ETHANOL WITHDRAWAL IN MICE GENETICALLY SELECTED FOR HIGH AND LOW ETHANOL WITHDRAWAL SEIZURES

by

Ann Kosobud

A THESIS

Presented to the Department of Medical Psychology
and the Graduate Council
of the Oregon Health Sciences University
School of Medicine
in partial fulfillment of the requirements for the degree of

Master of Science

March, 1986

APPROVED:

[redacted]

(Professor in charge of thesis)

[redacted]

(Chairman, Graduate Council)

TABLE OF CONTENTS.

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

My thanks go to John Crabbe, a good advisor and a good friend. Thanks also to Emmett Young, Brenda Tam and Nancy Johnson, for assistance in the laboratory at various stages of these experiments and for moral support throughout. These experiments were supported by grants AA 05828, AA 06243 and AA 06498 from the National Institutes on Alcohol Abuse and Alcoholism; by the Veterans Administration; by a grant from the Medical Research Foundation of Oregon, and by a fellowship from the N.L. Tartar Research Foundation to Ann Kosobud.

AN ABSTRACT OF THE THESIS OF

Ann Kosobud for the M.S. degree in Medical Psychology

Date of receiving this degree_____

Title: Ethanol Withdrawal in Mice Genetically Selected for High and Low Ethanol Withdrawal Seizures.

Approved_____

(Professor in charge of thesis)

We are engaged in a selective breeding program developing lines of mice which differ in severity of withdrawal convulsions following ethanol treatment. Withdrawal seizure prone (WSP) mice show greater handling-induced convulsion scores than withdrawal seizure resistant (WSR) mice following three days of ethanol intoxication. In the present experiments, we sought to characterize these mice further as a model of genetic susceptibility to ethanol dependence and withdrawal.

Symptoms of withdrawal following ethanol treatment have been observed in many species including man (Friedman, 1980). In general, ethanol withdrawal symptoms have been determined by observation and description, while the means by which ethanol treatment results in a withdrawal syndrome remains poorly understood. It is not known, for instance, whether the various symptoms which appear during ethanol withdrawal are the result of a single action or multiple actions of ethanol. By genetically selecting mouse lines for severe and mild handling-induced convulsions during ethanol withdrawal, we also have

selected these mice for severe and mild appearance of any other withdrawal symptoms that have a similar physiologic basis as handling-induced convulsions. Thus the first information these mice can offer us is whether the causes of ethanol withdrawal are single or multiple.

My primary goal was to compare these mice during ethanol withdrawal on a variety of withdrawal symptoms, and determine which are genetically related to handling-induced convulsions. It is possible that in selecting mice for differences in ethanol withdrawal, we have also selected mice for differences in metabolism of ethanol. In particular, our WSR line may metabolize ethanol faster than our WSP line, resulting in lower blood ethanol concentrations for the WSR line during treatment, and accounting for the difference in withdrawal severity which is observed between these lines of mice. In the present experiments, I examined metabolism of ethanol following acute and chronic treatments, and withdrawal from ethanol following acute and chronic treatments, in the WSP and WSR mice. I determined that the WSP and WSR mice did not differ in maximum blood ethanol concentration achieved, or in elimination of ethanol, following a single injection of ethanol. WSP and WSR mice also did not differ in blood ethanol concentration during three days of ethanol intoxication with pyrazole treatment (an alcohol dehydrogenase inhibitor), nor did they differ in elimination of ethanol following this intoxication. Six to 10 hours after an acute injection of ethanol, WSP and WSR mice showed elevated handling-induced convulsions. This elevation was more pronounced in

WSP than in WSR mice. WSP mice also showed slightly more severe convulsions than WSR mice when treated with saline or pyrazole alone. During withdrawal after chronic treatment with ethanol, WSP mice displayed more severe handling-induced convulsions and also more tremor than WSR mice. Several other measurements of ethanol withdrawal failed to distinguish WSP from WSR mice.

In summary, mice that have been selected for genetic differences in susceptibility to one ethanol withdrawal sign, HIC, and given identical doses of ethanol differ in some, but not all, symptoms of ethanol withdrawal measured in these experiments. This suggests that the actions of ethanol which result in the appearance of HIC and tremor during ethanol withdrawal are genetically distinct from those underlying other signs of withdrawal. The WSP and WSR mice thus constitute a population in which to study one component of the complex phenomenon of ethanol withdrawal.

INTRODUCTION

Alcoholism develops as the result of a complex set of physiological and environmental factors, the elements of which are largely unknown. Differences in experience and genetic make-up affect the response of a given individual to ethanol. How then can one judge how seriously someone will be impaired in a given drinking episode, or what his or her long term potential is for suffering the social and biomedical consequences of alcoholism? The ability to predict an individual's liability for developing alcoholism could reduce the personal and social cost of alcohol abuse.

Research utilizing animals to study alcoholism must necessarily limit itself to questions that can be addressed in animals. Because there are social and psychological aspects of alcoholism which make a complete animal model of alcoholism unattainable, most animal research has been directed at modelling specific components of human alcoholism, with much success (Deitrich and Spuhler, 1984). The research undertaken here is concerned with the physiological effects of ethanol, indeed, with those which can be shown to have a genetic basis. My research is intended to further an understanding of physical dependence on ethanol, which is one component of the alcoholic syndrome (Crabbe et al., 1985). Lines of mice have been genetically selected for differing susceptibility to handling-induced convulsions during withdrawal from chronic ethanol treatment. The Withdrawal Seizure Prone (WSP) and Withdrawal Seizure Resistant (WSR) mice comprise a useful model in which to study the genetic basis of ethanol physical dependence and the

physiological mechanisms underlying physical dependence and related actions of ethanol. Studies with these lines should facilitate prediction of how a given mouse will respond to ethanol. Ultimately, the goal is to extrapolate to humans to identify those individuals who carry a high genetic risk of developing alcoholism. This thesis begins with a description of the metabolic processes involved in the elimination of ethanol, the behavioral effects of ethanol during intoxication and withdrawal, and some current theories of the physiological bases of these effects, and is followed by a discussion of genetic selection techniques. The remainder of the paper describes four experiments concerned with ethanol's action in the WSP and WSR selected lines, with a discussion of the results obtained and the implications of the results for the study of ethanol effects.

Absorption, distribution and elimination of ethanol.

Ethanol is a small, hydrophilic molecule which moves readily through the mucosal epithelial layers of the stomach, small intestine and colon, and into the blood. In vapor form, it can be absorbed through the lungs. It distributes initially into the extracellular fluid, but equilibrates with total body water within 60 minutes (Theiden et al., 1983). Equilibration of ethanol concentration in various tissues is dependent on blood supply. Brain, which is highly vascularized, equilibrates with arterial blood almost immediately, but venous blood, which continues to lose ethanol to less vascularized tissues, can take an hour or more to equilibrate with arterial blood following an i.p. injection of ethanol (Goldstein, 1983). The lipid-water partition coefficient of ethanol is close to 0.1; ethanol

concentrations in body lipids tend to be about 10 times lower than in body water.

Only about 10-15% of an administered dose of ethanol is eliminated through expired air or urine. The rest is oxidized to acetaldehyde and then to acetate, which enters a variety of metabolic pathways (Amir et al., 1980). Most of the oxidation of ethanol occurs in the liver, although other tissues have some oxidative capacity. The brain has almost no measurable ethanol metabolizing capacity (Goldstein, 1983). Three enzymes are responsible for the oxidation of ethanol in the liver. These enzymes are alcohol dehydrogenase, the microsomal ethanol-oxidizing system, and catalase.

The most important is thought to be alcohol dehydrogenase, (ADH), which requires NAD as a cofactor. Human liver ADH is a dimer, made up of one of six possible combinations of three subunits. Each of these subunits in turn has variations, and additionally several other forms of ADH have been identified, each with its own activity (Von Wartburg and Bühler, 1984). The microsomal ethanol oxidizing system (MEOS), which requires the presence of NADPH and molecular oxygen, plays a smaller role in the metabolism of ethanol (Goldstein, 1983), although it may contribute as much as 35-70% of ethanol metabolism at high blood ethanol concentrations (30-40 mM or 2.4-4.2 mg/ml) (Takagi et al., 1985). The third enzyme, catalase, uses hydrogen peroxide as a cofactor in the oxidation of ethanol. Catalase is present in huge quantities in the liver, where it has a protective role in preventing tissue damage from hydrogen peroxide created as a byproduct of certain reactions. Catalase has been shown to be capable of metabolizing ethanol in vitro, but the available evidence suggests that its role in

ethanol oxidation *in vivo* is small, and limited by the availability of hydrogen peroxide (Leiber *et al.*, 1975). The variation in ethanol metabolic rate in humans is very large, and genetic differences in enzymes account for about half of this variation (Kopun & Propping, 1977).

Ethanol is oxidized by ADH to acetaldehyde, which is rapidly oxidized to acetic acid by the enzyme aldehyde dehydrogenase (ALDH), which also requires NAD as a cofactor. This is also a dimer and exists in several variant forms (Jörnvall, 1985).

Ethanol is one of the least potent of the general central nervous system depressants. Consequently, the amount of this drug routinely consumed constitutes a massive load for the liver, and results in considerable disruption of ongoing metabolism. At blood ethanol concentrations that are only moderately intoxicating, the liver must devote most of its oxidative capacity to the metabolism of ethanol. The available supply of NAD is rapidly exhausted, and must be constantly regenerated. Consequently, during the metabolism of ethanol the ratio of NAD to NADH in the liver becomes smaller, and metabolism is shifted towards reductive pathways (Goldstein, 1983). The kinetics of ethanol metabolism are quite complicated. Metabolism of ethanol follows first order Michaelis-Menten kinetics. Above blood concentrations of about 0.3 mg/ml (5mM or 0.03 mg%), and after absorption of ethanol is complete, the decline of ethanol is for all practical puposes linear. The apparent linear portion of the ethanol elimination curve has been explained as a natural consequence of first order Michaelis-Menten kinetics (Wilkinson, 1980), or as the result of the exhaustion of the available supply of NAD, so that the regeneration

of NAD from NADH is the limiting factor determining the rate of ethanol degradation (Israel et al., 1970; Scholz & Nohl, 1976; Thurman et al., 1976). Because of this linear decline, the initial elimination of ethanol can be treated as zero-order (Jones, 1984) though not necessarily dose independent (Wendell and Thurman, 1979).

Different tissues display varying abilities to oxidize ethanol and acetaldehyde. The primary site of metabolism of ethanol is in the liver, but recent studies indicate that within other tissues, particular cell types may have significant ADH and ALDH levels, although enzyme activity when measured in the organ as a whole may be quite low (von Wartburg and Bühler, 1984). This could have great significance in the pharmacological or toxic effects of alcohol and acetaldehyde. Both ethanol and acetaldehyde have pharmacological effects, and both are capable of damaging tissue, acetaldehyde being more toxic. Within a cell type, the local level of ethanol and acetaldehyde may be determined by enzymes present in that cell, and the local level of ethanol or acetaldehyde might be quite different than that suggested by the concentration of ethanol in blood. Certain specificities of action, in particular the susceptibility of certain cell types to damage during chronic ethanol consumption, could be explained by such regional differences in concentrations of metabolic enzymes (von Wartburg and Bühler, 1984).

