in rpm, from BASELINE. Initial sensitivity was assessed between CS and CE treatment groups on Day 9. A between-groups assessment of tolerance was determined from change scores on Day 19. The extent of tolerance development in each line was examined by one factor, repeated measure ANOVA, as described above.

The data are presented for each replicate line for consistency, except where noted, to demonstrate the effects in each replicate since they are considered to be separate genetic lines.

## EXPERIMENTS

### EXPERIMENT 1: Initial sensitivity to ataxia -- screen test

#### *Methods and Materials*

This test was used to measure ataxia thirty minutes after ethanol administration. An untreated mouse can ambulate around the screen in either position for an indefinite amount of time without falling. Pilot studies suggested

that the vertical position was better for detecting ethanol-induced ataxia; therefore, this position was used in this experiment.

Female mice (ages 57-90 days) from selected generations 31 and 32 were used in this experiment. Seven doses of ethanol were used in order to establish a dose-response curve and a saline group (0.0 g/kg) was included as well. These were 1.9, 2.1, 2.3, 2.5, 2.7, 2.9, and 3.1 g/kg ethanol. Four to ten mice per line and replicate were used per dose. The smallest numbers of mice were from the saline and 1.9 g/kg ETOH group.

***Procedure***

Mice were brought into the test room, weighed, and allowed to sit undisturbed for at least 30 minutes. Mice were injected approximately one minute apart, one cage at a time. Individual mice within a cage were randomly assigned to a particular dose. The order of cages was randomized. Thirty minutes after injection, a mouse was placed on top of the horizontally-positioned screen . The screen was slowly rotated to the vertical position. At this point the timer was started. The latency to fall off, up to a maximum of 60 seconds, was recorded. Mice were then returned to their homecage.

***Results***

All mice given saline were able to stay on the vertical screen for the 60 second limit. Since there was no variance associated with the saline group, this group was removed from the statistical analyses but the data were included in Figure 2. Ethanol produced a dose-dependent decrease in ability to remain on the screen that was equivalent in both selected lines (Figure 2). No significant effects were detected for LINE [$F(1, 142) = 1.1$, n.s.].

The dose-response relationship was supported by a significant effect of TREATMENT [$F(6, 142) = 5.2$; $p = .0001$]. Mice given lower doses of ethanol were able to remain on the screen longer than mice given the highest doses of

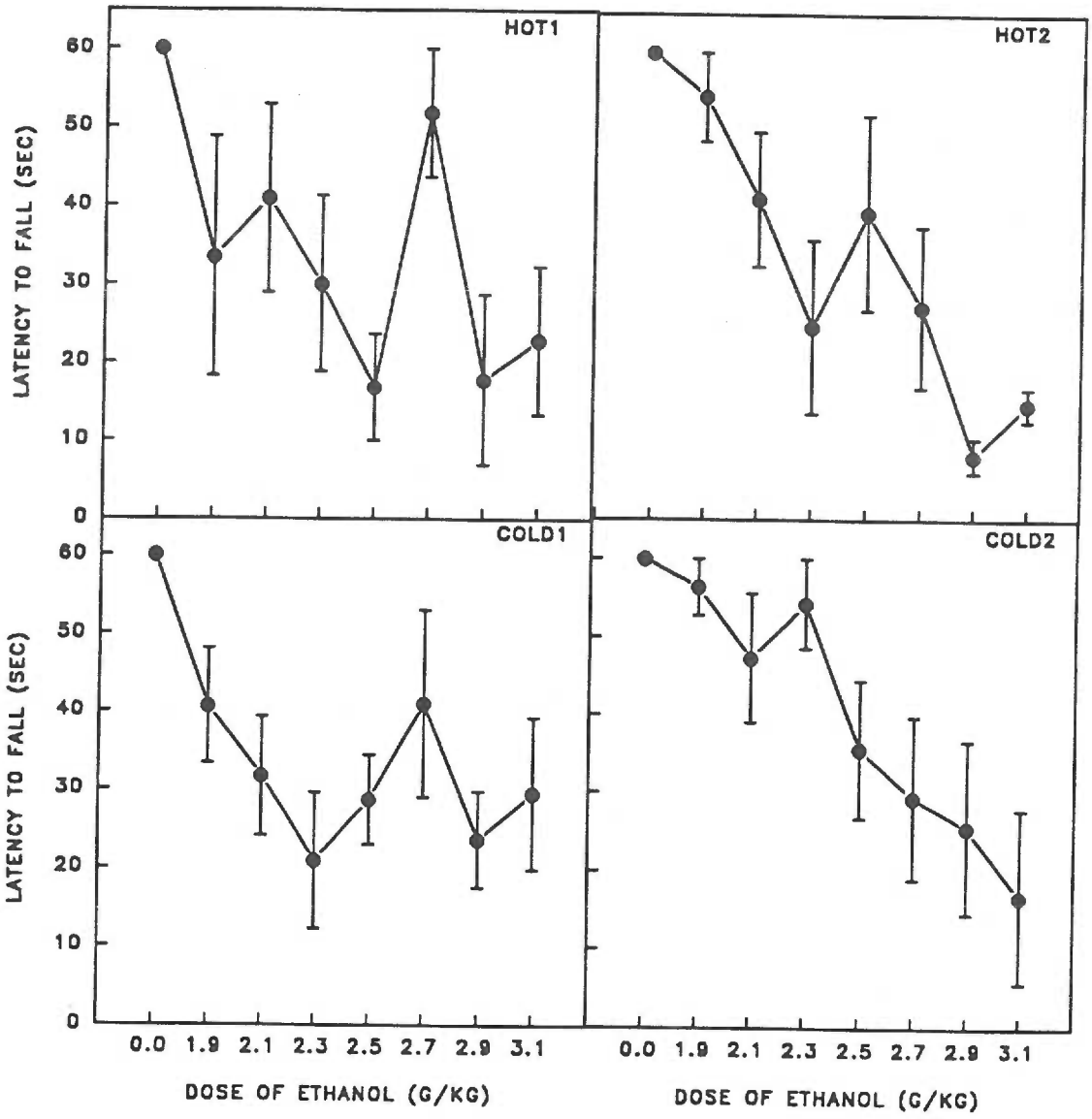


Figure 2. Dose-response curve for ethanol-induced ataxia measured by the screen test in HOT and COLD mice as mean latency to fall ± SEM for groups of 4-9 mice per dose. 0.0 g/kg dose represents saline group.

ethanol. Post-hoc analysis indicated that the mean latency for groups of mice receiving 1.9 g/kg or 2.1 g/kg differed significantly from the mean latencies for groups receiving 2.9 or 3.1 g/kg ethanol (Newman-Keuls, p's < 0.01). In addition, mice given 2.7 g/kg had significantly longer latencies than mice receiving 2.9 g/kg (Newman-Keuls, $p < 0.05$).

The replicate sets of lines showed sensitivity differences. In particular, Replicate 1 mice remained on the screen significantly longer than Replicate 2 mice at 2.7 g/kg ethanol. A different dose-response effect appeared in the Replicates as indicated by a significant interaction of TREATMENT x REPLICATE [$F(6, 142) = 2.3$; $p < 0.05$]. Simple main effects analysis of this interaction indicated that the latency to fall was greater in Replicate 2 at doses of 1.9 g/kg and 2.3 g/kg ethanol (p's < .01) whereas Replicate 1 mice had longer latencies than Replicate 2 at 2.7 g/kg ethanol ($p < .01$). At all other doses of ethanol, the replicate lines did not differ in latency to fall.

***Discussion***

The screen test failed to detect differences between HOT and COLD mice across a wide range of ethanol doses. A clear dose-response relationship was produced indicating that this was a sensitive test of ataxia. Mice given 2.5 g/kg ethanol had a mean latency to fall of 30 seconds which was half of the time required to stay on the vertical screen, suggesting that this would be an effective dose to use in further experiments.

This test probably combines measures of motor incoordination as well as muscle weakness (grip strength). It is unclear to what extent ataxia may be influenced by muscle weakness. Ethanol does produce changes in grip strength (Nevins, Nash, and Beardsley, 1993) and in nerve conduction velocities (Reed, 1980), which ultimately affect muscle function. The expression of ataxia therefore could be influenced by muscle weakness. Informal

observation of the behavior during testing suggests that at the highest doses of ethanol used (2.9 and 3.1 g/kg), when latencies were short, some mice were unable to grip the screen as the test was starting. At lower doses, when latencies were longer, mice were able to grip the screen. It seems that changes in grip strength could have had a greater influence on latency at higher doses of ethanol than at lower doses.

The amount of activity of the mouse also appears to have had some influence on latency to fall. Although the amount of locomotor activity was not explicitly determined in this study, informal and subjective observations of gross motor activity during the test was noted. The group of mice receiving 2.7 g/kg ethanol showed an increase in latency to fall as compared to the 2.5 and 2.9 g/kg doses of ethanol. The potentially higher activity of the mice in the 2.5 g/kg group versus mice in the 2.7 g/kg might have contributed to the lower latencies recorded for the 2.5 g/kg group. The increase in latency score was more prominent in Replicate 1 (in both HOT and COLD mice) than in Replicate 2 mice. It is accepted that ethanol produces dose-dependent effects on locomotor activity. A dose-dependent response is observed such that at low doses of ethanol ($\leq$ 2 g/kg for mice) there is a stimulation of activity and depression of activity at higher doses (Pohorecky, 1977). This effect of ethanol on activity may have influenced the performance on the screen apparatus.

Although this test was a sensitive measure of ataxia, it is most likely influenced by additional effects of ethanol. In addition, the combination of high variability, uncertainty regarding the contribution of muscle relaxation/grip strength to falling, and lack of support for a differential genetic influence on ataxia suggests that this test would not be an especially good choice for measuring tolerance to the ataxic effects of ethanol.

## EXPERIMENT 2: Initial sensitivity to ataxia onset -Rotarod

Pilot studies suggested that HOT and COLD mice might differ in onset to ataxia (E. Gallaher, personal communication; Schafer and Crabbe, unpublished observations). In these studies, mice were given 2 or 2.5 g/kg ethanol and immediately tested on a fixed-speed rotarod (5 rpm). BrEC were measured at the time of fall. The results yielded conflicting results with respect to treatment dose. Using 2 g/kg, COLD mice appeared more sensitive than HOT mice, whereas using 2.5 g/kg, HOT mice appeared slightly more sensitive than COLD mice. However, the results were confounded by a lack of ability to perform the task by undrugged mice. Experiment 2 attempted to measure the onset to ataxia using 2.5 g/kg while incorporating practice sessions into the design to ensure that all mice were able to remain on the rotarod for at least 2 min.

### *Methods and Materials*

Ten to twelve naive male mice from each line and replicate, 70-90 days old, from selection generations 33 and 34 were tested. The dose of ethanol used was 2.5 g/kg, i.p. Saline-treated mice received the equivalent volume of saline.

### *Procedure*

On day 1, mice were given three 30 second practice trials (thirty seconds between trials) on the fixed-speed rotarod (5 rpm). No injections were given. Mice were returned to their home cages. On the morning of Day 2, the mice were given additional practice (three 30 second trials), weighed, and then allowed to rest in home cages for at least one hour before testing started. The first mouse was injected with 2.5 g/kg ethanol, put in a holding cage for 10 seconds then placed on the rotarod. When the mouse fell, it was immediately euthanized by cervical dislocation and the brain rapidly removed in a fume hood and frozen on dry ice. The rest of the mice were tested in the same

fashion. One mouse from each cage was designated as a control and received a saline injection. The control group was tested to see if they could remain on the rotarod for 3 minutes, which was expected to be sufficiently longer than any ethanol-treated mouse could remain on the rotarod. The latency to fall was recorded.