Behavioral actions of ethanol

The symptoms of ethanol intoxication show a progression as dose increases. Chu (1983) described the behavioral signs of intoxication during the rising phase of blood ethanol concentrations. Fifteen to

thirty minutes after i.p. administration of ethanol (20% v/v) in rats, Chu reported that doses of 0.5 g/kg resulted in gait disturbances and impaired landing responses. One g/kg caused mild to moderate incoordination, 2 g/kg resulted in gross incoordination and stupor, and 3 g/kg made animals semi-conscious with marked ataxia. Lethal doses vary, but would be expected to be in the range of 5.0 to 8.0 g/kg. Ethanol in low doses provokes an initial excitatory action on the EEG (Kalant, 1975; Murphree, 1973), and on evoked potentials (Himwich and Callison, 1972). Low doses of ethanol also stimulate locomotor activity (Pohorecky, 1977) and facilitate responses on operant tasks (Goldman and Docter, 1966; Holloway and Wansley, 1973). In humans, performance on cognitive tasks may actually be improved by small doses of ethanol (McNamee, 1980). However, Barry (1979) reported that at blood ethanol concentrations (BEC) of .05% (.5 mg/ml), humans showed impaired attention to two simultaneous tasks, failure to perceive infrequent stimuli, and swaying of body when standing. BEC of .10% (1.0 mg/ml) resulted in deficits in sensorimotor tracking, arithmetic calculations, and short term memory, as well as staggering when blindfolded. BEC of .15% (1.5 mg/ml) led to staggering with eyes open, slurred speech, slower responses to signals, and slower arithmetic calculations. At BEC of .20% (2.0 mg/ml) responses to sensory stimuli, at .25% (2.5 mg/ml) consciousness, and at .5% (5.0 mg/ml) breathing were abolished.

It is well known that humans who routinely consume ethanol are able to tolerate increased amounts while displaying reduced signs of intoxication. Similarly, animals repeatedly exposed to ethanol can be shown to be tolerant to ethanol by a variety of measures of

intoxicating effect. Tolerance to ethanol is said to have developed when the dose effect curve is shifted to the right. This means not only that there is a decreased effect at a previously given dose, but also that given a higher dose, the previous level of effect can be restored. Therefore, the drug is still having the same effect on the target organ, but the relationship between drug dose and effect has been altered.

Several types of tolerance can be distinguished. Functional tolerance occurs when an organism shows a reduced effect of the drug at blood concentrations which previously elicited a greater effect, as described above. Metabolic tolerance occurs when the rate of absorption, distribution or elimination of ethanol changes. This form of tolerance violates the above description in that the relationship between ethanol concentration at target organ and effect remains the same, while a given dose results in a lower concentration at target organs. Finally, learned or behavioral tolerance is tolerance which involves higher processes integrating information from past experience in order to behave appropriately. These three types of tolerance can be described in terms of the physiological processes which might be involved in each type of tolerance. Metabolic tolerance is mediated through induction of drug eliminating processes (such as enzyme induction, increase of hepatic blood flow, etc.), functional tolerance through alterations in cell function (changes in receptor number or affinity, changes in vesicular storage, membrane adaptations, etc.), and learned tolerance through activation of memory patterns like those one might use in order to ride a bicycle or walk in high-heeled shoes. All of these processes could contribute simultaneously to a given

instance of tolerance.

Physical dependence on ethanol develops during chronic ethanol exposure, as evidenced by the appearance of a withdrawal syndrome as blood ethanol concentration falls. Withdrawal is by definition an indirect effect of ethanol. Withdrawal signs have been observed following a single exposure to ethanol (Sanders, 1980; Mucha and Pinel, 1979; McQuarrie and Fingl, 1958). The severity of the withdrawal syndrome increases with increasing dose and duration of administration (Goldstein, 1972). The ethanol withdrawal syndrome is characterized by nervous system hyperexcitability. Victor and Adams (1963) studied the severe ethanol withdrawal syndrome in 266 patients in a hospital in Boston. They found that tremulousness appeared in the first few hours, followed by benign hallucinations. After 24 hours convulsions occurred, ranging from short spasms to full tonic-clonic seizures with loss of conciousness. Within 3 or 4 days, delirium tremens (confusion, disorientation, severe hallucinations) began, accompanied by severe autonomic overactivity; sweating, nausea, vomiting, diarrhea, and fever.

Animal models of the ethanol withdrawal syndrome have utilized measures similar to those seen in the human withdrawal syndrome as well as some unique to animals. Friedman (1980) summarized the withdrawal syndrome as observed in man, monkey, chimpanzee, dog, cat, mouse and rat. All species displayed tremor, and a variety of other motoric symptoms including muscle fasciculations and spastic rigidity. Behavioral symptoms included stereotypy, irritability, aggression, fright, and both increases and decreases in activity and reactivity. Sleep disorders and spontaneous vocalizations were common, as well as autonomic symptoms including mydriasis and photophobia, temperature

changes, sweating, salivation, and piloerection. Convulsions occurred in all species, both tonic-clonic with recovery, and fatal. No equivalent of delirium tremens has been demonstrated in animals, although hallucination-like behaviors have been observed in monkeys, dogs, and rats (Friedman, 1980; Barry, 1979). Because the physiological mechanisms responsible for the withdrawal syndrome are unknown, any unusual behavior which reliably appears in the hours following cessation of ethanol treatment and which disappears as the animal recovers meets the criteria for an ethanol withdrawal sign.

Physiological bases for the behavioral actions of ethanol

Ethanol is included in a broad category of general central nervous system depressants and anesthetics which share as their site of action the biological cell membrane (Seeman, 1972). Drugs in this category include, among others: barbiturates; inhalation anesthetics such as nitrous oxide, xenon, and ether; and a number of simple organic molecules, in addition to ethanol, such as paraldehyde and chloral hydrate. As early as 1900, E. Overton and H. H. Meyer independently observed that anesthetic potencies of a number of compounds were directly related to their lipid solubilities (Meyer, 1937). They proposed that the site of action must be some lipid portion of the cell. It was suggested that the cell membrane might be the important part of the cell, but it was not clear whether the anesthetics were acting within the membrane or just moving through it. Mullins (1954) noted that if one took into account molar volumes of the anesthetics, the relationship between potency and concentration of the anesthetic in the lipid phase was improved. This suggested that the amount of space

occupied by anesthetics within the membrane was a factor in their potency, and that the site of action was the membrane itself.

The membrane normally exists in an equilibrium between a fluid and gel state. The insertion of the anesthetic into the lipid bilayer results in an expansion of the membrane (Seeman, 1974). Through this expansion, general CNS depressants are thought to alter the phase transition temperature of the membranes of cells and cell organelles such that they are in a more fluid, or disordered state. This expansion can be accounted for by the molar volume of the anesthetic in model membranes, but in biological membranes the expansion is even greater than that predicted by molar volume, suggesting that conformational changes in proteins contribute to the expansion (Seeman, 1974). All cells in all tissues are potentially affected by anesthetics, but the function of excitable cells appears to be most disrupted (Seeman, 1972).

Additional support for the notion that anesthetics act by disordering membranes came from research demonstrating that increased pressure, which is known to increase the order of membranes _in vitro_ (Chin _et al._, 1976), reverses the anesthesia produced by the general CNS depressants _in vivo_ (Halsey and Wardley-Smith, 1975). Disordering of the membrane has consequences for many aspects of cellular function. Passive and active transport can be either increased or decreased by anesthetics, while facilitated flow appears to be universally decreased (Seeman, 1972). Endocytosis and exocytosis (or neurosecretion) may be altered: Ethanol increases miniature end plate potentials recorded at the neuromuscular junction (Okada, 1967; Gage, 1965), but decreases acetylcholine release within the central nervous

system (Carmichael & Israel, 1978; Sinclair & Lo, 1978). The function of proteins embedded in the membrane might be disrupted, due to conformational changes ("unfolding": Eyring et al, 1973), failure of proteins to undergo normal conformational changes (Trudell, 1977), disruption of protein coupling (Lee, 1976), and/or disruption of receptor-ligand interactions (Heron, 1980).

It is apparent then that even if ethanol acts solely as a nonspecific depressant through its membrane fluidizing effect, there are a number of mechanisms which result in specific actions of this drug. First, polysynaptic networks may be more affected by depressant drugs than monosynaptic pathways (Himwich and Callison, 1972), by a multiplicative effect: the response of a neuron to a given stimulus is depressed, thus it transmits an attenuated signal to a neuron similarly depressed, and so on. More complicated behaviors, requiring multiple synapses, should be most sensitive to the effects of ethanol, and this appears in general to be true. Second, certain membrane embedded proteins may depend more on fluidity for proper function than others, or interactions involving the coupling of several proteins within the membrane may be more affected by increased fluidity than those requiring the interaction of only two proteins. Third, each anesthetic may have in addition to its effect on cell membranes a unique action of its own. An example of this for ethanol would be the possible role of its metabolite, acetaldehyde, in some of the effects seen following ethanol administration (discussed below).

If the equilibrium of the fluid/gel state is artificially disrupted, mechanisms may exist to alter the composition of the

membrane (the ratio of certain lipids) such that the desired fluid/gel state is recovered (Stubbs and Smith, 1984). This compensatory response has been proposed to explain both tolerance to ethanol, and the appearance of a withdrawal syndrome (Hill and Bangham, 1975). Functional tolerance could occur through a number of mechanisms: One might be increased rigidity through alteration in lipid composition of the membrane - incorporation of different, "stiffer" lipids, such as cholesterol, into the membrane. A second might be incorporation of lipids which exclude the drug from the membrane. Again, cholesterol is thought to do this (Goldstein, 1983). A third possibility is alterations in the activity of the various functional units of the membrane (the receptors, transport proteins, etc.) such that they are able to function normally in the disrupted membrane. Once these compensatory responses have occurred, the disappearance of ethanol could result in a transient hyperactive state before return of the system to normal. In this view, physical dependence and tolerance are progressive aspects of a single phenomenon.

Experimental evidence supports the idea that exposure to ethanol causes changes in cell membranes. Specifically, repeated exposure to ethanol _in vivo_ results in brain membranes that are less sensitive to the fluidizing effect of ethanol _in vitro_, although showing no basal fluidity difference (Chin & Goldstein, 1977; Harris _et al_, 1984a; Lyon & Goldstein, 1982). This is exactly the sort of adaptive process one would predict - the membrane maintains normal fluidity but becomes resistant to the particular disruption to which it has been exposed. However, evidence for involvement of membrane adaptation in the development of tolerance to other general CNS depressants is less

clear. Synaptic membranes from mice rendered tolerant to phenobarbital did not differ in baseline fluidity from those of nontolerant mice, nor did they differ in the increase in fluidity produced by the in vitro addition of ethanol or phenobarbital (Mitchell et al., 1985). Furthermore, there has been limited success in linking any changes in membrane composition with a withdrawal syndrome. The sort of membrane effect one would predict to underly the withdrawal syndrome is an alteration in membrane fluidity, specifically a stiffening of the membranes, which can be observed in the waning phase of intoxication, and is present through the time period when withdrawal signs are normally observed. Crews et al. (1983), studied changes in brain membranes from rats made physically dependent on ethanol and still intoxicated, rats made physically dependent and 4-6 hr withdrawn, rats treated acutely with ethanol, and water-treated control rats. They measured temperature-induced changes in viscosity, and cholesterol:phospholipid molar ratio of the synaptosomal plasma membranes. Ethanol-dependent rats showed larger increases in membrane fluidity, and a higher cholesterol:phospholipid molar ratio, than either acutely ethanol treated or water treated rats, but these changes were more extreme in intoxicated rats than in withdrawing rats. In other studies, mice made dependent and showing a robust withdrawal syndrome have failed to show any reliable alteration in membrane fluidity or composition (Chin & Goldstein, 1977; Harris et al., 1984b), and brain membranes from mice made dependent on phenobarbital showed no alteration in intrinsic membrane fluidity or in susceptibility to the fluidizing effect of ethanol or phenobarbital (Mitchell et al., 1985), but these studies looked at mice only at time

of withdrawal, while the mice were still intoxicated. There are two ways to interpret these findings. On one hand, the lack of correlation between the membrane changes and the appearance of the withdrawal syndrome suggests that membrane changes do not underly withdrawal symptoms. On the other hand, ethanol itself may block expression of the withdrawal symptoms in the early phase of withdrawal, so that the membranes have partially recovered from ethanol treatment while ethanol is still masking withdrawal symptoms.

Acetaldehyde, the major metabolite of ethanol, has pharmacologic actions of its own and has been implicated in some actions of ethanol. Most of the evidence is against a direct role of acetaldehyde in physical dependence. Physical dependence has been demonstrated following treatment of animals with acetaldehyde (Ortiz et al., 1973), however no ADH inhibitor was employed to prevent creation of ethanol from acetaldehyde. Physical dependence also arises following treatment with t-butanol, which is not metabolized to acetaldehyde (Wallgren et al., 1973; McComb and Goldstein, 1979), and physical dependence occurs in animals treated with ethanol and pyrazole, an ADH inhibitor which would lower or eliminate acetaldehyde accumulation (Goldstein and Pal, 1971). Manipulations which increase blood levels of acetaldehyde tend to reduce voluntary consumption of ethanol (Schlesinger et al., 1966; Lindros et al., 1975). Peripheral administration of acetaldehyde in humans causes an extremely unpleasant reaction which includes facial flushing, vasodilation, palpitations, respiratory difficulties and nausea (Asmussen et al, 1948; Raby, 1954). However, when acetaldehyde is administered directly into the cerebral ventricles the story is reversed: Naive rats will learn to self-administer acetaldehyde, but

not ethanol (Amit et al., 1977), and while peripherally administered acetaldehyde can be used to produce conditioned taste aversions, centrally administered acetaldehyde cannot (Brown et al., 1978). Acetaldehyde may also play an indirect role in the production of unusual metabolites, tetrahydroisoquinolines (TIQs) and ß-carbolines, from catecholamine and indoleamine metabolism, respectively (Davis and Walsh, 1970; Cohen et al, 1976). The metabolism of ethanol itself may also assist in the production of these compounds, by shifting metabolism towards reductive pathways, which favors the creation of TIQs and ß-carbolines (Goldstein, 1983). Some of these compounds have been shown to have opiate activity, and may play a role in the rewarding effects of ethanol. Administration of the TIQs tetrahydropapaveroline (THP) and salsolinol into rat cerebral ventricles increases voluntary ethanol consumption (Myers and Melchior, 1975; Duncan and Deitrich, 1980). This increase in ethanol consumption was suppressed by administration of naloxone, an opiate antagonist (Myers and Critcher, 1982).

Application of genetic techniques to the study of ethanol physical dependence

While many descriptions exist of the behavioral and physiological effects of ethanol, their value in predicting the reaction of any particular individual is limited. For many drugs, it is exactly the reaction of a given individual which is of interest. By use of a number of techniques, it is possible to sort out the genetic and environmental factors which contribute to sensitivity to a particular drug effect (independent of understanding the mechanism of that

effect). Then subject populations can be identified which are genetically distinct in their drug reaction, and these populations can in turn be used to study the mechanism underlying the difference, and also to identify indicators that allow prediction of a given individual's response to the drug.

One way to identify possible indicators is to look for correlations between a trait of interest and a trait which appears to accompany it. Given two characters measured in each individual within a population and found to be correlated, these characters are said to show a phenotypic correlation. A phenotypic correlation is composed of genetic and environmental factors. Genetic correlations are of particular importance because they suggest a physiologic basis for the correlation rather than an environmental one. A genetic correlation between two characters suggests that they are influenced by many of the same genes. If a single gene, this is an example of pleiotropy; one gene which influences several different characters. Most behavioral characters are influenced by multiple genes, and the genetic correlation may involve all or a subset of the genes which modulate expression of the two characters. Nonetheless, the implication is that these genes are acting as a functional group: that the basis for the genetic correlation is a physiological relationship between the two correlated characters.

In an experimental situation, a single manipulation, for example administration of a drug, may result in two observed effects which are found to covary. These effects may be independent - the drug may have two distinct mechanisms of action. Alternatively, the drug may have act through a single mechanism, but have two apparently unrelated

effects. For example, a single injection of ethanol (2.0 g/kg in rodents) results in reduction of body temperature and ataxia. These effects may both be the result of a single action of ethanol (for instance, depression of the action potential due to the fluidizing effect of ethanol of neuronal membranes) or due to two independent actions of ethanol (one response might be mediated through an effect of ethanol on catecholamines in the hypothalaums, while the other is mediated through the depression of synapses at the neuromuscular junction). If these effects are due to a single action of ethanol, then it should be possible to demonstrate a genetic correlation between the two effects. If they are mediated through different actions of ethanol, it should be possible to demonstrate genetic independence of the two effects. Of course, other factors may make demonstration of genetic correlations difficult. For instance, if one character shows little or no genetic variability, it will be impossible to demonstrate a genetic correlation between that character and another.

One technique useful for distinguishing genetic from phenotypic correlations can be found in studies with inbred mice. Inbred strains are developed by many generations of brother/sister mating, resulting in animals that are virtually genetically identical, and homozygous at all gene loci. An inbred strain is somewhat analogous to a population of clones, in that all genetic variability between individuals has been eliminated. If members of an inbred strain are tested on a given measure, variability on that measure reflects environmental effects alone. When tested under constant environmental conditions, differences between means for several inbred strains are thus attributable to differences in genetic make-up. In the simplest case,

two inbred strains can be identified which represent the extremes of susceptibility to a certain drug effect. One inbred strain may show a very high sensitivity to the drug while another is very resistant. One can examine these strains for the purpose of identifying some physiologic factor that accounts for the observed difference. But two inbred strains will display any number of additional differences, say in body weight or brain size or blood chemistry, arising from the random fixing of the gene pool which occurred when the strains were developed. One or more of these differences may be related to the drug response, but in comparing only two inbred strains, it is impossible to tell which. However, the addition of several more inbred mouse strains which show a range of responses to the drug allows true genetic correlations to emerge.

Inbred strains have been used to explore the genetic relationships between measures of initial sensitivity to, tolerance to and withdrawal from ethanol. In a study using 20 inbred strains, Crabbe and associates (1983c) recorded eight measures of initial sensitivity, tolerance to ethanol-induced hypothermia, and withdrawal following chronic ethanol administration. Of these measures, hypothermia and hypothermic tolerance showed a significant negative genetic correlation with withdrawal severity. Thus, by measuring hypothermia, one could predict an animal's withdrawal score: A large drop in body temperature would lead one to expect a low degree of withdrawal, while a small drop in body temperature would predict a severe degree of withdrawal.

Inbred strains, then, constitute a great resource available to be applied to any question. But studies requiring the use of a minimum of eight or ten different strains are a problem. If a particular question

is to be studied extensively, a subject population can be created in which true genetic correlations are not confounded by chance associations of genes. The technique of artificial genetic selection has been used to produce lines of animals that are genetically invariant with regard to the genes determining a particular trait, while remaining freely variant at all other gene loci.

Because the technique of genetic selection is relatively unfamiliar, I will describe it in some detail. To begin with, one must have an outbred population of animals with demonstrated variability in the trait of interest for selection to act upon. Two genetically heterogeneous (HS: Heterogeneous Species) strains of mice have been developed by intercrossing mice from eight inbred strains with widely divergent origin. These mice constitute particularly good starting populations for a genetic selection because the genetic composition of the parent strain can be examined in detail. The first step in establishing selected lines is to test a base population of mice for the trait of interest. Breeding pairs are then selected at random to form a selection control line. Then individuals are chosen on the basis of test scores for maximum expression of the trait of interest, and these mice are used to establish a maximum expression line. The same strategy is used to create a minimum expression line. In succeeding generations, offspring within the control line are randomly selected to continue the control line, offspring within the maximum line are selected for maximum expression of the trait to continue the maximum line, and offspring from the minimum line are selected to continue the minimum line.

The purpose of the control line is twofold: First, practical

considerations limit the number of breeding pairs used to maintain a selection line. The mice chosen to begin a selection line can only contain a subset of the genetic information available in the genetically heterogenous (HS) mice. Therefore if one wishes to assess how selection pressure has altered the minimum and maximum lines relative to their original genetic composition, one must have a control line chosen from the same subset of mice that the minimum and maximum lines are chosen from. Second, as selection proceeds the animals will unavoidably become partially inbred at some gene loci unrelated to the selected character. The effects of this trait-irrelevant inbreeding will be reflected in the control line, and should be taken into account when interpreting the effects of selection.

A second important control in a genetic selection is that the entire selection should be replicated: A second subset of mice should be tested, and a second control, minimum and maximum line established. This replication also controls for the accidental fixing of genes unrelated to selection which can occur in the initial choice of breeding pairs or in inbreeding later in the selection. This allows distinction of true genetic correlations between the trait selected for and a correlated trait, as the genetic correlation will appear in both replications. It is very unlikely that random or spurious correlations would appear in both replications. Similarly, inbreeding may result in the appearance of a trait in one replication which appears to be a result of the selection, but is unlikely to produce the same correlation in the other replication. Thus a difference between both replicates of the maximum and minimum lines in a trait in addition to the primary trait on which the lines differ is strong evidence that a

genetic correlation exists between the traits (Deitrich and Spuhler, 1984; DeFries, 1981).

We are currently developing a model of differing sensitivity to the development of ethanol withdrawal by genetically selecting lines of mice on the basis of handling-induced convulsions (HIC) after withdrawal from chronic ethanol treatment (the selection procedure is described in detail beginning on p. 25). Mice are made dependent by 72 hours of ethanol inhalation with daily injections of pyrazole, an alcohol dehydrogenase inhibitor. While there is no way to measure the development of dependence directly, the appearance of a withdrawal syndrome following cessation of chronic ethanol treatment implies that physical dependence on ethanol has developed. By selecting for withdrawal severity, we have evolved lines of mice which differ principally in the allelic frequency for those genes relevant to withdrawal severity, and show no systematic differences for other gene loci. The genes which are involved in withdrawal severity may have pleiotropic effects; in other words they may have several physiological consequences. Thus any other characteristic which differentiates these lines of mice can be attributed to the expression of those genes which determine withdrawal severity, and, by implication, severity of physical dependence on ethanol.

We have achieved marked success in our selection study. In the fifth selected generation, a 5-fold difference in HIC between the WSP and WSR lines after identical ethanol exposure was found (Crabbe et al., 1983a), and in the eleventh selected generation, this had increased to a ten-fold difference in severity of HIC (Crabbe et al., 1985). This result confirms that there is a strong genetic influence

on withdrawal severity as measured by HIC. One of the first applications of these mice has been to the question of the genetic relationship between withdrawal susceptibility and ethanol-induced hypothermia: WSP and WSR mice from the second selected generation were tested for hypothermia following a single administration of ethanol, and in both replications WSP mice tended to show less hypothermia than WSR mice, as predicted by the genetic correlations estimated from inbred strain means (Crabbe et al., 1983c). Results with later generations have been equivocal: An experiment using mice from the fourteenth and fifteenth generations showed WSR mice to be more sensitive to the hypothermic effect of ethanol, while an experiment with the fifteenth and sixteenth generations showed equal sensitivity in the WSP and WSR mice (unpublished). It may be that the base populations of mice from which the two replicate selection lines were created were not sufficiently genetically variant with regard to hypothermic sensitivity, and so no selection pressure could be applied (indirectly through the selection for withdrawal severity) to that trait. A study of the WSC lines could reveal whether this is the case. Alternatively, the estimate of genetic correlations using inbred strain means may have been biased, by the choice of inbreds tested or by the small number of animals tested, to reveal a higher correlation than would be found in the HS mice from which the selected lines were developed. A study of the genetic correlation between hypothermic sensitivity and withdrawal in the eight inbred strains used to establish the HS mice, and the HS mice themselves, would be more relevant to the prediction of genetic correlations in the WSP and WSR mice.