***Results***

In this experiment two measures of sensitivity were determined: latency to become ataxic and the concentration of ethanol in the brain at the time of ataxia onset. Control mice were able to stay on the rotarod for the established criterion and there were no differences between saline-treated HOT and COLD mice, supporting the use of practice trials to achieve an equivalent, stable baseline performance in both selected lines.

Figure 3 depicts the latencies for the ethanol-treated groups for each line. HOT and COLD mice did not differ in the latency to fall from the rotarod. Neither the main effect of LINE or REPLICATE was significant [both F's (1, 37) = 0.3, n.s] nor was there a significant LINE x REPLICATE interaction [F(1,37) = 0.3, n.s].

Brain ethanol concentrations at ataxia onset are shown in Figure 4. HOT and COLD mice showed an equal sensitivity to ethanol. ANOVA did not reveal any significant effects for any factor or interaction [all F's (1,37) < 2.5, n.s].

***Discussion***

The results from this experiment demonstrated that HOT and COLD mice are equally sensitive to the ataxic effects of a 2.5 g/kg dose of ethanol. No pharmacokinetic differences (i.e., differences in absorption, distribution, elimination of ethanol) between the selected lines can be assumed to be involved since HOT and COLD mice did not differ in their latency to fall from the rotarod or in brain ethanol concentrations at the time of ataxia onset.

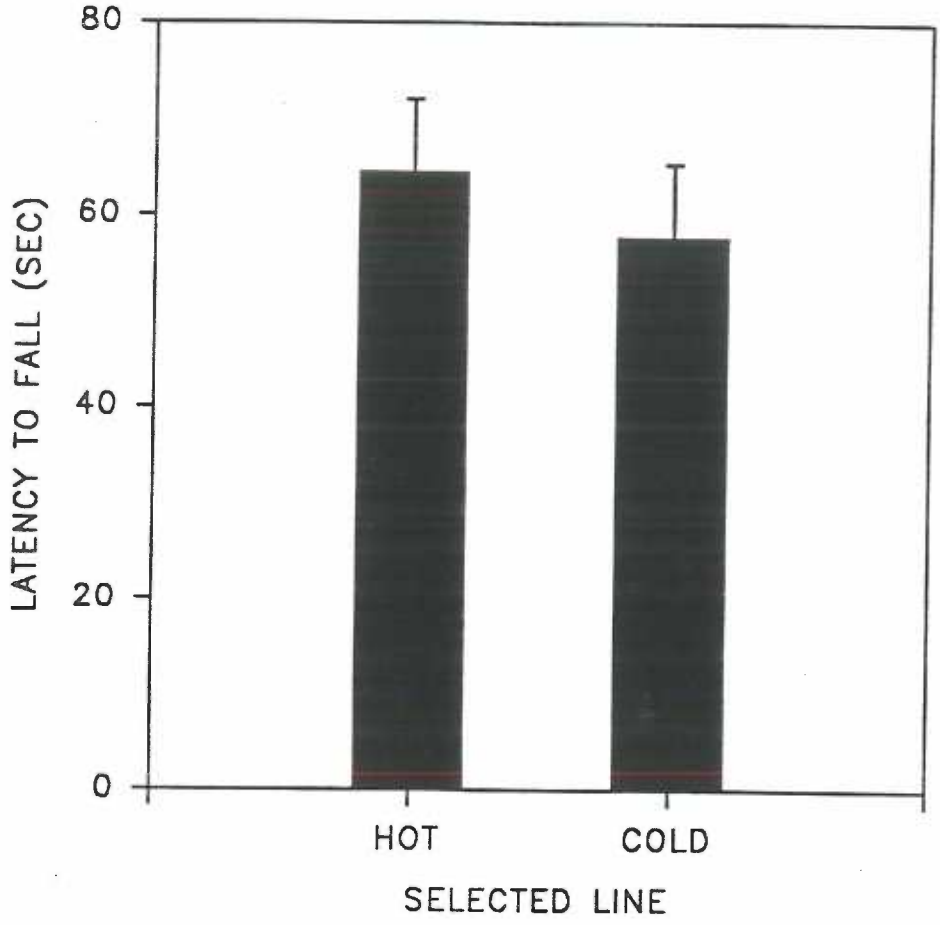


Figure 3. Ataxia onset measured by latency to fall from a fixed-speed rotarod (5 rpm) after 2.5 g/kg ethanol. Bars represent mean latency to fall (in seconds) ± SEM for groups of 21 and 20 HOT and COLD mice, respectively. Data are collapsed over Replicate since there were no significant differences.

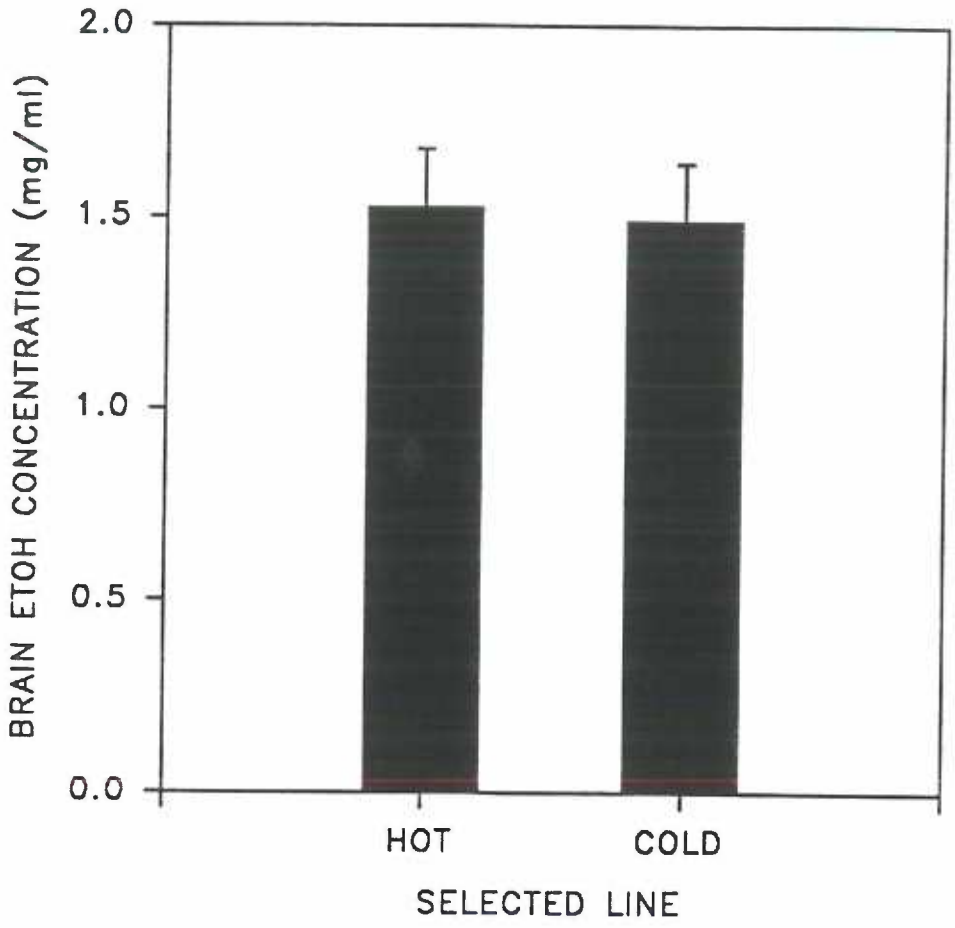


Figure 4. Brain ethanol concentration at time of fall from fixed-speed rotarod (5 rpm) after 2.5 g/kg ethanol. Bars represent mean BrEC (mg/ml) ± SEM for groups of 21 and 20 HOT and COLD mice, respectively. Data are collapsed over Replicate.

## Experiment 3: Initial sensitivity and tolerance to ataxia -- Grid test

### *Methods and Materials*

Ten to twelve male mice per line and replicate per group, from selection generations 35 and 36, were tested at ages 65-80 days of age. The mice had to be tested in two passes due to scheduling conflicts. In each pass, half of the mice were designated as saline controls and half were assigned to the ethanol group (see below). Caution was taken to keep timing of runs, and all other procedures, constant between the passes. Due to experimenter errors, some data were lost for a few mice on various test days. If either error counts or activity counts were not recorded for the test session, no Ratio was calculated and that subject was removed for that day. If activity was recorded, but no error counts, the subject was maintained in the data set for the activity analysis but no Ratio was calculated.

### *Procedure*

Ethanol was administered at a dose of 2.5 g/kg; test duration was 10 minutes. There were two treatment groups: a chronic saline group (CS) and a chronic ethanol group (CE). Mice were transported from the colony room to the procedure room and allowed to sit undisturbed for at least thirty minutes. Mice were weighed on each injection/test day ten minutes prior to injection and placed in individual holding cages before the test. Immediately after injection mice were placed into the testing apparatus for a 10 min. test. The daily schedule of procedures is outlined in Table 1. Immediately after completion of the test on the last day (Day 11), a ROS blood sample was taken from each mouse.

**Table 1.** Outline of test procedure for Expt. 3. On days 4, 6, 8, and 10 no injections or testing occurred. All mice were tested with ethanol on Day 11. CS = chronic saline; CE = chronic ethanol.

| Group | Day1 | Day2 | Day3 | Day4 | Day5 | Day6 | Day7 | Day8 | Day9 | Day10 | Day11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CS | Sal test | Sal test | Sal test | -- | Sal test | -- | Sal test | -- | Sal test | -- | EtOH test |
| CE | Sal test | Sal test | EtOH test | -- | EtOH test | -- | EtOH test | -- | EtOH test | -- | EtOH test |

## *Results*

Initial Sensitivity (Day 3)

RATIO

The results for each replicate are shown in Figure 5. Mice given ethanol for the first time on Day 3 had a greater RATIO (errors to activity counts) than mice given saline. This observation was supported by a significant main effect of TREATMENT [$F(1,67) = 205.8$, $p < .0001$]. No other factors or interactions were significant [all $F$'s $< 2.0$, n.s.]. These results indicated that ethanol-treated mice were sensitive to ethanol but the degree of impairment (measured by the magnitude of the Ratio) did not differ between HOT and COLD mice.

ERRORS

Ethanol impaired the motor performance of HOT and COLD mice on the grid test (Figure 6). Analysis of the data indicated significant main effects of LINE [$F(1,67) = 15.6$, $p < .001$], REPLICATE [$F(1,67) = 8.9$, $p < .01$], and TREATMENT [$F(1,67) = 153.7$, $p < .0001$]. TREATMENT interacted with both LINE [$F(1,67) = 16.4$, $p < .001$] and REPLICATE [$F(1,67) = 8.2$, $p < .01$] and the three-way interaction LINE x REPLICATE x TREATMENT was significant [$F(1,67) = 5.6$ $p < .05$]. A separate analysis for each replicate follows.

For Replicate1, there were significant main effects of LINE [$F(1,32) = 11.5$, $p < .01$] and TREATMENT [$F(1,32) = 70.4$, $p < .0001$] and there was a significant interaction of LINE x TREATMENT [$F(1,32) = 12.4$, $p < .01$]. Simple main effects analysis indicated HOT1-CE mice made significantly more missteps than COLD1-CE mice [$F(1,32) = 26.90$, $p < .0001$]. Replicate 2 mice did not differ in the number of errors made due to ethanol treatment; however, ethanol produced a significant impairment as compared to saline-treated mice.