EXPERIMENTS

Rationale.

The WSP and WSR lines of mice are intended as a general tool for research concerned with processes of physical dependence and withdrawal. In the experiments reported here, I explored several issues related to the utility of these lines of mice as a model of differing genetic susceptibility to developing physical dependence on ethanol. First, one of the most obvious ways the WSP mice and WSR mice may differ is in pharmacokinetic factors in metabolism of ethanol. While such a difference could in itself be of interest, we are primarily interested in differences in neurosensitivity between these mice, independent of dose. In Experiments 1 and 2, I measured metabolism of ethanol in the lines following acute and chronic treatment with ethanol to determine if there was any evidence that these lines of mice differ in the blood ethanol concentration they achieve or in their rate of elimination. Similar elimination rates suggested that the basis for the observed differences in withdrawal severity between the lines lies primarily in differing sensitivity of target organs to the drug.

In Experiment 3, withdrawal following an alternative method of intoxication was examined. It was posssible that some aspect of the treatment of the WSP and WSR mice other than the ethanol influenced the expression of withdrawal. For instance, pyrazole is known to increase HIC (Crabbe et al., 1981), to deplete brain norepinephrine (Macdonald, 1976; Brown et al., 1978b) and to cause cellular damage to the liver

(Lieber et al., 1970). The inhalation procedure may produce side effects from the exposure of the lungs to ethanol, and from reduced food and water intake due to constant intoxication (e.g. irritation of lung tissue, weight loss, dehydration, disruption of hormonal systems). Animals may differ in genetic susceptibility to these parameters and therefore may have been selected on these bases in addition to ethanol sensitivity. Thus, a method of ethanol intoxication that did not require the use of pyrazole or involve inhalation was desirable.

McQuarrie and Fingl (1958) reported that mice given a single administration of ethanol (by i.p. injection) showed evidence of a rebound hyperexcitability, as measured by increased sensitivity to several convulsants, in the period following clearance of ethanol. Other investigators have performed similar single administration studies and reported elevated withdrawal signs after acute administration, suggesting that some degree of physical dependence develops rapidly (Goldstein, 1972; Sanders, 1980; Mucha and Pinel, 1979). Because i.p. injections of ethanol are a very simple procedure, used frequently in our lab for studies of sensitivity and tolerance to ethanol, we chose this method of ethanol administration. In Experiment 3, WSP and WSR mice were given a single injection of ethanol and scored for handling-induced convulsions to compare acute withdrawal in the selected lines.

Experiment 4 is concerned with the generality of the withdrawal syndrome displayed by these mice. Withdrawal is defined by a number of behavioral and physiological symptoms, of which susceptibility to induced convulsions is only one (Friedman, 1980). If ethanol acts to

induce physical dependence in many genetically distinct physiological systems, it would be expected that the WSP and WSR mice will differ in severity of withdrawal only in those signs of withdrawal which share a genetic basis with handling-induced convulsions. If, however, only a single physiological system is responsible for all components of the withdrawal syndrome, then WSP and WSR mice should prove to be equally divergent regardless of the symptom used to assess withdrawal. Most likely, some symptoms of withdrawal arise from systems which have no genetic connection with the appearance of HIC during withdrawal, while other signs may share a common genetic basis. Thus we should be able to identify a constellation of withdrawal signs attributable to the same physiological mechanism responsible for HIC. In the fourth experiment, animals chronically treated with ethanol were assessed on a variety of withdrawal signs in addition to HIC.

Methods

General Methods.

Subjects. Mice used in these experiments were bred at the VA Medical Center, Portland, Oregon. Beginning with two independent groups of nine families each from the genetically heterogeneous HS/Ibg strain, we have selected two WSP and two WSR lines. Each line consists of nine families, and within-family selection as described in DeFries et al. (1978) is practiced; one male and one female mouse are selected from each family. This system of selection doubles the effective breeding population size, thus minimizing inbreeding, and the maintenance of replicate lines with relatively large breeding populations in a

bidirectional selection program increases the likelihood that differences between the lines are due to genetic pleiotropism rather than trait-irrelevant drift (Falconer, 1960). In addition, two randomly selected control lines (WSC) are maintained as a control for inbreeding effects. In these experiments, WSP and WSR mice from both replicate mouse lines were used. Since there were no differences between replicates of any mouse line, (e.g. both the WSP-1 and WSP-2 lines responded very similarly), the data were collapsed across replication for analysis. Mice from the WSC lines were not tested in these experiments. Data from WSC lines yields information about the contribution of accidental fixing of genes and inbreeding to a measurement in the WSP and WSR mice, and also about the magnitude and direction of response to selection. However, use of the replicate lines also provides information about inbreeding and accidental fixing of genes. Because we were interested principally in absolute differences between WSP and WSR mice, and because these experiments are very time consuming, the decision was made to exclude WSC mice. In all colony and test rooms, temperatures ranged from 24.0 to 25.5 ^{o}C and lights were on from 0600 to 1800 hr. Except where noted, mice were housed in polycarbonate boxes (28 x 17 x 11.5 cm) containing wood chips as bedding (3-6 mice per cage), and food (Rodent Laboratory Chow™ #5001, Ralston Purina Co.) and water were available ad libitum.

Induction of ethanol dependence. Mice were made dependent by an inhalation procedure as described by Goldstein (1972), with some modifications (Crabbe et al, 1983b). On the first day, each mouse was weighed and given an i.p. loading dose of ethanol (1.25 g/kg, 20% v/v) and pyrazole HCl (1 mmol/kg), an alcohol dehydrogenase inhibitor. The

mice were then placed in wire mesh cages (18 x 18 x 15 cm) in a plastic chamber (105 x 65 x 70 cm) into which ethanol and air were introduced. Ethanol vapor concentrations within the chamber were monitored with a gas chromatograph. Temperatures in the chambers averaged 29°C. At 24 and 48 hr, the mice were removed from the chamber, weighed, and injected with pyrazole. The mice were replaced in the chamber, and the vapor concentration of ethanol in the chamber was raised. At 72 hr, 24 hr after the final pyrazole injection, the mice were removed from the chambers, weighed, had blood samples drawn for blood ethanol concentration determinations. The mice were then returned to clean home cages and scored for ethanol withdrawal symptoms over several hours post-withdrawal. Food and water were available ad libitum throughout intoxication and withdrawal.

Determination of blood ethanol concentration. For analysis of BEC, a 20µl blood sample was drawn from the tip of the tail. The blood sample was added to 50µl of 3N $ZnSO_4$, shaken, and stored on ice until all blood samples were drawn. 50µl of $Ba(OH)_2$ (5%) and 300µl ice cold distilled water were then added to each sample, and the samples were shaken and centrifuged for 4.5 min at 3000 rpm. The supernatant was analyzed for ethanol concentration using gas chromatography (Crabbe et al., 1982).

Handling-induced convulsions. HIC were scored using a procedure modified from that developed by Goldstein (1972). Each mouse was lifted by the tail and observed for seizures. If no seizure occurred when the mouse was lifted, the mouse was spun gently 180° and again scored for seizures. Possible scores ranged from 0-4, as follows: 4: Severe tonic-clonic convulsion when lifted by the tail, with quick

onset and long duration, often continuing for several
mouse is released. 3: Tonic-clonic convulsion when
tail, often with onset delayed by as much as 1-2 se
convulsion when lifted by the tail or tonic-clonic convulsion
gentle spin through a 180° arc. 1: No convulsion when lifted by the tail, but tonic convulsion elicited by spin. 0.5: Only facial grimace following spin. 0: No convulsion. Interrater reliability for scoring HIC has been estimated for three observers in our lab to be greater than 0.8 (unpublished).

Experiment 1

In this study, we monitored blood ethanol concentrations following an acute injection of ethanol to assess metabolism of ethanol in WSP and WSR mice. Seventy-eight naive male and female mice from the fourth selected generation of the WSP and WSR lines were used (17 to 21 mice per group). These mice were 73 to 84 days old at the time of the test. Beginning between 0700 and 0800 hr, the mice were weighed, injected with ethanol (3 g/kg i.p., Aaper Chemical Co., 20% v/v in physiological saline), and returned to their home cages. Tail blood samples were taken at 1, 2, 3, and 4 hr post injection and analyzed for ethanol concentration (mg ethanol/ml whole blood).

Although ethanol metabolism is most appropriately described by Michaelis-Menten kinetics (Wilkinson, 1980), the treatment of the declining blood ethanol curve as linear, as first described by Widmark (1933, as quoted in Wilkinson, 1980), is sufficient for the detection of gross differences in the initial, zero-order phase of ethanol elimination (Jones, 1984). We calculated β_{60}, the apparent linear decline in blood ethanol concentration (mg/(mlxhr)), as the slope of

the BEC curve using least-squares linear regression. BEC_0, the theoretical concentration of ethanol in blood at time 0 (assuming instantaneous absorption and distribution of a dose of ethanol), was calculated by extrapolating the BEC regression curve to time 0. Vd, the apparent volume of distribution (ml) was calculated as dose (mg)/ BEC_0(mg/ml). Vd/BW is apparent volume of distribution expressed as a proportion of body weight.

Experiment 2

In this experiment, we monitored BEC during induction of ethanol dependence and subsequent withdrawal to see whether WSP mice display more severe withdrawal than WSR mice due to differences between the lines in exposure to ethanol. Severity of dependence was assessed by measuring HIC during withdrawal. Sixty-eight naive male and female mice (9 to 12 per group) from the seventh selected generation of WSP and WSR mice were used. The mice were 50-60 days old at time of test. Dependence was induced in one group of mice (Ethanol Dependent/withdrawn) in an inhalation chamber as outlined above. Ethanol vapor concentrations within the chamber averaged 5.7, 6.6 and 10.0 mg/l on Days 1, 2 and 3, respectively. Control mice were given similar treatment, including pyrazole injections, but no ethanol was introduced into the otherwise identical chamber in which they were housed. Tail blood samples were collected at 24 and 48 hr after initial alcohol injection, just prior to the pyrazole injection. Additional blood samples were collected at time of withdrawal (72 hr), when the mice were returned to their home cages, and at 1 and 3 hr after withdrawal. All mice were scored for HIC at hours 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 23 and 25 post withdrawal. Scoring was without

knowledge of the line (WSP or WSR) or condition (Ethanol Dependent/withdrawn or Control).