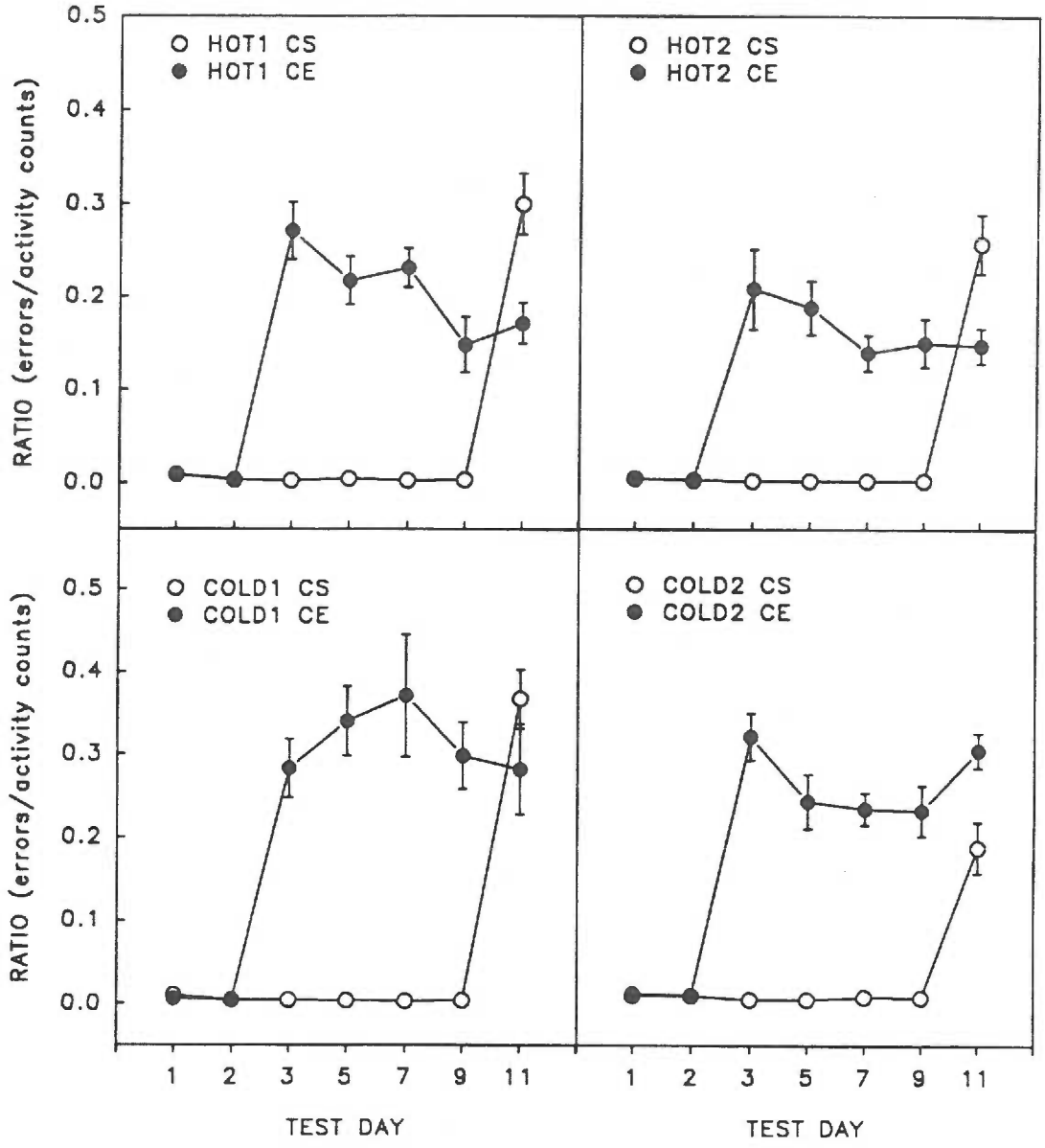


Figure 5. Mean Ratio (± SEM) of missteps to activity counts in the Grid test. Data were cumulated over the ten minute test following injection of saline (CS) or ethanol (CE) are presented for each test day. Data presented were for each Replicate line.

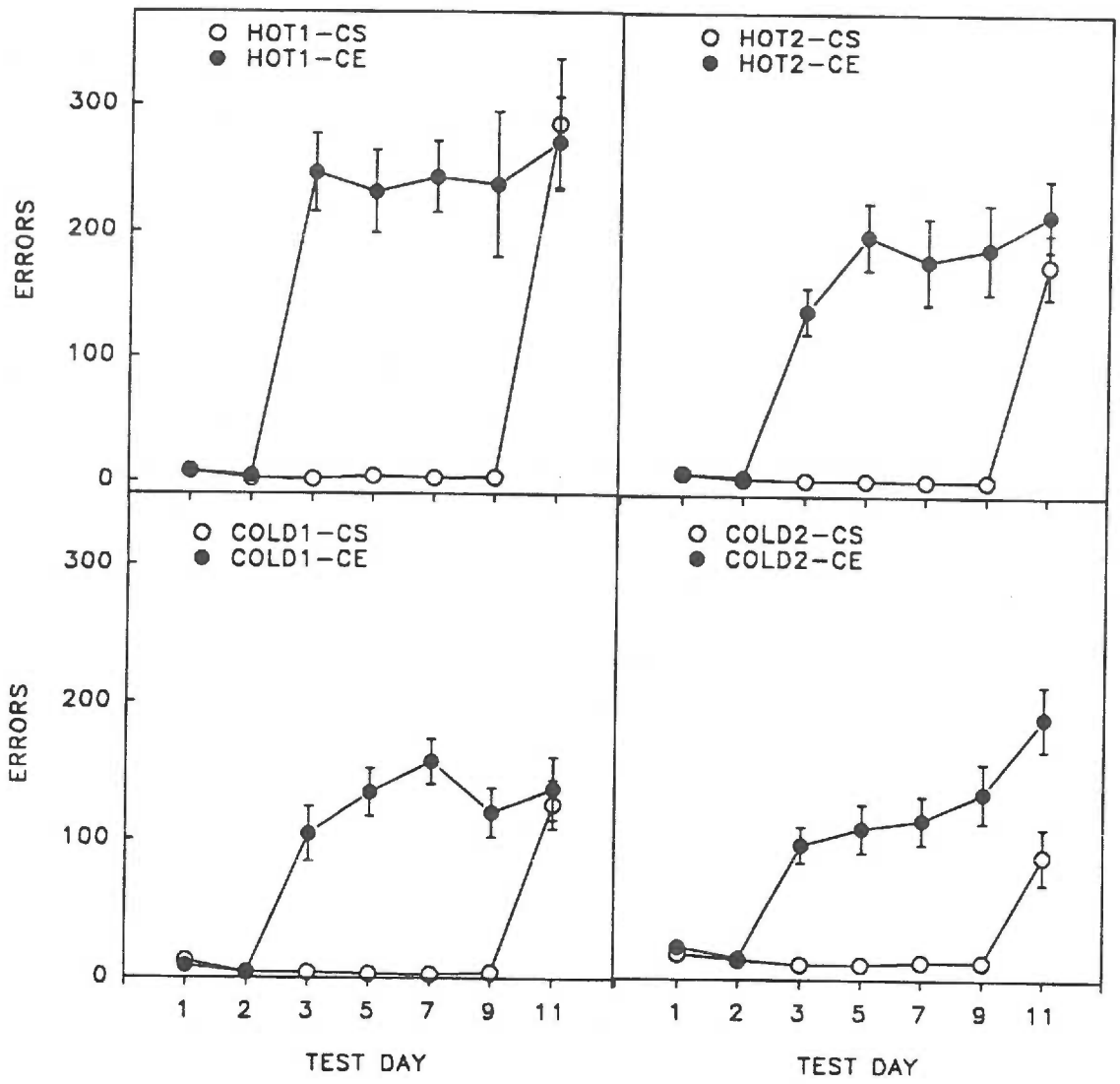


Figure 6. Mean Errors (± SEM) for the Grid test. Data were cumulated over the ten minutes following injection of saline (CS) or ethanol (CE) for each test day for each Replicate line.

These observations were supported by a significant TREATMENT effect [$F(1,32) = 101.8$, $p < .0001$]. LINE and LINE x TREATMENT did not reach significance [both F's$(1, 32) < 4$, $p > .05$]. That is, HOT2 and COLD2 mice had equivalent number of missteps.

ACTIVITY

Figure 7 depicts the acute effect of 2.5 g/kg ethanol on locomotor activity. Activity counts were corrected for baseline (day2) activity. Ethanol depressed locomotor activity in both replicates of COLD mice, whereas ethanol did not alter the locomotor activity of HOT mice. There were significant main effects of LINE [$F(1, 68) = 15.1$, $p < .001$] and TREATMENT [$F(1, 68) = 8.6$, $p < .01$] as well as a significant LINE x TREATMENT interaction [$F(1,68) = 12.9$, $p < .001$]. Simple main effects analysis of this interaction indicated that COLD mice were significantly affected by ethanol but HOT mice were not. The REPLICATE x TREATMENT interaction [$F(1, 68) = 4.5$, $p < .05$] was significant; simple main effects analysis indicated that Replicate 2 mice exhibited an overall decrease in locomotor activity after ethanol which was not observed in Replicate 1. The main effect of REPLICATE, the LINE x REPLICATE interaction and the interaction of LINE x REPLICATE x TREATMENT were not significant [all F's$(1,68) > .05$, n.s.].

Tolerance: Day 11

RATIO

Tolerance was analyzed by comparing treatment groups (CS vs. CE) on day 11 (refer to Figure 5). The results indicated that, in addition to significant effects of LINE [$F(1,66) = 6.1$, $p < .05$], REPLICATE [$F(1,66) = 9.1$, $p < .01$], and TREATMENT [$F(1,66) = 5.9$, $p < .05$], significant interactions of LINE x TREATMENT [$F(1,66) = 8.6$, $p < .01$] and REPLICATE x TREATMENT [$F(1,66) =$

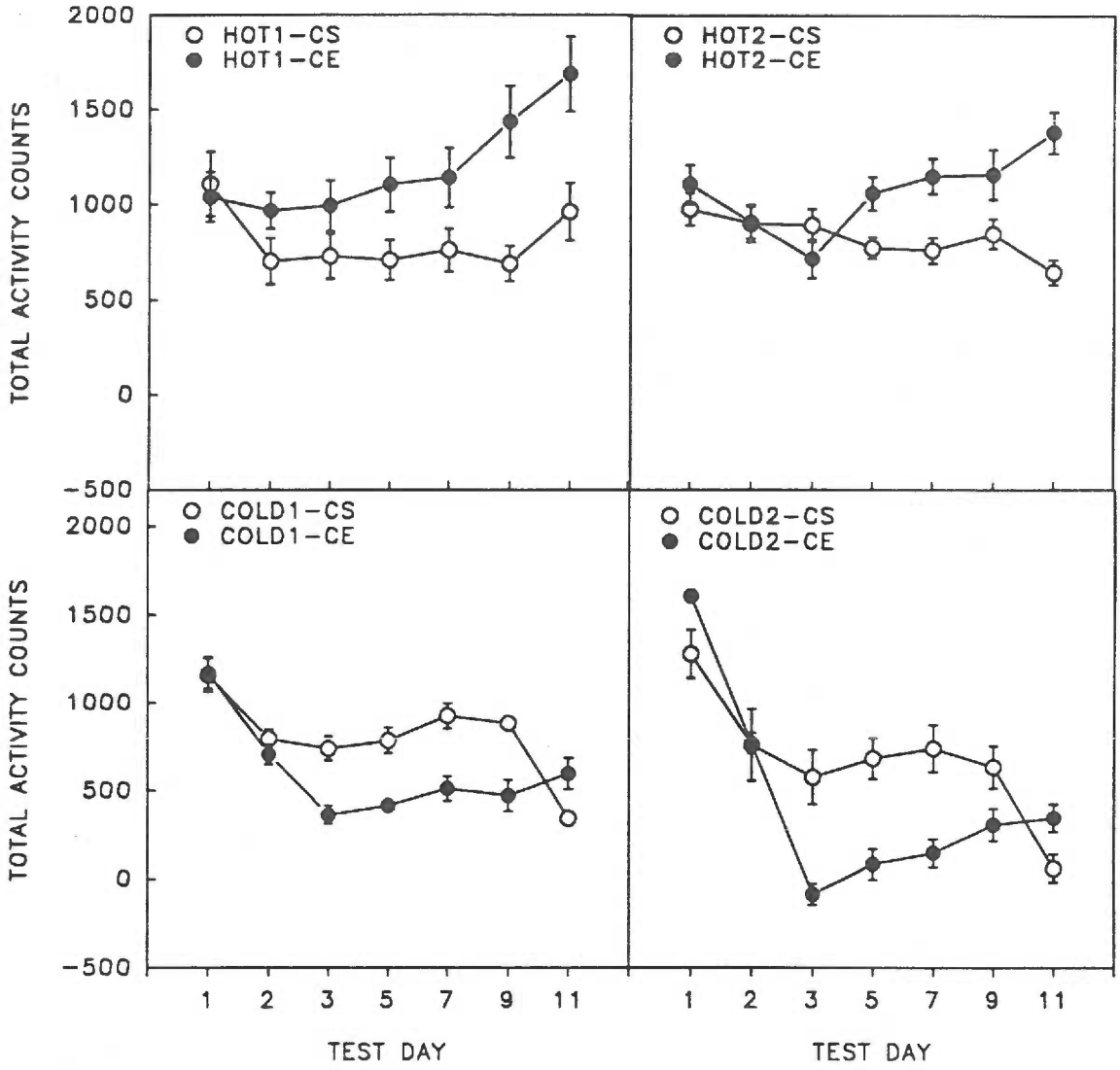


Figure 7. Mean activity counts (± SEM) for each treatment group (CS or CE) for each test day are presented for each Replicate line. Activity was recorded for ten minutes after injection.

5.6, $p < .05$] were found but not a LINE x REPLICATE interaction [$F(1, 66) = 2.3$, n.s.]. Since the three-way LINE x REPLICATE x TREATMENT interaction was very close to significance [$F(1, 66) = 3.8$, $p = .057$], these data were reanalyzed separately for each Replicate.

Tolerance to the ataxic effects of ethanol developed to an equivalent degree in both selected lines of Replicate 1. This was supported by a significant TREATMENT effect [$F(1, 31) = 8.0$, $p < .01$] but no LINE x TREATMENT interaction [$F(1, 31) = 0.3$, n.s]. This result can be seen in Figure 5a (upper panel) where the reduction of Ratio in the CE group versus the CS group was not significantly different in HOT1 and COLD1 mice. Regardless of treatment, HOT1 mice had lower RATIOS than COLD1 mice. This was supported by a significant LINE effect [$F(1, 31) = 5.5$, $p < .05$].

In Replicate 2, neither LINE nor TREATMENT was significant [$F(1, 35) < 1.0$, n.s.] but the LINE x TREATMENT interaction was significant [$F(1, 35) = 18.6$, $p < .001$]. Simple main effects analysis revealed that the selected lines differed in the CS group [$F(1, 35) = 5.9$, $p < .05$] and in the CE group [$F(1, 35) = 13.7$, $p < .001$] and that in HOT2 mice, repeated ethanol treatment resulted in a smaller ataxic response than from acute ethanol treatment [$F(1, 35) = 9.8$, $p < .01$], whereas a potentiation of the initial ataxic response was observed in COLD2 mice [$F(1, 35) = 8.9$, $p < .01$]. Although the mean RATIOs of the treatment groups and of each LINE were equivalent, tolerance was observed in HOT2 mice only. On the other hand, the increase in RATIO observed in COLD mice suggests these mice might have sensitized to ethanol as a function of repeated ethanol.

ERRORS

The analysis of ERRORS on Day 11 indicated that the number of missteps made after five ethanol treatments did not differ from the number of

missteps made after the first ethanol treatment (see Figure 6). There were significant main effects of LINE [$F(1,66) = 22.19$, $p < .0001$] and REPLICATE [$F(1,67) = 5.18$, $p < .05$] and a significant LINE x REPLICATE [$F(1,66) = 5.61$, $p < .05$] but no effects of TREATMENT were significant [all $Fs < 3.03$, n.s.]. These results indicated that although neither selected line was affected by repeated ethanol treatment, there was a differential response between the sets of replicate lines. However, since TREATMENT was not statistically different between the groups, the interaction of LINE x REPLICATE was not further analyzed. Analysis of this interaction would not provide any additional information related to tolerance.

ACTIVITY

ACTIVITY counts were corrected for baseline activity. HOT mice were significantly more activated than COLD mice. Significant LINE [$F(1, 67) = 42.3$, $p < .001$], REPLICATE [$F(1, 67) = 7.4$, $p < .01$] and TREATMENT [$F(1, 67) = 19.3$, $p < .001$] effects were found. No interactions were significant [all $F's < 1.0$, n.s.]. These results indicate that, regardless of number of ethanol treatments, HOT mice were more activated than COLD mice. Figure 7 showed that both HOT and COLD mice had higher activity counts after the last ethanol treatment than the first ethanol treatment.

Tolerance: Days 3 - 11

The between groups analyses of tolerance did not allow for an investigation of the effects of repeated ethanol on a day to day basis. In order to accurately determine if tolerance, or sensitization, developed at some time point other than the last day, independent repeated-measures ANOVA were run on the data from each line in the CE treatment group.

RATIO

The results from independent ANOVA indicated a significant effect of repeated ethanol treatment in two lines only. There was a significant effect of DAYS in HOT1-CE mice [$F(4, 36) = 4.1$, $p < .01$] and in COLD2-CE mice [$F(4, 32) = 2.8$, $p < .05$]. In HOT1-CE mice, DAYS 9 and 11 yielded significantly lower RATIO scores than DAY 3 ( p's < .05), suggesting tolerance developed over the course of ethanol exposure. Post-hoc testing of COLD2-CE data did not detect changes in RATIO over DAYS; it is possible that a less conservative test would detect differences between each day of treatment. No significant effect of DAYS was apparent in HOT2-CE [$F(4, 24) < 1$, n.s.] or COLD2-CE mice [$F(4, 32) < 1$, n.s.].

ERRORS

Analysis of the data for each selected line on ERRORS detected a significant change in ERRORs as a function of repeated exposure to ethanol in COLD2-CE mice only [$F(4,32) = 10.1$, $p < .0001$]. Post-hoc analysis revealed that more ERRORS were committed on Day 11 than on any other day (p's < .05). No other line had any significant changes in ERRORS as a consequence of repeated ethanol exposure [all F's < 3, n.s.].

ACTIVITY

Examination of activity in each line exposed to repeated ethanol treatments revealed significant changes in all four lines. Significant effects of DAYS was revealed in HOT1-CE [$F(4, 45) = 8.7$, $p < .0001$], HOT2-CE [$F(4, 40) = 7.8$, $p < .0001$], COLD1-CE [$F(4, 35) = 4.8$, $p < .005$], and COLD2-CE [$F(4, 45) = 30.6$ $p < .0001$]. Post-hoc analysis indicated that HOT1-CE mice were more activated by ethanol on Days 9 and 11 ($p < .05$). HOT2-CE mice had significantly higher activity counts on Day 11 than saline (Day 2), Day 3, 5, or 7 ($p < .05$). COLD1-CE did not differ as a result of ethanol treatment over days

(Newman-Keuls, $p > .05$), but day 2 (saline) yielded significantly higher activity scores than all other days. For COLD2-CE mice, all ethanol treatments resulted in significantly lower activity counts than saline treated mice. Day 3 activity counts were significantly less than all other ethanol-treatment days ($p's < .05$] and Days 9 and 11 produced significantly greater activity than Day 3, 5, or 7.

Blood Ethanol Concentration

BECs, measured after the last day of treatment, were not different between HOT and COLD mice in either TREATMENT group. No differences in BECs were detected for any factor [all $F's = 0.0 - 3.0$, n.s] (Figure 8). This indicates that the differences in ACTIVITY observed between HOT and COLD mice, and in HOT mice between treatment groups, was not due to pharmacokinetic mechanisms but rather an alteration in the sensitivity of the brain (i.e., target tissue).

## *Discussion*

Using the ratio of errors per activity counts to assess ataxia, the results indicated that the initial ataxic response to ethanol did not differ in HOT and COLD mice. Both selected lines were equally impaired by 2.5 g/kg ethanol. When tolerance was compared between line and treatment groups on the last day (Day 11), HOT mice showed tolerance but COLD mice did not. That is, the RATIOs for HOT mice given repeated ethanol treatment were significantly lower than either COLD mice given repeated ethanol or HOT mice exposed to ethanol for the first time. COLD mice given the chronic ethanol treatment did not differ from one another suggesting no tolerance developed with repeated ethanol exposure. Tolerance was assessed within each selected line to determine if, in

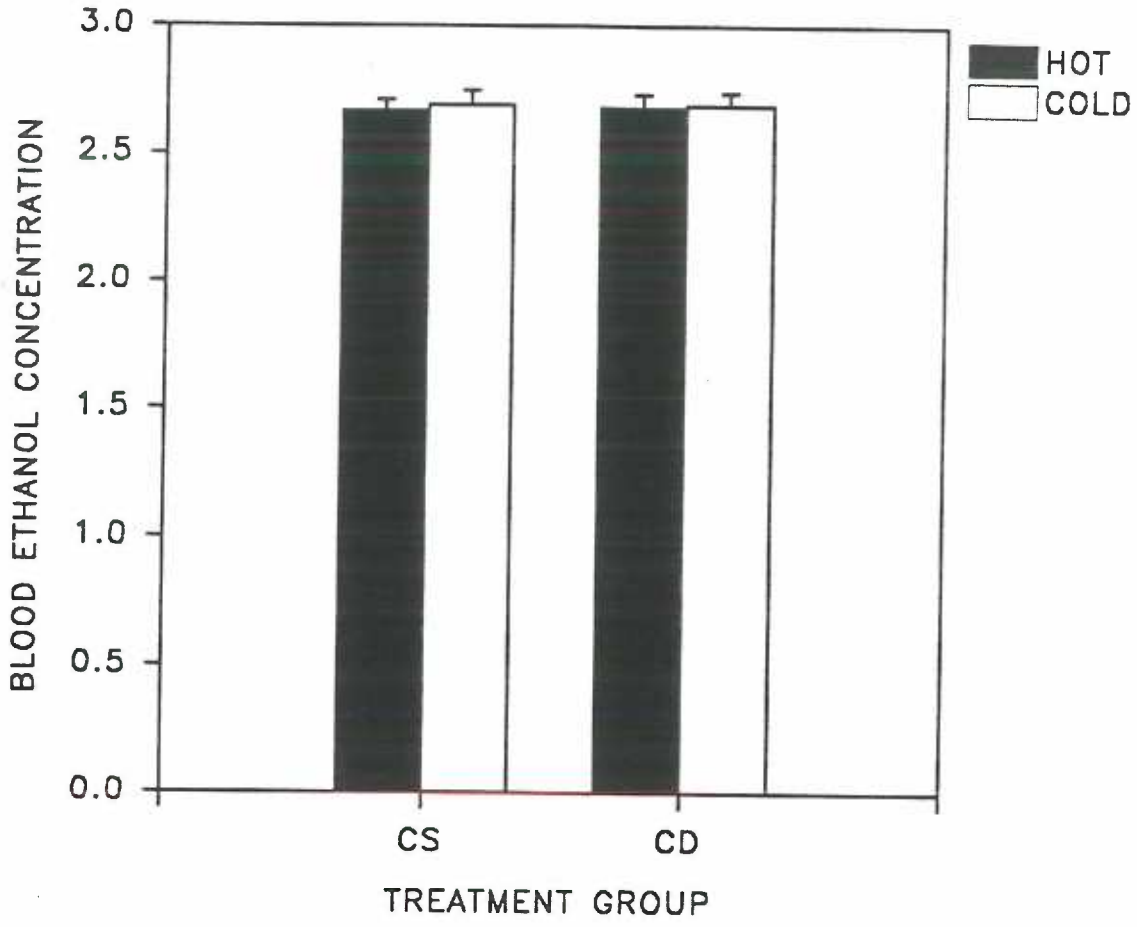


Figure 8. Blood ethanol concentrations from mice receiving the first (CS) or fifth (CE) ethanol injection. Data are mean BEC (mg/ml) ± SEM for each line. Blood was sampled immediately following the ten minute test. Data are collapsed over replicate since there were no significant differences.