Experiment 3

In Experiment 3, we examined the effect of a single dose of ethanol on handling-induced convulsions in the WSP and WSR lines. There is evidence suggesting that a rebound hyperexcitability follows an animal's first exposure to ethanol (McQuarrie and Fingl, 1958). This test allowed us to measure a possible difference between WSR and WSP mice in initial sensitivity to ethanol and also to test ethanol's ability to produce withdrawal seizures in the absence of pyrazole treatment. One hundred and three naive male and female mice from the twelfth selected generation of the WSP and WSR lines were used (22-29 mice per group). The mice were 130 days old at the time of testing. All mice were were scored for HIC, as described above, at 0800 and 1000 hr to establish baseline HIC scores. Following the second scoring, each mouse was weighed and injected with 4 g/kg ethanol (Aaper, 20% v/v in physiological saline) or saline. The mice were then scored for HIC at 2, 4, 6, 8, 10, 12, 22, and 24 hr post injection. Scoring was done without knowledge of line (WSP or WSR) or drug condition.

Experiment 4

In the fourth experiment, we studied the generality of the withdrawal differences in these lines following three days of treatment with ethanol and pyrazole, or pyrazole alone. Handling-induced convulsions were measured at intervals over the first 25 hr of withdrawal. In addition to HIC, withdrawal was assessed using protocols from two different laboratories. First, mice were observed on an open platform for a number of behaviors chosen for ease of

evaluation and distinctness from any behavior seen in control mice (Belknap et al., 1977). Second, mice were tested for their activity in the hole-in-wall apparatus (DeFries et al., 1970) and on a vertical screen. These two tests comprise a portion of a battery of tests used to assess withdrawal in a similar selection project in progress at the Institute for Behavioral Genetics, Boulder, Co. (Allen et al., 1983). All testing was performed under dim illumination. Forty-three female mice from the eighth and ninth selected generation of the WSR and WSP lines were used (8-13 mice per group). These mice had been used in an ethanol drinking test in which they were water deprived 24 hours and then were offered 10% v/v ethanol as the only fluid source for 24 hr. The drinking test was performed when the animals were 49 to 83 days old, and the experiments reported here were performed 25 days later. Ethanol dependence was induced as described above. Concentrations of ethanol in the inhalation chamber averaged 6.1, 8.1 and 11.9 mg/l on Days 1, 2 and 3, respectively. Control mice were given similar treatment, but no ethanol was introduced to their chamber. A tail-blood sample was drawn from each animal at the time of withdrawal.

Scoring of withdrawal:

Handling-induced convulsions. Mice were scored for HIC as described above. Testing occured at 0, 1, 5, 7, 10, 12, 13, 24 and 25 hr post-withdrawal.

Behavior on an open platform. Each mouse was placed on a stainless steel platform (25x47 cm, raised 25 cm above the table top) and observed for 2 min at hours 5, 7, 10, and 12 post-withdrawal. Withdrawal signs were scored according to the method of Belknap et al. (1977). Tremor was defined as a high-frequency trembling

involving at least the head and shoulders of the animal. Straub tail was scored as an arching of the tail over the animal's back. In addition to these signs, we had observed in pilot experiments that some mice walked backward during ethanol withdrawal. This behavior has been reported as an ethanol withdrawal sign (Freund, 1969). Thus, we also scored the presence of backward walking, defined as continuous backward movement covering at least 10 cm. Mice were scored for the presence of any or all of these signs in each observation period.

Vertical Screen. This apparatus was similar to one used by Hutchins et al.(1981). It consisted of a 1.22 m x 2.44 m piece of hardware cloth (3.2 mm mesh) mounted vertically on a wooden frame. The screen was divided into 5.1 cm squares by string suspended behind the screen, and a foam rubber pad was placed under the screen in case the mouse fell. A mouse was placed in the middle of the screen and number of squares crossed in 3 min was recorded. Mice were tested between 7 and 10 hr post withdrawal, prior to testing in the hole-in-wall test.

Hole-in-wall. This apparatus was similar to one described by DeFries et al., (1970), and consisted of a clear plastic box, 31.8 x 31.0 x 15.2 cm high, divided into four equal triangular compartments by two plastic panels running from corner to corner. The compartments were connected by four holes, 4.2 cm in diameter and 1 cm above the floor, one in the center of each wall. Two opposing compartments were lighted by diffuse light through the floor of the apparatus, and had white walls. The other two compartments were not illuminated and had black walls. The subject

was placed in the center of one of the lighted compartments and confined in a transparent cylinder (overturned 250 ml beaker) for a 90 sec adjustment period, then released for a 3 min test. Number of crossings (forepaws touching the floor of an adjacent compartment), head pokes (inserting head through hole into another compartment without crossing), rearings, time spent in dark, and latency to first crossing were recorded. Mice were tested between 7 and 10 hr post-withdrawal, following testing on the vertical screen.

Results

Experiment 1. In this study, we monitored blood ethanol concentrations following an acute injection of ethanol to assess metabolism of ethanol in WSP and WSR mice. Following an acute injection of ethanol, BEC of male and female mice of the WSP and WSR lines were equivalent at 1, 2, 3, and 4 hr post injection (Figure 1). Initial blood ethanol concentration (BEC_0), $ß_{60}$, apparent volume of distribution (Vd) and Vd divided by body weight were calculated (Table 1). Differences in these parameters, in BEC at Hour 1, and in bodyweight were assessed using 2-way ANOVAs (line by sex). No significant differences between WSP and WSR mice were observed. Thus, the selected lines did not differ in metabolism of ethanol in their first exposure to the drug. Female mice, both WSP and WSR, showed a significantly smaller ($F(1,69) = 10.95$, $p < .001$) Vd than male mice. This difference was eliminated when Vd was corrected for bodyweight (Table 1). Female mice showed a significantly lower bodyweight ($F(1,69) = 11.10$, $p < .001$).

Experiment 2. In this experiment, we monitored BEC during induction of ethanol dependence and subsequent withdrawal to see whether WSP mice

FIGURE 1: Mean BEC in male and female mice of the WSP and WSR lines from Experiment 1 following a single i.p. injection of ethanol (3 g/kg, 20% v/v). See text for analysis.

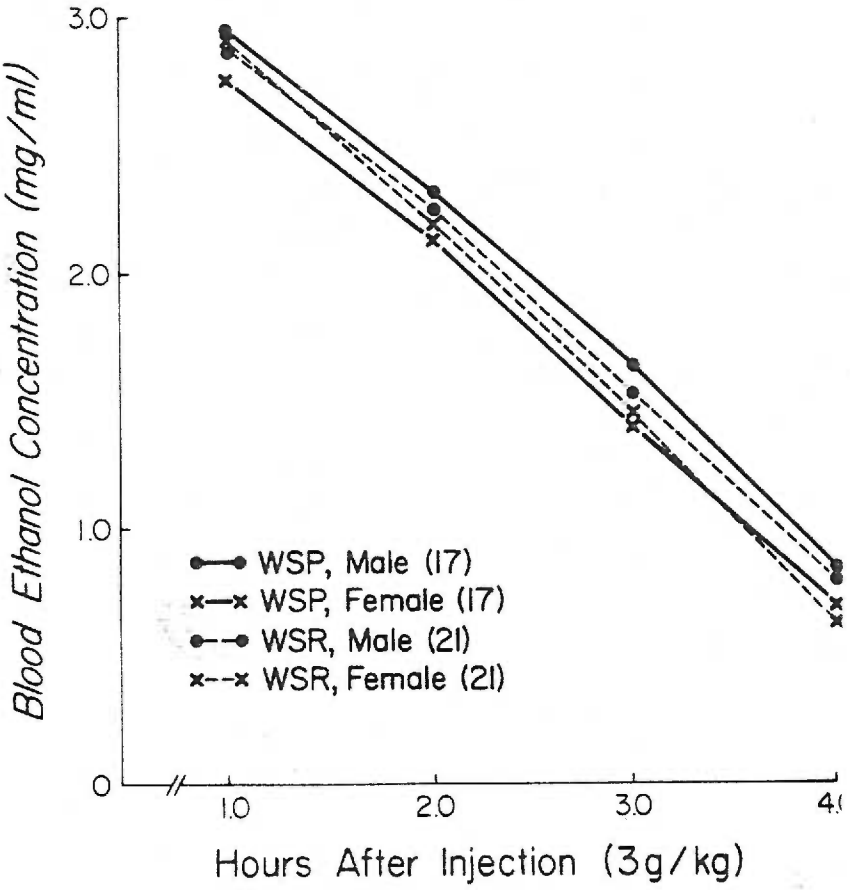

3.0
2.0
1.0
0
Blood Ethanol Concentration (mg/ml)
WSP, Male (17)
WSP, Female (17)
WSR, Male (21)
WSR, Female (21)
1.0
2.0
3.0
Hours After Injection (3g/kg)

TABLE 1

Metabolism of an acute injection of ethanol. Means ± S.E. are shown for mice from Experiment 1. For a description of statistical tests, see text.

	WSR		WSP	
	Male (N=21)	Female (N=21)	Male (N=17)	Female (N=17)
Bodyweight (g)	29.0 ± .7	25.5 ± .6**	28.7 ± .8	23.9 ± .5**
BEC hour 1 (mg/ml)	2.89 ± .05	2.92 ± .06	2.95 ± .06	2.94 ± .11
BEC_0 (mg/ml)	3.59 ± .06	3.68 ± .08	3.62 ± .08	3.49 ± .14
Vd (ml)	24.5 ± .8	20.9 ± .7**	24.0 ± .8	21.5 ± 1.4**
Vd/BW (ml/g)	0.84 ± .02	0.82 ± .02	0.84 ± .02	0.88 ± .04
β_{60} (mg/(mlxhr))	0.69 ± .02	0.75 ± .02	0.68 ± .02	0.70 ± .03

** $p<.001$ for the difference between males and females.

displayed more severe withdrawal than WSR mice due to differences between the lines in metabolism of ethanol. Figure 2 shows BEC during 3 days of chronic ethanol inhalation (Left Panel) and during withdrawal from chronic treatment (Right Panel). There were no significant differences between same-sex WSP and WSR mice at any time BEC was measured. The data collected during acquisition of physical dependence were analyzed using a 3-way repeated measures ANOVA (line by sex by time). There was a significant interaction of time and sex ($F(2,66) = 15$, $p < .0001$). A test of simple effects contributing to the interaction (Keppel, 1973) indicated that male mice achieved a higher BEC than female mice at 48 and 72 hours of inhalation. No differences in BEC between WSP and WSR mice were detected. β_{60} was calculated for each mouse from BEC following removal from the inhalation chambers as the slope of the regression line. As almost all female mice had BEC below .01 mg/ml by hour 3, β_{60} values were calculated using hours 0 and 1 only for female mice. Only male mice having BEC of .01 mg/ml or greater at hour 3 were included in the calculations. Values were as follows [mean mg/(mlxhr) and S.E. (N)]: Male mice: WSR = 0.42 + .03 (8); WSP = 0.46 + .02 (8). Female mice: WSR = 0.77 + .12 (9); WSP = 0.54 + .05 (11). There were no differences between same-sex WSP and WSR mice in β_{60}. Female mice showed a significantly faster β_{60} than male mice ($F(1,33) = 10.1$, $p < .01$).

Figure 3 illustrates the progressive development of HIC during withdrawal from ethanol intoxication for male and female WSP and WSR mice. The area under the HIC curve was calculated for each mouse to assess severity of withdrawal. This 25 hr HIC area is the index of withdrawal severity upon which the genetic selection is based (Crabbe

FIGURE 2: Mean BEC in male and female mice of the WSP and WSR lines from Experiment 2 during 72 hr of ethanol inhalation, and after withdrawal. See text for analysis.