fact, tolerance had developed at any point during the treatment. Using this procedure, it was determined that HOT1 and COLD2 mice (in the CE group) had become tolerant, but this effect had disappeared by Day 11 in COLD2 mice. A similar trend has been observed in COLD mice during chronic hypothermia tolerance testing at relatively high ethanol doses (> 2.5 g/kg) (Crabbe, 1994). It was quite clear that multiple exposures to ethanol resulted in an attenuation of the initial response. The decrease in RATIO observed in HOT1-CE and COLD2-CE mice appeared to be due to an increase in locomotor activity.

No tolerance developed to the occurrence of errors in any of the selected lines. In general, there were no changes in the number of missteps as a function of repeated ethanol treatment except in COLD2-CE mice, where the number of errors increased with each day of treatment. This might be expected to happen since locomotor activity increased each day and this can increase the likelihood of making errors. However, as discussed below, all lines increased activity as a function of repeated ethanol exposure, but a simultaneous increase in errors did not occur. Thus, COLD2 mice appeared to sensitize to the incoordinating effects of ethanol, as measured by errors.

The effect of an acute ethanol injection on locomotor activity was quite different in HOT versus COLD mice. Locomotor activity in COLD mice was significantly decreased from baseline by 2.5 g/kg ethanol whereas HOT mice remained relatively unaffected by this dose of ethanol. Analysis of locomotor activity as a function of repeated exposure revealed sensitization in HOT mice and tolerance to the initial depressant effect in COLD mice. Essentially the same response was observed in HOT and COLD mice after repeated ethanol exposures, that is, an increase in locomotor activity. However, since the initial effect of 2.5 g/kg was quite different between these mice, it cannot be assumed

that the same neurobiological mechanisms were involved in producing tolerance and sensitization.

## EXPERIMENT 4: Initial sensitivity and tolerance to ataxia-- Accelerating Rotarod

The purpose of this experiment was to provide a measure of tolerance in a design that incorporated initial sensitivity as well. Based on pilot studies, it was determined that HOT and COLD mice required prior experience with the accelerating rotarod before beginning ethanol treatment to achieve a stable basal performance level. Therefore, this study was divided into two parts: a practice phase, to stabilize performance, followed by the testing phase.

### *Methods and Materials*

Male mice from the HOT and COLD selected lines (generation 36) were tested. Ages ranged from 60-80 days old at the start of the experiment. Mice were given 2.5 g/kg ethanol (i.p.) or an equivalent volume of saline. Ten mice per line and replicate were tested in two treatment groups.

### *Procedure*

The experimental procedure is diagrammed in Table 2.

Practice Phase (Days 1-5): Mice were injected with saline (volume equivalent to 2.5 g/kg EtOH), placed into individual holding cages for thirty minutes, and then tested on the accelerating rotarod. A trial involved placing the mice on the rod surface, starting the accelerator and timer and measuring latency to fall and revolutions per minute (RPM) at time of fall. Mice were given three consecutive trials, the first of which was not counted. Performance on the second and third trials was averaged to obtain a score for that day. Testing

**Table 2.** Outline of experimental procedures for Expt 4. This experiment was divided into three parts: Practice, Baseline and Test phases. During Practice and Baseline phases and Postbaseline test all mice were given saline. CS = chronic saline; CE = chronic ethanol.

| | PRACTICE | BASELINE | TEST DAYS | | FINAL TEST | POSTTEST BASELINE |
|---|---|---|---|---|---|---|
| Day: | 1-5 | 7-8 | 9, 11, 13, 15, 17 | 10, 12, 14, 16, 18, 20 | 19 | 21 |
| Group | | | | | | |
| CS | SAL Test | SAL Test | Sal Test | -- | EtOH Test | SAL Test |
| CE | SAL Test | SAL Test | EtOH Test | -- | EtOH Test | SAL Test |

proceeded in this manner for five days by which time performance had reached a plateau (Figure 9). Mice were given a 48 hours respite before the test procedure resumed.

Test Phase (Days 7 - 19): Mice were given two days of saline testing (as described above), separated by 24 hours (DAYS 7 and 8). These scores were averaged and constitute the BASELINE or pre-drug performance. Twenty-four hours after baseline determination (DAY 9), mice were divided into two groups. The CS group was given saline and the CE group was given ethanol (CE). Mice were tested 30 minutes after injection. This procedure was repeated for four additional sessions, each separated by 48 hours (DAYS 11-17). On the sixth test day (DAY 19), all mice were tested after ethanol. On DAY 19, after completion of the test, a retro-orbital sinus blood sample (ROS) was taken from each mouse and processed for ethanol content. On Day 21, all mice were tested after a saline injection. This last test was used to determine if mice improved their undrugged performance as a result of repeated exposure to the apparatus and testing procedure.

### *Results*

Practice: Days 1 - 5

A two-way ANOVA, grouped by LINE and REPLICATE, indicated that COLD mice were able to perform on the rotarod better than HOT mice [$F(1,72)= 31.6$, $p < .0001$]. The mean score for COLD mice was 25.5 ± 0.7 rpm and 17.5 ± 0.5 rpm for HOT mice. There were no differences in Replicate [$F(1,72) = 1.1$, n.s.].

Baseline: Days 7 and 8

During this part, all mice received saline injections prior to rotarod

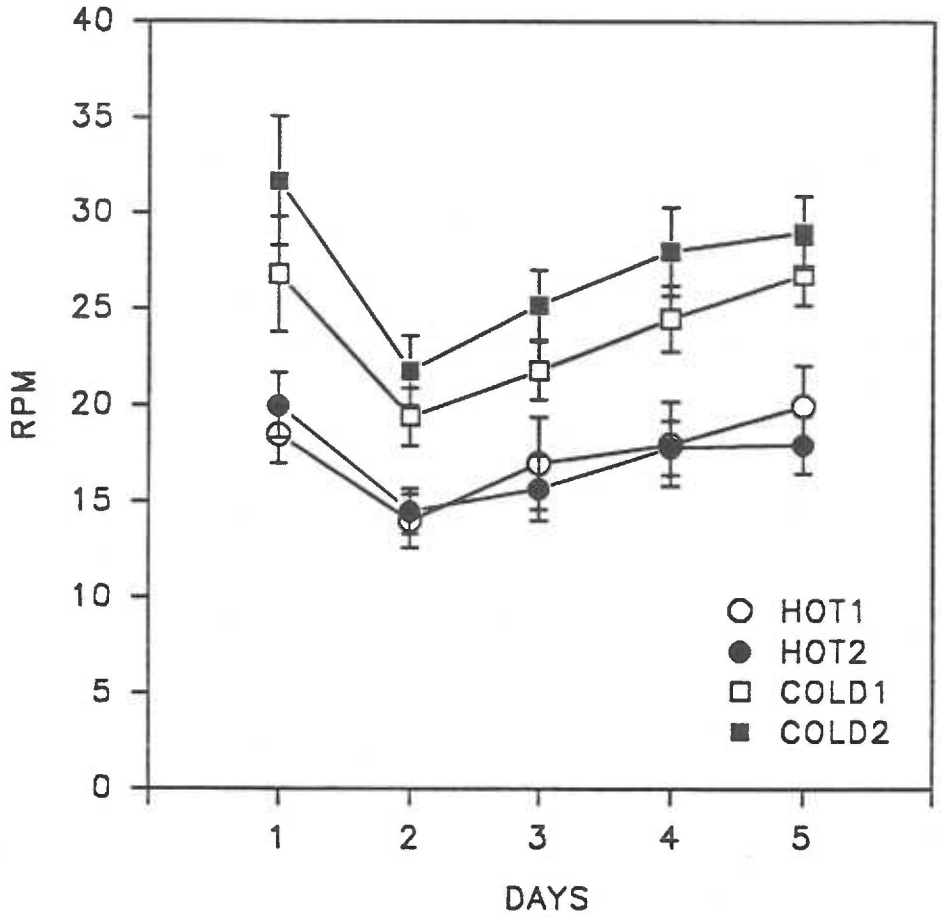


Figure 9. Performance on the accelerating rotarod during the practice phase. Mean speed of rotarod (in rpm) at time of fall (± SEM) is presented for each line and replicate. All mice were given saline and tested 30 minutes following injection for five days.

testing. Baseline performance was indexed by averaging performances from Days 7 and 8. HOT and COLD mice differed in their baseline performance on the accelerating rotarod. Each Replicate is presented separately in the graph. Despite previous experience with the accelerating rotarod, HOT and COLD mice were not able to perform at the same level. COLD mice performed better than HOT mice under saline treatment conditions. These data were analyzed by a three-way ANOVA that included grouping on LINE, REPLICATE, and TREATMENT. TREATMENT was included to assess whether there were any *a priori* differences in performance before ethanol treatment began. A three-way ANOVA revealed a significant main effect of LINE [$F(1, 68) = 8.2$, $p < .01$], supporting the observation that COLD mice were able to reach higher rotarod speeds before falling. No significant main effects of REPLICATE or TREATMENT [$F$'s $(1, 68) < 3.0$, n.s.] emerged. A marginally significant interaction of REPLICATE x TREATMENT was found [$F(1, 68) = 4.0$, $p = .05$]. Since there were no significant main effects involving either of these factors no further analysis on this interaction was performed. No other interactions were significant [all $F$'s $< 2.0$, n.s.].

Initial Sensitivity: Day 9

To index sensitivity and tolerance, difference scores [Day 9 - baseline, in rpm] were used to control for the line difference in baseline performance. A negative change in RPM indicated that the performance on the test day was worse than performance on BASELINE, while a positive change reflected performance on a test day which was better than baseline performance.

The first ethanol injections were given on Day 9, with only the CE group given ethanol; the CS group was given saline. The results for each Replicate from this day can be seen in Figure 10. The degree of sensitivity did not differ between the replicate lines of mice.

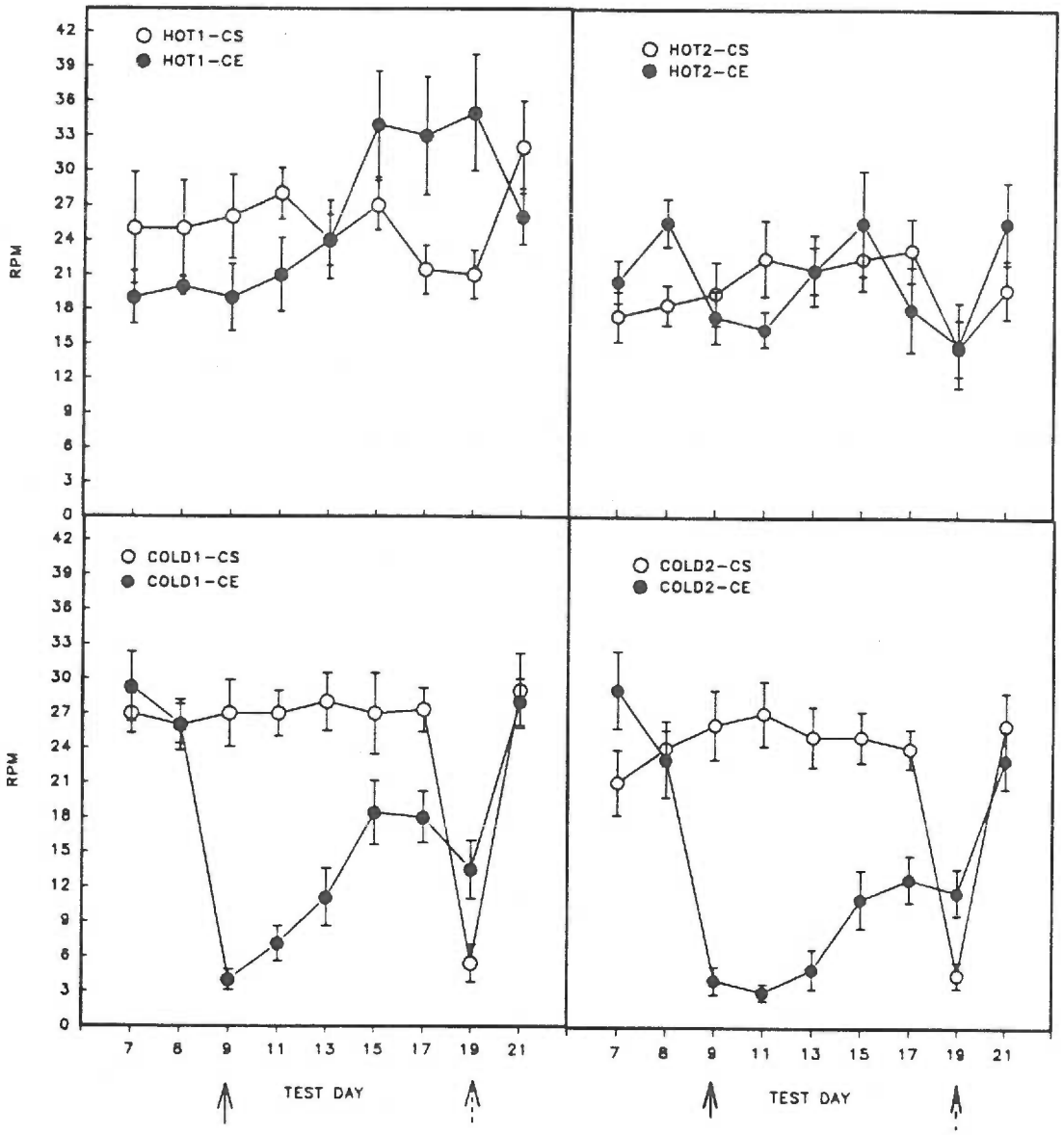


Figure 10. Performance on the accelerating rotarod 30 minutes after injection during the course of treatment for each Replicate Line. Speed in rpm at time of fall from the rotarod (mean ± SEM) are given. See Table 2 for outline of injection schedule. Solid arrow represents first ethanol injection for CE group; dashedarrow represents EtOH for CE and CS groups. On days 7, 8 and 21, all mice were given saline.

Initial sensitivity differences between HOT and COLD mice were revealed by significant main effects of TREATMENT [$F(1, 68) = 66.8$, $p < .0001$], LINE [$F(1, 68) = 29.3$, $p < .0001$] and significant LINE x TREATMENT interaction [$F(1,68) = 33.6$, $p = .0001$]. Simple main effects analysis indicated that ethanol produced a significantly greater change from baseline than saline in COLD mice but not HOT mice [$F(1, 68) = 97.9$, $p < .01$; $F(1, 68) = 3.3$, n.s., respectively]. Additionally, COLD mice were more sensitive to ethanol than HOT mice [$F(1, 68) = 61.4$, $p < .0001$] as indicated by their relative decrease in performance (see Figure 9), but the lines did not differ after saline treatment [$F(1, 68) = 0.1$, n.s.]. There was no significant effect of REPLICATE [$F(1,68) < 1.0$, n.s.] nor any significant interactions involving REPLICATE [$F$'s $< 1.5$, n.s.]. These results indicated that initial sensitivity was observed in both Replicates to approximately the same degree.

Tolerance: Day 19

Tolerance was indexed in a between-subjects design as the difference in performance between the CS and CE Treatment groups. A three-way ANOVA yielded significant main effects of LINE [$F(1, 65) = 53.4$, $p = .0001$], REPLICATE [$F(1, 65) = 4.4$, $p < .05$], and TREATMENT [$F(1, 65) = 5.8$, $p < 0.5$]. Significant interactions were found for LINE x REPLICATE [$F(1, 65) = 6.8$, $p < .05$], REPLICATE x TREATMENT [$F(1, 65) = 9.3$, $p < .01$], and LINE x REPLICATE x TREATMENT [$F(1, 65) = 5.4$, $p < .05$] but not for LINE x TREATMENT [$F(1, 65) = 0.3$, n.s.]. To simplify the interpretation of the three-way interaction, independent two-way ANOVAs were run for each REPLICATE using LINE and TREATMENT as between-subjects factors.

Tolerance was observed in Replicate 1 but not Replicate 2, as reflected as a significant difference between CS and CE groups. In both replicates HOT

mice were less impaired than COLD mice despite treatment condition. Analysis of Replicate 1 indicated main effects of LINE and TREATMENT were significant [$F(1, 31) = 43.0$, $p < .0001$ and $F(1, 31) = 13.0$, $p < .01$, respectively]. The interaction was not significant ($p = .065$). In Replicate 2, the main effect of LINE was significant [$F(1, 34) = 12.7$, $p < .01$]. The TREATMENT main effect and LINE x TREATMENT interaction were not significant [$F < 1$, n.s.]. The LINE difference indicated that HOT were less impaired than COLD mice, regardless of TREATMENT or REPLICATE.

Tolerance: Days 9 - 19 (CE group)

To examine the extent of tolerance development in the CE group mice, independent one-way ANOVA were run on the data for each line in this group. There was no significant effect of DAYS for HOT2-CE mice [$F(5, 40) = 2.2$, n.s.]. In HOT1-CE mice, the significant effect of DAYS [$F(5, 35) = 8.8$, $p < .0001$] was further analyzed by Newman-Keuls. Performance on Days 15, 17, and 19 was greater than on Days 9, 11, and 13 (Newman-Keuls, p's $< .05$). There was a significant effect of DAYS [$F(5, 40) = 8.9$, $p < .0001$] in COLD1-CE mice. Post-hoc analysis indicated that performance on the first ethanol test (Day 9) was significantly worse than on Days 13, 15, 17, and 19 (Newman-Keuls, p's $< .05$), performance on Day 15 was better than on Day 9, 11, and 13 (Newman-Keuls, p's $< .05$), and Day 17 performance was better than on Day 11. A significant of DAYS was detected in COLD2-CE [$F(5, 40) = 16.4$, $p < .0001$] and post-hoc testing indicated that on Days 15, 17, and 19, performance were significantly better than on Days 9, 11, and 13 (Newman-Keuls, p's $< .05$).

Saline Performance Test (Day 21)

On this day all mice were given saline and tested. The aim of this test was to determine whether baseline performance was altered by the repetitive

testing experience. Performance did not change between baseline measurement (days 7 and 8) and the post-ethanol baseline test (day 21). The pretreatment BASELINE averages were 22± 2 rpm, 27± 1 rpm, 20± 1 rpm and 24± 2 rpm for HOT1, COLD1, HOT2 and COLD2 mice, respectively. On Day 21 performances were 28± 2 rpm, 29± 2 rpm 22 ± 2 rpm, and 24± 2 rpm for HOT1, COLD1, HOT2 and COLD2 mice, respectively.

Blood Ethanol Concentration

Blood ethanol concentrations were measured after testing on day 19; the results are depicted in Figure 11. Significant main effects of LINE [$F(1, 64) = 31.2$, $p = .0001$] and for REPLICATE [$F(1, 64) = 11.6$, $p = .001$] were observed. There were no differences between treatment groups and no significant interactions [all $F's < 2$]. The difference in BEC observed between the selected lines was ~0.3 mg/ml ethanol (HOT1 = 2.30± .05 mg/ml; HOT2 = 2.39± .03 mg/ml; COLD1 = 2.47± .03 mg/ml; COLD2 = 2.64±.04 mg/ml) which is probably not enough produce the behavioral difference observed between the lines of mice.

***Discussion***

The accelerating rotarod had some advantages over the fixed-speed rotarod which made it amenable to this experimental design. Using the accelerating rotarod undrugged performance could be quantified for each mouse since there was an individual limit to how long each mouse could remain on the rod (i.e., how fast the mouse can run). Undrugged mice are able to stay on the fixed-speed rotarod indefinitely.

The design of this test was able to incorporate measures of initial sensitivity and tolerance to the ataxic effects of ethanol. The results indicated a marked difference in sensitivity to ethanol between HOT and COLD mice; COLD