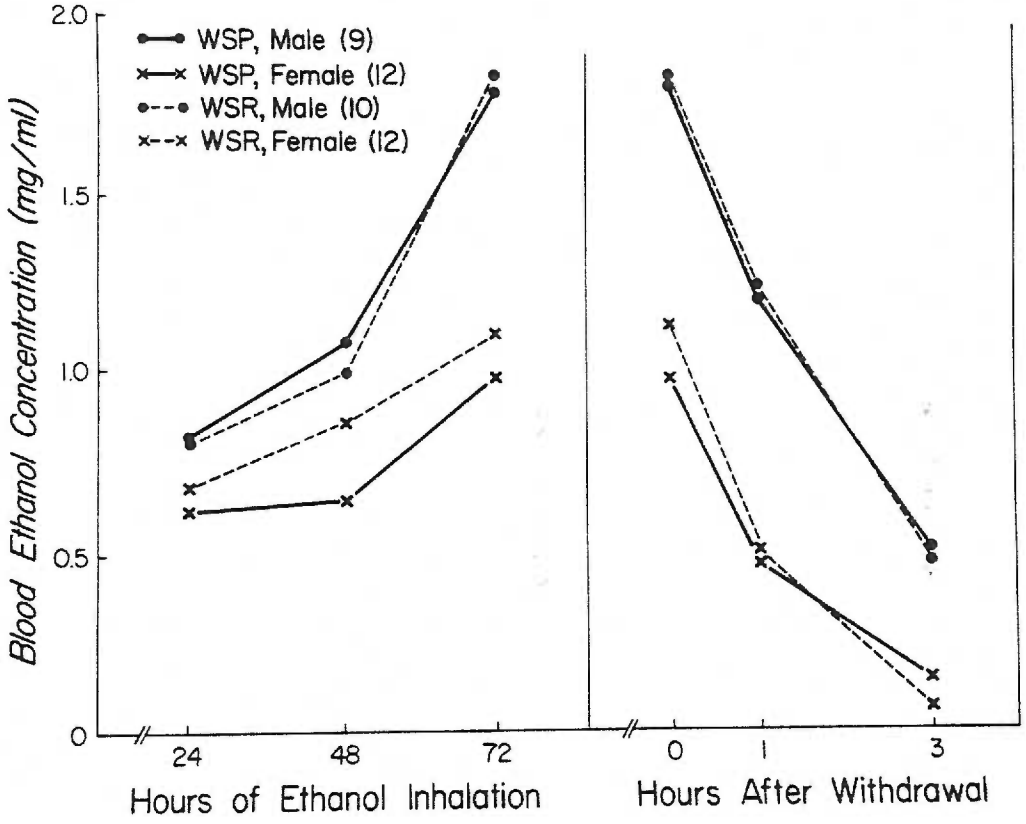
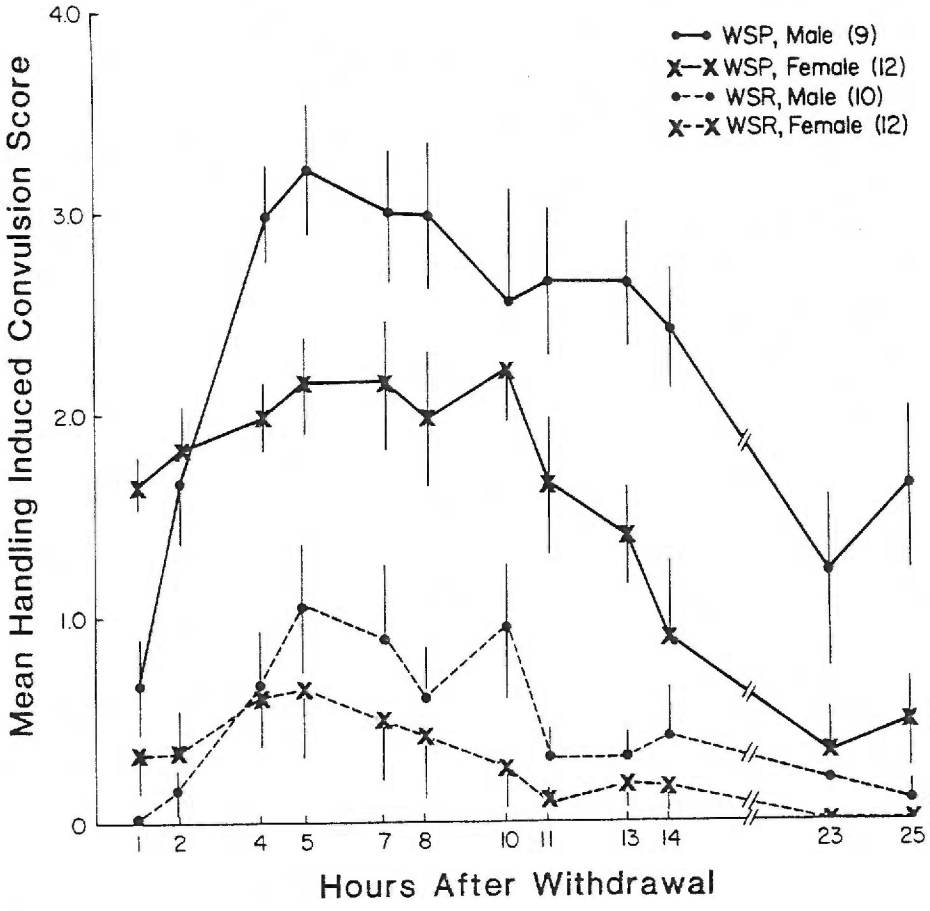

WSP, Male (9)
WSP, Female (12)
WSR, Male (10)
WSR, Female (12)
Blood Ethanol Concentration (mg/ml)
2.0
1.5
1.0
0.5
0
24
48
72
0
1
3
Hours of Ethanol Inhalation
Hours After Withdrawal

FIGURE 3: Handling-induced convulsions for male and female mice of the WSP and WSR lines from Experiment 2, during 25 hr of withdrawal following 72 hr of ethanol intoxication. Means ± S.E. are shown. See text for discussion.


WSP, Male (9)
WSP, Female (12)
WSR, Male (10)
WSR, Female (12)
Mean Handling Induced Convulsion Score
4.0
3.0
2.0
1.0
0
1
2
4
5
7
8
10
11
13
14
23
25
Hours After Withdrawal

et al., 1983a). Figure 4 shows the mean 25 hr area for WSP and WSR male and female mice, both Ethanol Dependent/withdrawn and Control. Differences were assessed using a 3-way ANOVA (drug by line by sex). Male mice of both the WSP and WSR lines showed more severe withdrawal than female mice ($F(1,52) = 8.92$, $p < .004$), consistent with their elevated blood levels. A significant interaction of drug and line was found ($F(1,52) = 16.5$, $p < .001$). A test of the simple effects (Keppel, 1973) contributing to the interaction revealed that: 1) Ethanol significantly elevated HIC in both WSP and WSR mice as compared to controls ($F(1,64) = 31.3$, $p < .001$); and 2) WSP mice showed higher HIC than WSR mice for both the Dependent/withdrawn group and for the Controls ($F(1,64) = 56.5$, $p < .05$).

Experiment 3. In Experiment 3, we examined the effect of a single dose of ethanol on HIC in the WSP and WSR lines. Figure 5 shows HIC scores pre (-2, 0 hr) and post (2 - 24 hr) injection with ethanol or saline. Comparisons between ethanol- and saline-treated mice within each line were made using Student's t-tests (unpaired, two-tailed). Because WSR mice showed no baseline HIC, WSP and WSR mice could not easily be compared directly. Saline-treated WSP mice showed a baseline HIC averaging about 0.7 on the scale of 0-4. Ethanol initially suppressed this baseline almost to 0 at the 2 hour test ($t(44) = 5.9$, $p < .001$). By six hr post injection, ethanol-treated WSP mice displayed a marked elevation in HIC score over saline-treated WSP mice, which continued through hr 12 ($t(44) = 2.25$, $p < .05$). WSR mice showed little or no basal HIC, hence it was not possible to assess suppression of HIC by ethanol in these mice. Ethanol-treated WSR mice showed significant elevation of HIC scores only at hour 10 ($t(44) = 2.25$, $p < .05$).

FIGURE 4: Mean ± S.E. area under the 25 hr withdrawal curve for male and female mice of the WSP and WSR lines from Experiment 2 following 72 hr of inhalation of ethanol (Ethanol Dependent/withdrawn), or air without ethanol (Control). See text for analysis.

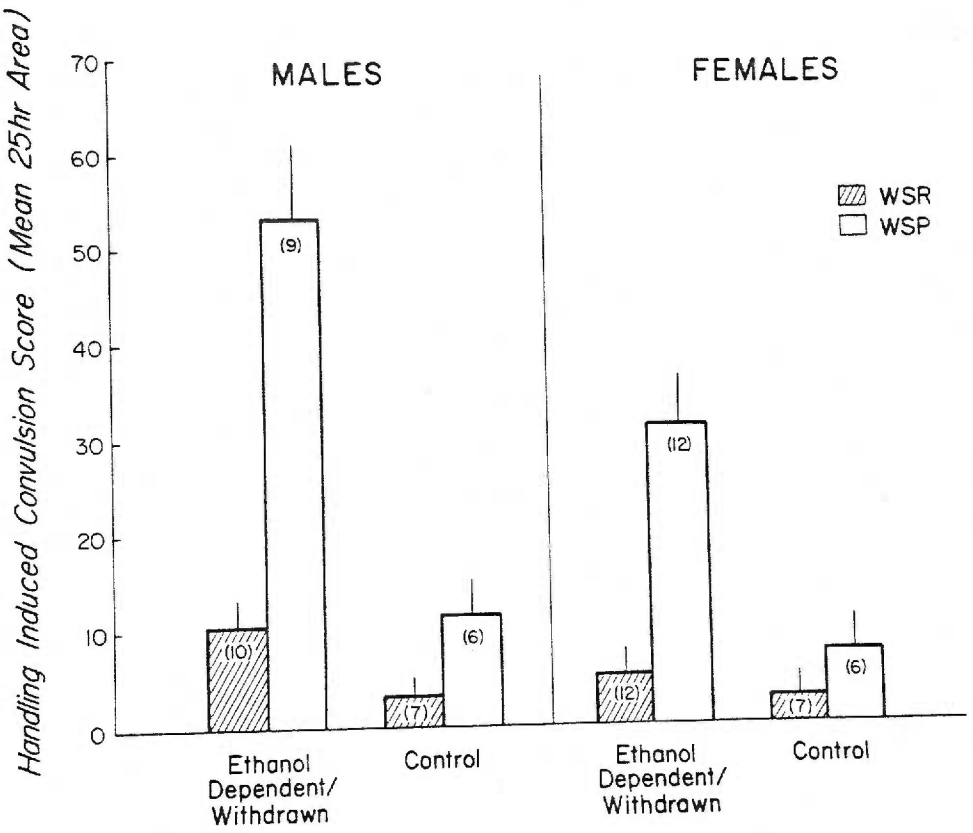
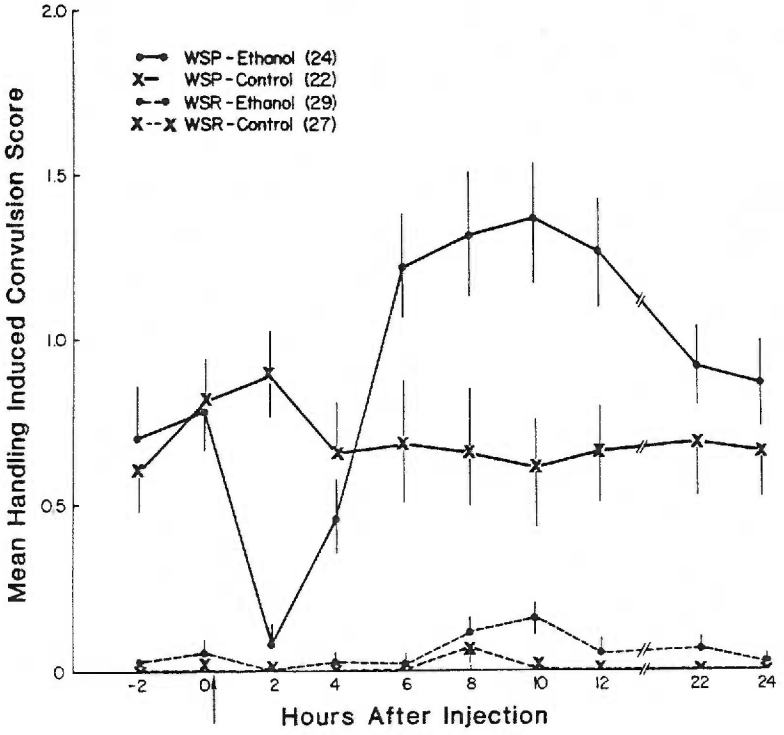