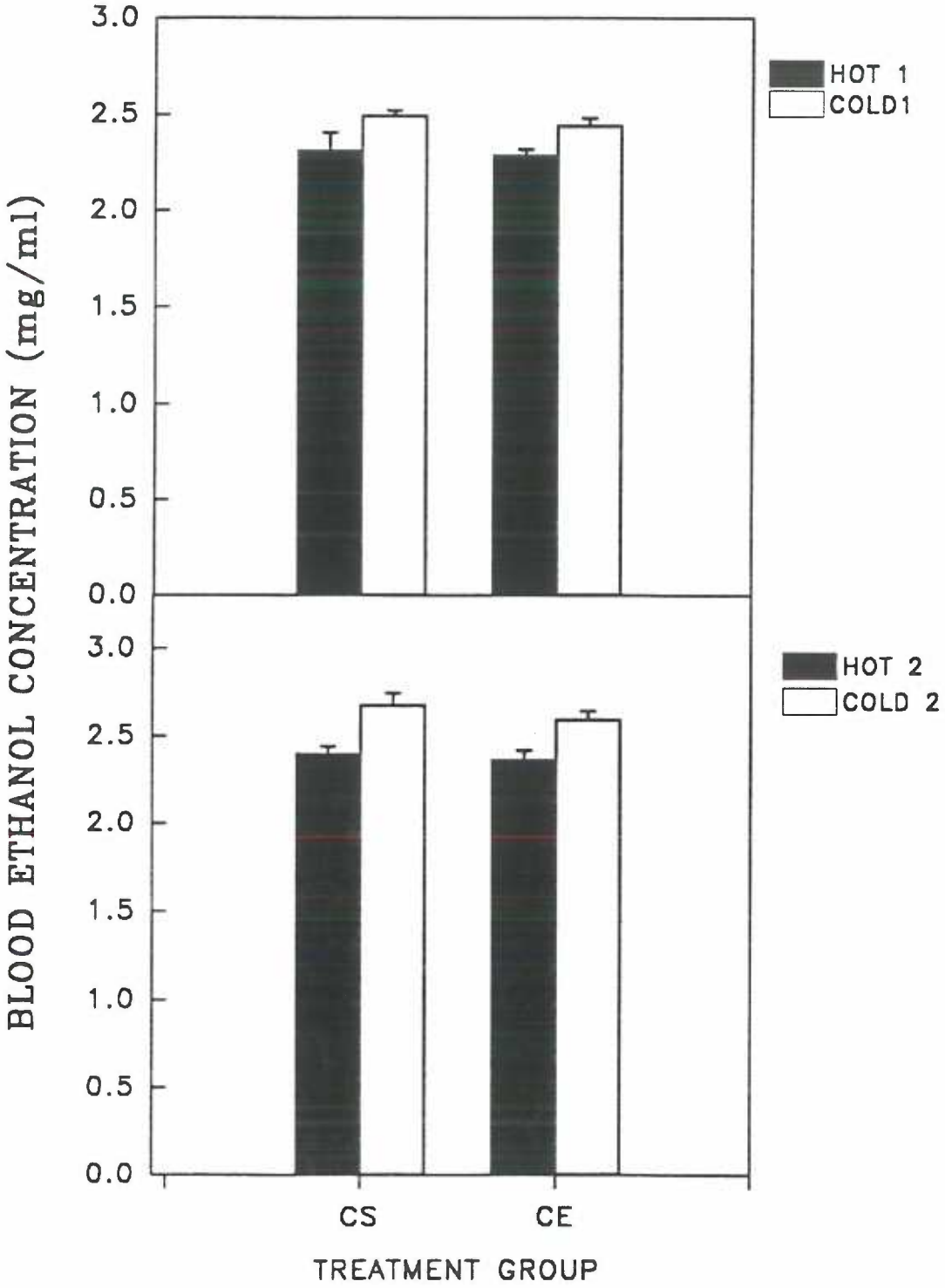


Figure 11. Blood ethanol concentration on Day 19. BEC values are presented as mean (mg/ml) ± SEM. Blood was sampled from retro-orbital sinus at time of fall from accelerating rotarod. Each replicate line is presented.

mice were more sensitive to ethanol than HOT mice. This difference was apparent when determined on Day 9 (after the first ethanol exposure in CE group) and on Day 19 when only the CS group was compared (see Figure 10).

Tolerance was not as clearly apparent. Although the selected lines differed in performance on Day 19, these differences did not appear in the treatment groups of each replicate. It is interesting that the Treatment effect did not appear in Replicate 2 but was apparent in Replicate 1. The nearly significant interaction in Replicate 1 was due to a better than baseline performance in HOT1-CE mice while COLD -CE mice were still performing worse than their baseline ability. In Replicate 2, this trend was not observed. Instead of HOT2-CE mice improving performance by the last day of treatment, these mice actually had slightly lower scores than did their HOT2-CS counterparts. The COLD mice had similar changes in performance in the respective treatment groups across Replicates.

The between-groups comparison controls for handling, injection, and testing procedures so that the two treatment groups differ only in number of ethanol exposures. Of course, how performance was affected by each successive ethanol exposure cannot be extracted from this type of comparison, but it can be determined from the repeated measure analysis. When each line was assessed individually for the development of tolerance, it was apparent that there was a significant effect of repeated ethanol exposure. Tolerance clearly developed in both COLD lines (see Figure 10a, b). However, since neither HOT line was initially impaired, assessment of tolerance, per se, was not warranted. However, the effects of repeated ethanol treatment in HOT1 mice produced a sensitization-like behavior. These mice improved performance on the accelerating rotarod, from BASELINE, with repeated ethanol exposure.

## SUMMARY AND CONCLUSION

An important goal of ethanol research is to define and determine the mechanisms of action of ethanol. Given the profuse actions of ethanol in the CNS and its ability to interact with many neurotransmitter systems, behavioral responses to ethanol are likely to be elicited by many mechanisms. Some of these responses may be under the same control; others may be completely unrelated. The present experiments attempted to determine whether ethanol-induced hypothermia and ataxia share common genetic control mechanisms. It was predicted that COLD mice would be more sensitive to ataxia than HOT mice if these two responses were influenced by the same genetic mechanisms. In addition, the relationship between ethanol-induced locomotor activity and hypothermia was also investigated. The design of these experiments allowed a comparison among different tasks measuring ataxia, a determination of locomotor activity, and a comparison of the estimates of the degree of correlation between the responses of hypothermia and ataxia in inbred mice and in selected lines. The results addressed the following questions: were the tests employed here measuring the same behavior? and were the responses of hypothermia and ataxia sensitivity that were initially observed to be correlated in inbred mice also correlated when measured in selected lines?

These data did not support the general hypothesis that ethanol-induced hypothermia and ataxia are genetically related. The hypothesis was based on significant phenotypic correlations (Crabbe et al., 1981) and genetic correlations (Crabbe et al, 1982; Crabbe, 1983). However, not all correlations between hypothermia and ataxia measures were significant. Studies of heterogeneous stocks of mice, which provide information on the genetic and environmental influence, and of inbred mouse strains, which provide an

estimate of genetic variability, suggested that hypothermia and ataxia sensitivity were phenotypically and genetically correlated. A modest phenotypic correlation between LORR and hypothermia sensitivity and a small correlation between LORR and hypothermia tolerance was detected in genetically heterogeneous mice (Crabbe et al., 1981). Although LORR might not be considered ataxia *per se*, it represents an additional depressant effect of ethanol, and may therefore be correlated. That study provided evidence for a phenotypic correlation between hypothermia and LORR. Crabbe and colleagues (1982, 1983, 1994) tested inbred strains of mice for responses to ethanol including hypothermia, ataxia and activity to determine the extent of a genetic association between responses to ethanol. A positive correlation between homecage ataxia and hypothermia was found (Crabbe et al., 1982) but no other ataxia measures [grid test (Crabbe, 1983) or rotarod (Crabbe et al., 1994)] yielded significant correlations with hypothermia. A nonsignificant positive or negative correlation does not necessarily imply that the genetic correlation does not exist. For example, large numbers of inbred strains are often necessary to detect a significant correlation; however, this was achieved in the aforementioned inbred strains. There might be a large number of genes involved but each with weak influence, making it difficult to detect significant correlations. Given the variability of results of correlations between hypothermia and ataxia from inbred strains, it is not surprising that hypothermia and ataxia were not genetically related to any significant degree in HOT and COLD mice. Table 3 summarizes the results from the present experiments as a comparison between HOT and COLD mice for each measurement. It should be noted that ACTIVITY represents sensitivity to the depressant effects and an increase from the initial response for the effect of repeated drug administration.

**Table 3.** Summary of Ataxia test results for initial sensitivity and tolerance for each replicate line (test dose and time of testing are indicated for each experiment).

| TEST | DOSE (g/kg) | TEST TIME (after Inject) | Acute Response | Effect of Repeated EtOH |
|---|---|---|---|---|
| **Screen** (Expt. 1) | 1.9 -3.1 | 30 min | HOT1 = COLD1<br>HOT2 = COLD2 | nd<br>nd |
| **Fixed-speed Rotarod** (Expt. 2) | 2.5 | immediate | Latency to Fall<br>HOT1 = COLD1<br>HOT2 = COLD2 | <br>nd<br>nd |
| **Grid** (Expt. 3) | 2.5 | 0-10 min | Ratio<br>HOT1 = COLD1<br>HOT2 = COLD2 | Decreased Ratio<br>HOT1 = COLD1<br>HOT2 > COLD2 |
| | | | Errors<br>HOT1 > COLD1<br>HOT2 = COLD2 | Decreased Errors<br>No change<br>No change |
| | | | Increased Activity<br>HOT1 < COLD1<br>HOT2 < COLD2 | Increased Activity<br>HOT1 > COLD1<br>HOT2 > COLD2 |
| **Accel. RPM Rotarod** (Expt. 4) | 2.5 | 30 min | RPM at Fall<br>HOT1 < COLD1<br>HOT2 < COLD2 | Increased<br>HOT1 = COLD1<br>HOT 2 < COLD2 |

'>' indicates HOT line more affected; '<' indicates COLD line more affected.
nd = not determined

If other hypothermia-sensitive and resistant lines were available, similar investigations of this problem could be made. Unfortunately, no other rat or mouse lines have been selected for the hypothermic response to ethanol, but several rat lines have been selected for motor incoordination and subsequently tested for hypothermia. Similar investigations of these responses have been performed in rat selected lines described earlier. When tested for sensitivity to hypothermia after an i.p. injection of 4 g/kg ethanol, no differences between the AT and ANT lines were detected except at 60 minutes after injection, when ANT rats were 0.6°C colder than AT rats (Eriksson and Sarviharju, 1984). Although times of hypothermia testing were not explicitly given in the report, it appears that 60 minutes was the earliest time point measured. It is possible that a greater difference may have been present at earlier times after ethanol administration. In contrast to these results, MA rats were found to be more sensitive to ethanol-induced hypothermia than LA rats (Mayer et al., 1983). Again, there are mixed results in selected lines regarding the CR of hypothermia and ataxia suggesting that there is not a strong genetic relationship between these responses, if at all.

In addition to not supporting the general hypothesis, the results from the individual experiments with HOT and COLD mice did not reveal similar results between them (see Table 3 for summary). All of the tasks assessed sensitivity to ataxia: however, three of the four tests of ataxia did not detect genetic differences. This suggests that the ability of the tasks to detect sensitivity to ataxia was not equivalent or that the tasks also measured sensitivity to different responses that may be related to ataxia. The three tests (Expt. 1, 2, and 3) which produced equivalent sensitivity in HOT and COLD mice might not have been able to measure subtle genetic differences. Alternatively, Expt. 4, which

did detect a line difference in sensitivity, might not have solely measured ataxia. Additionally, several environmental factors can influence the expression of sensitivity and tolerance, such as test dose, measurement system, time of measurement, and schedule of ethanol administration. It is reasonable to conclude that these factors could have contributed to the discrepancy in correlated response results between inbred mouse studies and the present experiments.

***Ataxia measurement***

The results from the different tests of ataxia suggest that some of these tasks may be more capable for detecting sensitivity to ataxia or detecting subtle genetic differences in sensitivity. In humans, ataxia is a general term used to describe myriad motor and sensory afflictions. There are many neuroanatomical origins of ataxia including cerebellar, spinal, or vestibular lesions. Chemical-induced ataxia, such as that produced by ethanol, may arise from a mechanism shared by one of the naturally occurring ataxias. The seemingly complex nature of the ataxic phenotype adds to the difficulty of determining the most sensitive method for measuring ataxia in animal models. Accordingly, the tasks chosen to measure ataxia in the present set of experiments may be measuring sensitivity to other effects of ethanol that could lead to a masking of ataxia differences in HOT and COLD mice. As was discussed previously (Expt. 1), the screen test appeared to detect alterations of grip strength. Although HOT and COLD mice have not been tested for grip strength changes after ethanol, it is possible that these mice do not differ in the effects of ethanol on grip strength. Thus, if the screen test measured grip strength preferentially to ataxia, then this is what would have been reflected in the results. Unfortunately, the experiments were not designed to dissociate muscle weakness from ataxia. However, it appears less likely that grip strength influenced the results of the screen test to

such an extent; one might expect muscle weakness to affect the rotarod tasks as well.