Handling Induced Convulsion Score (Mean 25hr Area)
70
60
50
40
30
20
10
0
MALES
FEMALES
WSR
WSP
(9)
(10)
(6)
(7)
(12)
(12)
(6)
(7)
Ethanol Dependent/ Withdrawn
Control
Ethanol Dependent/ Withdrawn
Control

FIGURE 5: Effect of a single i.p. injection of ethanol on HIC in WSP and WSR mice from Experiment 3. Mean ± S.E. HIC scores are shown for 2 hr prior to ethanol injection, and at intervals for 24 hr post injection. See text for analysis.


WSP - Ethanol (24)
WSP - Control (22)
WSR - Ethanol (29)
WSR - Control (27)
Mean Handling Induced Convulsion Score
2.0
1.5
1.0
0.5
0
-2
0
2
4
6
8
10
12
22
24
Hours After Injection

Experiment 4. In the fourth experiment, we studied the generality of the withdrawal differences in these lines following three days of treatment with ethanol and pyrazole, or pyrazole alone. Table 2 shows BEC at withdrawal, mean 25 hr HIC area, bodyweight on Day 1, and change in bodyweight between Day 1 and time of withdrawal for mice following three days of ethanol intoxication. Data were analyzed using two way ANOVAs (line by drug). No significant differences were detected between groups for body weight or weight loss. WSP and WSR mice showed equivalent BEC at time of withdrawal. Withdrawal convulsion severity over the 25 hr of withdrawal measured was higher for WSP mice than for WSR mice ($F(1,37) = 55$, $p < .001$), and ethanol elevated HIC scores to a greater degree than pyrazole alone ($F(1,37) = 18.7$, $p < .001$).

Figures 6, 7, and 8 show the results for tremor, straub tail and backward walking, respectively. The left panel shows the percent incidence at each hour tested and the right panel shows mean score over the four tests. As no Control mice showed any of these three behaviors, and given that scoring was blind, we eliminated control data from the analysis. The data were not normally distributed, thus the differences in mean score between withdrawing WSP and WSR mice were assessed using Fisher's exact test. WSP mice showed significantly more tremor ($p = .005$) than WSR mice, but did not differ significantly in incidence of straub tail ($p = .43$). WSR mice showed a trend toward more backward walking than WSP mice ($p = .08$).

Analysis of data from the hole-in-wall and vertical screen tests revealed inhomogeneity of variance between the Control and Dependent/withdrawn groups. This discrepency in variance arose because all withdrawing animals were much less active than control animals.

TABLE 2

Body weight, weight loss, blood ethanol concentration and mean 25 hour area HIC score for WSP and WSR mice following 72 hr of ethanol intoxication. (Mean ± S.E. for mice from Experiment 4.)

	Ethanol Dependent/withdrawn		Control	
	WSR	WSP	WSR	WSP
Number of animals	13	12	8	8
Body weight Day 1 (g)	23.7 ± .7	23.2 ± .7	23.8 ± .9	22.8 ± .8
Weight loss Day 1-4 (g)	1.2 ± .3	1.3 ± .3	0.5 ± .5	0.9 ± .4
BEC at withdrawal (mg/ml)	2.30 ± .20	2.64 ± .23	----	----
Mean 25 hour area	9.4 ± 2.2	41.6 ± 3.7	1.6 ± .8	20.0 ± 5.3

FIGURE 6: Appearance of tremor during withdrawal after 72 hr of ethanol intoxication for mice from Experiment 4. The left panel shows percent incidence (number of animals showing sign/total number of animals) at 5, 7, 10 and 12 hr of withdrawal. The right panel shows mean ± S.E. for scores summed over the 4 hr. See text for analysis.

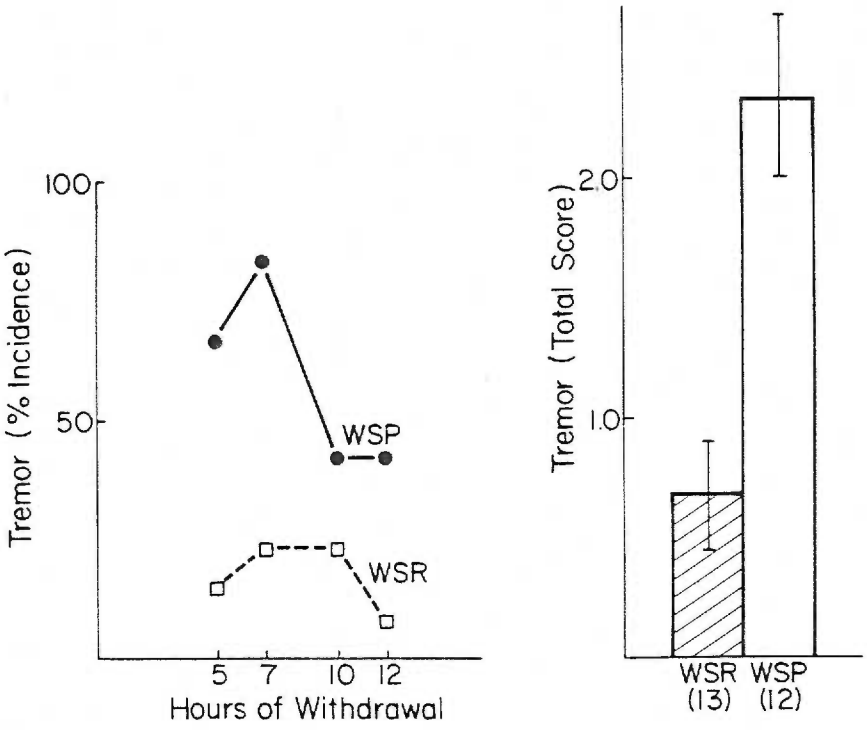

100
50
Tremor (% Incidence)
WSP
WSR
5 7 10 12
Hours of Withdrawal
2.0
1.0
Tremor (Total Score)
WSR (13)
WSP (12)

FIGURE 7: Appearance of straub tail during withdrawal after 72 hr of ethanol intoxication for mice from Experiment 4. See caption to Figure 6.

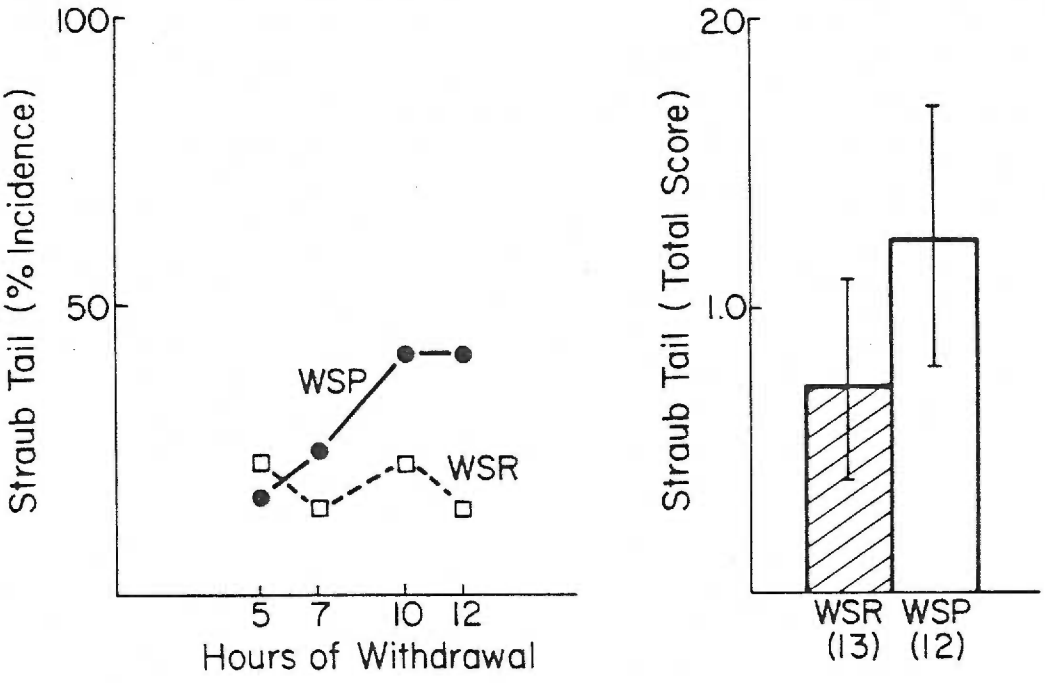
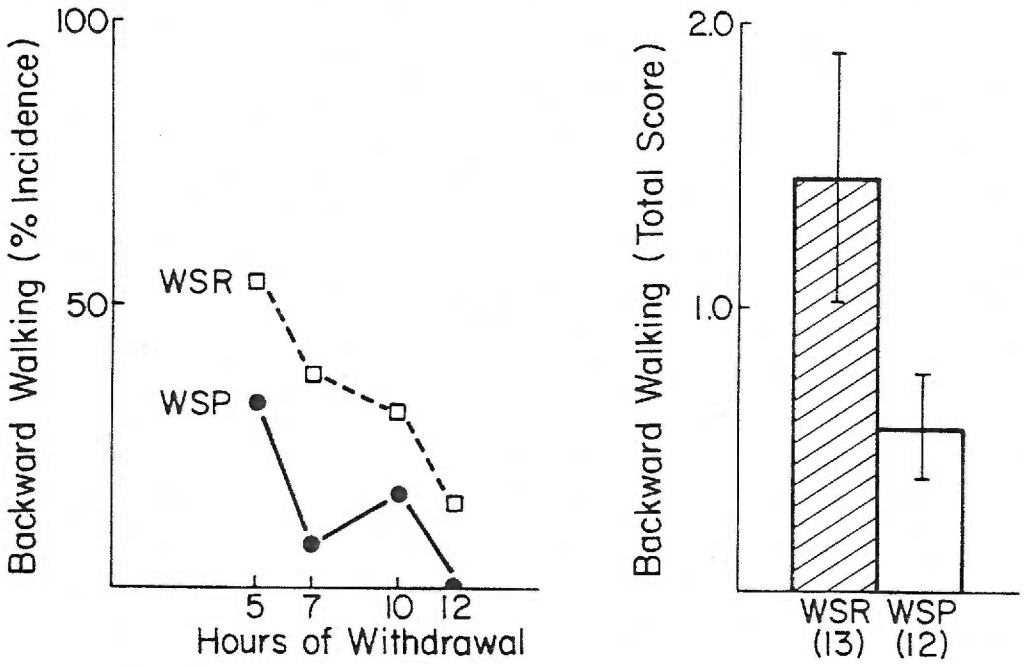

100
50
Straub Tail (% Incidence)
WSP
WSR
5
7
10
12
Hours of Withdrawal
20
1.0
Straub Tail (Total Score)
WSR
(13)
WSP
(12)

FIGURE 8: Appearance of backward walking during withdrawal after 72 hr of ethanol intoxication for mice from Experiment 4. See caption to Figure 6.