Experiment 4 was the only experiment in which a significant difference was found in sensitivity to ataxia between HOT and COLD mice. The pattern of results from the accelerating rotarod mirrored the activity results of Expt. 3 (Fig. 7 vs. Fig 10), in which HOT and COLD mice differed in sensitivity to the effects of ethanol on locomotor activity (see Table 3). That is, baseline locomotor activity of COLD mice was markedly decreased by 2.5 g/kg ethanol and COLD mice fell from the accelerating rotarod at a much lower speed after an acute 2.5 g/kg injection than baseline. For HOT mice, on the other hand, locomotor activity and accelerating rotarod performance were unaffected by the initial administration of ethanol. The similarity of results suggests that the accelerating rotarod might have been highly sensitive to locomotor activity as opposed to ataxia.

***Dose-response effects***

Due to the dose-specific nature of the ethanol response, the use of a single dose of ethanol to measure ataxia or hypothermia limits the interpretation of results. Low doses of ethanol typically elicit an activating response, whereas higher doses elicit the characteristic CNS depressant effects (Pohorecky, 1977). The same dose of ethanol (2.5 g/kg) was used to test HOT and COLD mice in the present studies allowing these results to be directly compared to one another. If different doses of ethanol had been used to test HOT and COLD mice in these experiments, this might have had an effect on the nature of the response. For example, testing HOT and COLD mice with the dose of ethanol used during selection (3 g/kg) might have yielded slightly different results in ataxia. However, results from the screen test (Expt 1) demonstrated that the same degree of ataxia was produced by 3 g/kg in both HOT and COLD mice. A

similar argument applies to each of the tests. The strain difference in sensitivity between C57BL/6J and DBA/2J mice did not change as a function of dose when measured by the grid test with ethanol doses ranging from 1.5 to 2.5 g/kg (Belknap, 1975, 1982). There is conflicting evidence regarding the effects of ethanol dose on the performance on the rotarod test in LS and SS mice. At relatively low doses of ethanol (1.4 to 2.0 g/kg), LS and SS mice did not differ in sensitivity (Sanders, 1976). A later study found LS mice to be more sensitive than SS mice when tested at 2 g/kg ethanol (Stinchcomb, Bowers and Wehner, 1989). In that study, SS mice were also tested after 2.5 g/kg but LS mice were not because of the substantial degree of impairment caused by the lower dose. A floor effect most likely would have prevented the ability to detect line differences at the higher dose. The results from these two studies suggested that the fixed-speed rotarod may have a rather small window of sensitivity detection. The results from the rotarod test in HOT and COLD mice presented here suggested that there would still be room for increased impairment at higher doses (see Fig. 3). However, based on the results from other tests of ataxia (screen test, grid test), a differential response between HOT and COLD mice at a higher dose does not seem likely. If hypothermia and ataxia were genetically associated in HOT and COLD mice, a difference in sensitivity at this test dose would still be possible since COLD mice exhibit a decrease of 4°C in body temperature while HOT mice are unaffected at 2.5 g/kg (Crabbe, 1994).

It is more likely that locomotor activity results would be affected by the change in test dose. Line differences have been observed in other studies of locomotor activity using HOT and COLD mice at different doses of ethanol. After mice were given 2.0 g/kg ethanol and tested for open-field activity, HOT2 mice were more activated than COLD2 mice but no differences were observed between HOT1 or COLD1 (Phillips and Crabbe, 1991). At a slightly higher dose

of ethanol (2.25 g/kg) and using a different measuring apparatus, HOT mice showed a significant increase in activity up to thirty minutes following injection, whereas COLD mice showed an initial activation that dissipated after five minutes but never fell below that of saline-treated COLD mice (Cunningham et al., 1991). These studies suggest that HOT and COLD mice differ in sensitivity to the locomotor effects of ethanol in a dose-dependent manner. This apparent CR could be a result of the pleiotropic effects of a subset of genes that control ethanol-induced hypothermia, a selection for genes involved in mediating the depressant effects of ethanol in COLD mice, or chance fixation of genes unrelated to hypothermia.

The dose of ethanol used for the present experiments was not used in any of the studies involving inbred mice. The correlational studies by Crabbe et al. (1994, 1981, 1982) used ethanol doses ranging between 1 g/kg and 4 g/kg but never used 2.5 g/kg. It is unlikely that this would account for the lack of corroboration between results from the inbred strains and HOT and COLD mice. It should be noted that 4 g/kg ethanol was used to measure LORR sensitivity, which was significantly correlated with hypothermia sensitivity, but HOT and COLD mice do not appear to be differentially sensitive to this effect (Crabbe, unpublished). Since no significant correlation between hypothermia and locomotor activation or depression was obtained over the range of ethanol doses used in Crabbe et al. (1994, 1982), a positive relationship between hypothermia sensitivity and locomotor activity depression as determined from the current results was not expected.

***Time of behavioral measurement***

Another important determinant of sensitivity to ethanol is the time after injection when measurements are made. Although ethanol is pharmacologically classified as a CNS depressant, even high doses of ethanol produce

stimulant effects at early timepoints after injection (Pohorecky, 1977). For example, measuring cumulated locomotor activity after a large dose of ethanol over an extended period of time will reflect stimulant and depressant effects of ethanol. When measuring an acute response over an extended period of time acute tolerance may also result. Therefore, when making comparisons at a single timepoint after injection, a difference in sensitivity could reflect differences in the development of acute tolerance. LeBlanc, Kalant, and Gibbins (1975) showed a parallel shift to the right of the dose-response curve in rats thirty minutes versus ten minutes after an acute ethanol injection, which demonstrated the development of acute tolerance by this time point. Experiment 4 (accelerating rotarod) was the only experiment to detect a difference in sensitivity (Fig. 10 and Table 3). Since testing did not occur until thirty minutes after ethanol was administered, it was possible that acute tolerance developed in HOT mice but not in COLD mice. However, results from the other experiments do not suggest that there are differences in the development of acute tolerance. Experiment 1 (screen test) also measured ataxia thirty minutes after injection but revealed no difference in sensitivity at 2.5 g/kg or any other dose of ethanol. Likewise there were no differences detected for the ataxia measurement in Expt. 3 (grid test), which measured ataxia for the first ten minutes after injection. Locomotor activity did differ between HOT and COLD mice when measured for the ten minute period following injection and it is unlikely that this difference would be gone by thirty minutes (cf. Cunningham et al., 1991; Crabbe et al., 1988). Unpublished results from this lab suggest that acute tolerance to ataxia does not develop in either HOT or COLD mice. BEC at onset of ataxia (see Fig. 4) were not different from BEC at recovery from ataxia as measured by the fixed-speed rotarod (unpublished results). Thus, it appeared that the time of behavioral measurement with respect to

administration of ethanol was not a significant factor in the disparity of results in HOT and COLD mice. Likewise, it would not be able to explain the lack of correlation between hypothermia and ataxia.

***Tolerance***

Inconsistent results were generated from the experiments on tolerance to ataxia. When chronic ethanol-treated mice were compared with naive mice receiving their first ethanol injection, a differential tolerance response was elicited from the accelerating rotarod test: HOT1 mice were more tolerant than COLD1 mice, but no tolerance developed in either line of Replicate 2. The same magnitude of tolerance developed in HOT1 and COLD1 mice when tested with the grid test, whereas HOT2 mice developed tolerance but COLD2 appeared to sensitize to ataxia. When tolerance data were analyzed by repeated measures ANOVA, the development of tolerance was apparent in both HOT and COLD mice in the accelerating rotarod test (see Fig. 16).

As with sensitivity, tolerance development is specific to treatment dose and response measured. Chronic treatment of rats with 2 g/kg ethanol for 18 days led to the development of tolerance to the hypothermic effects but not to the cardioaccelerating effects (Peris and Cunningham, 1985). Lê, Khanna, and Kalant (1984) found tolerance development to the hypothermic effects of ethanol with three doses although the rate and magnitude of tolerance development varied with dose. A similar dose regime only produced a measurable amount of tolerance to ataxia with low to moderate doses of ethanol. In HOT and COLD mice tested at 22°C or 18°C, tolerance to hypothermia was only observed in COLD mice tested (Crabbe, Feller, and Dorow, 1989). The present results indicated that under similar treatment conditions and dose schedules, similar patterns of development of tolerance to ataxia resulted in both selected lines. That is, there tended to be a trend of decreasing impairment after 3-4

treatments, but then an attenuation of tolerance. However, since hypothermia was not simultaneously measured it is unclear how the applied treatment regime would affect the hypothermic response in HOT and COLD mice. Previous studies have indicated that there is an attenuation of the hypothermic response in COLD mice treated chronically with ethanol doses greater than 3 g/kg (Crabbe, 1994).

The effects of chronic treatment on locomotor activity were quite different from those observed for ataxia. HOT mice were unaffected by the initial administration of ethanol in both the accelerating rotarod and locomotor activity and sensitized to the activating effects with repeated ethanol administration, although sensitization was observed in both replicates for locomotor activation and only in Replicate 1 for the accelerating rotarod.

In summary, the overall pattern of results suggested that the accelerating rotarod was less specific for ataxia detection in HOT and COLD mice than the fixed-speed rotarod, screen test and grid test. This difference was not affected by the dose of ethanol used or the time of measurement of impairment. An alternative interpretation could be that the accelerating rotarod is the most sensitive of the tasks. However, the similarity of results from the accelerating rotarod and locomotor activity indicate that the former hypothesis is true. This produces a bit of conflict with its use in behavioral research because the accelerating rotarod has been used as a screen to test for the incoordinating effects of drugs (Hoffman, Tabakoff, Szabo, Suzdak, and Paul, 1987; Bogo, Hill, and Young, 1981). It may be that the method of testing (i.e., using a single timepoint that signifies neither onset nor recovery of impairment) was not the best way to measure sensitivity. It may have been better to take the same approach as the fixed-speed rotarod and start testing immediately after injection, or to have determined recovery from impairment.

***Future Directions***

The relative roles of other effects of ethanol on ataxia need to be investigated in order to determine if all the behavioral tasks used in these experiments are equally sensitive to ataxia. Along these lines, HOT and COLD mice need to be tested for the effects of ethanol on grip strength, for example, to determine if there is a sensitivity difference that would interfere with the expression of ataxia.

Locomotor activity appeared to be strongly correlated with hypothermia sensitivity in HOT and COLD mice. Although a full dose-response curve has not been generated, HOT mice appear to be more activated, or less depressed, by ethanol doses up to 2.5 g/kg. It is interesting that these two responses appear to be correlated when they were not correlated in inbred or heterogeneous stocks of mice. It would be interesting to see if alterations of locomotor activity sensitivity would have effects on locomotor activity and hypothermia in HOT and COLD mice. For example, dopamine antagonists are known to attenuate ethanol-induced locomotor activation (Imperato and DiChiara, 1986; Liljequist, Berggren, and Engel, 1981). However, pretreatment of HOT and COLD mice with some dopaminergic agents did not differentially affect sensitivity to hypothermia in HOT and COLD mice (Feller and Crabbe, 1991).

The ultimate goal of this work should be to determine if similar neuro-chemical and neuroanatomical substrates are involved in the determination of ethanol sensitivity. However, significant correlated responses need to be established. In HOT and COLD mice, locomotor activity appears to be a better response system than ataxia to investigate CR with hypothermia.

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