Backward Walking (% Incidence)
100
50
WSR
WSP
5
7
10
12
Hours of Withdrawal
Backward Walking (Total Score)
2.0
1.0
WSR
(13)
WSP
(12)

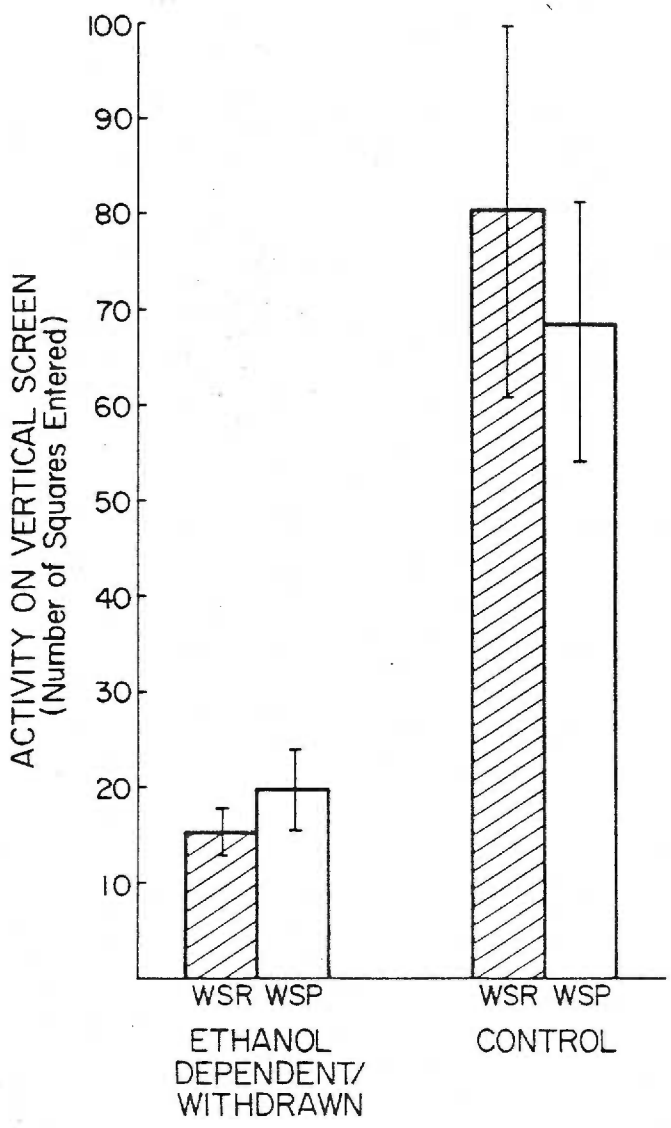
Therefore data from Control and Dependent/withdrawn groups were analyzed independently using Fisher's exact test (hole-in-wall) or Student's *t*-test (two-tailed, unpaired: vertical screen). In the hole-in-wall test, there were no significant differences between WSP and WSR mice on any variable in the control condition (Table 3), but withdrawing WSP mice tended to show greater reduction of activity on each measure than withdrawing WSR mice. Figure 9 shows total number of squares entered during the 3 min test on the vertical screen. Activity was severely reduced in animals made dependent and withdrawn, but WSP mice did not differ from WSR mice.

TABLE 3

Behavior in a hole-in-wall apparatus for WSP and WSR mice from Experiment 4 (Mean ± S.E.).

	Ethanol Dependent/withdrawn		Control	
	WSR	WSP	WSR	WSP
Number of crossings	.85 ± .36	.25 ± .18	10.5 ± 2.7	12.9 ± 1.6
Number of head pokes	5.4 ± 1.1	3.3 ± .3	27.8 ± 3.9	30.5 ± 2.9
Instances of rearing	.39 ± .18	.08 ± .08	8.4 ± 2.7	6.4 ± 2.4
Time in dark (sec)	32 ± 16	11 ± 9	89 ± 17	105 ± 12
Latency to enter dark (sec)	133 ± 18	162 ± 13	50 ± 20	53 ± 19

FIGURE 9: Mean ± S.E. for total number of squares crossed by mice from the WSP and WSR lines from Experiment 4 tested for 3 min on the vertical screen apparatus, between 7 and 10 hr of withdrawal. See text for analysis.


ACTIVITY ON VERTICAL SCREEN
(Number of Squares Entered)
100
90
80
70
60
50
40
30
20
10
WSR WSP
WSR WSP
ETHANOL
DEPENDENT/
WITHDRAWN
CONTROL

DISCUSSION

It was possible that in genetically selecting for high (WSP) and low (WSR) convulsions during ethanol withdrawal, we had inadvertently selected for pharmacokinetic factors, which might have resulted in differences in peak tissue concentration, altered distribution, or slower or faster metabolism of ethanol. Thus, part of the difference in withdrawal severity between WSP and WSR mice could have been accounted for by a difference in dose, rather than differences in tissue sensitivity to a given dose. We tested this possibility after acute treatment with ethanol, and during and after chronic treatment using ethanol and pyrazole. In Experiment 1, we found that same-sex WSP and WSR mice given a single injection of ethanol reached nearly identical blood ethanol concentrations one hour later, and showed similar rates of decline in BEC over the next four hours. In Experiment 2, we found that same-sex mice from the WSP and WSR lines, intoxicated chronically by ethanol inhalation, maintained nearly identical BEC while intoxicated and showed comparable rates of decline in BEC following removal from the inhalation chambers. Thus there is no evidence that WSP and WSR mice experience significantly different BEC during acute or chronic ethanol treatment.

Female mice from both lines showed significantly larger β_{60} following chronic treatment. This evidence of a more rapid initial decline in BEC in females than in males may explain the lower BEC experienced by the female mice during the development of dependence (see Figure 2, Panel A). β_{60} differed between Experiments 1 and 2,

with the chronically treated mice showing much slower rates of decline (compare Figure 1 with Figure 2, Panel B). There are a number of factors which influence β_{60} in the chronic versus the acute situation. Chronic treatment with ethanol may have induced ethanol metabolism (Wendell and Thurman, 1979), while residual ADH inhibition may have slowed metabolism. Additionally, as the initial rate of linear decline of BEC is dose dependent (Wendell and Thurman, 1979), β_{60} would not be expected to be the same in these two experiments.

In Experiment 1, we estimated apparent volume of distribution (Vd) of ethanol to see if there was evidence of a difference in distribution of ethanol between WSP and WSR mice. Male and female WSP and WSR mice did not differ significantly in Vd of an acute dose of ethanol when Vd was corrected for body weight. Because ethanol distributes primarily to body water, the value of Vd should approximate total body water (Goldstein, 1983). The values obtained for Vd in this experiment are high relative to published values of the body water content of mice (about 67% of body weight: Dawson, 1970). However, the values obtained agree with published data (85 - 89%, Phillips et al, 1984; 63 and 78%, Gilliam et al, 1983; see also Goldstein, 1983, p. 6). It may be that the values are large because the concentrations of ethanol used in calculating Vd in our experiments are concentrations in whole blood, rather than plasma. If our data are corrected for the solid content of whole blood, a factor of about 0.8 (York, 1982), the value for Vd is in the range that would be expected for for body water. However, the uncorrected Vd has been used successfully to detect differences between genetically selected lines of mice in the volume of distribution of ethanol (Gilliam et al, 1983), which was our purpose in these

experiments. The data we report in Experiments 1 and 2 suggest that WSP and WSR mice do not differ in distribution or rate of elimination of ethanol following acute or chronic treatment.

Handling-induced convulsions are a good measure for assessment of ethanol withdrawal in mice for a number of reasons. First, they can be measured repeatedly in the same mice, so the time course of the appearance and disappearance of withdrawal can be easily observed. Second, they are a very sensitive measure, appearing after a single administration of ethanol (Experiment 3, Figure 5) in WSP and, to a lesser degree, WSR mice. Third, they show dose-dependence; the severity of HIC is a function of the dose of ethanol given (Goldstein, 1972). Fourth, all species tested, including humans, display convulsions of some type as a symptom of withdrawal from ethanol dependence (Friedman, 1980). However, the HIC probably represents only part of the complex syndrome of ethanol withdrawal (Wilson et al., 1984). In Experiment 4, we found that WSP mice showed more severe withdrawal than WSR mice in amount of tremor observed, and also showed a tendency toward increased reduction of activity in the hole-in-wall apparatus. These results indicate that the difference in withdrawal between WSP and WSR mice is not limited to the single withdrawal sign, handling-induced convulsions. Some withdrawal signs revealed no difference between WSP and WSR mice. The genetic basis for these signs of withdrawal, therefore, may differ to some degree from that underlying handling-induced convulsions. By implication, the physiological mechanism by which ethanol treatment produces these withdrawal signs may be different from the mechanism by which it produces HIC and tremor.

A similar selection project is in progress at the Institute for Behavioral Genetics at the University of Colorado, Boulder, Colorado (Allen et al., 1983). These investigators are developing two Severe Ethanol Withdrawal (SEW) lines, two Mild Ethanol Withdrawal (MEW) lines, and two control (C) lines. This study uses as selection criterion a multivariate index derived from seven different measures of withdrawal severity, including handling seizure severity, body temperature, hole-in-wall crossings, hole-in-wall rearings, spontaneous seizures in the hole-in-wall, vertical screen crossings, and ethanol consumption. Significant response to selection has been reported in the measures of seizure severity and body temperature, while hole-in-wall seizures and vertical screen crossing do not distinguish the SEW and MEW lines as clearly (Wilson et al., 1984). Hole-in-wall crossings and rearings, and ethanol consumption are intermediate in separating the lines. The differences between individual measures of withdrawal appears to indicate some degree of genetic independence of these measures. This agrees with our finding that some, but not all measures of withdrawal are correlated with the HIC difference for which we have selected in the WSP/WSR mice.

The selection for WSP and WSR lines employs pyrazole in the inhalation method of inducing ethanol dependence. Pyrazole is known to increase HIC slightly (Crabbe et al., 1981) and susceptibility to this enhancement differs among inbred strains (Crabbe et al., 1983c). Thus, part of the selection pressure we have placed on the WSP and WSR mice has likely been applied to relative genetic sensitivity to pyrazole-induced HIC, in addition to relative genetic sensitivity to ethanol-induced HIC. The results of Experiment 2 (see Figure 4) suggest that this has played a minor role in the development of the

10-fold response to selection we report between WSP and WSR mice. In Experiment 3, we showed that following a single injection of ethanol, WSP mice showed much greater rebound elevation of HIC than WSR mice, independent of pyrazole treatment. Harris et al. (1984b) measured ethanol withdrawal in WSP and WSR mice using a liquid diet method of intoxication, which does not require the use of pyrazole. They also found increased severity of withdrawal seizures in WSP as compared to WSR mice. Thus, the difference between WSP and WSR mice is largely one in sensitivity to ethanol, not pyrazole.

During withdrawal from ethanol, mice placed on a horizontal surface walk backwards, a behavior that is seen rarely if ever in control mice (unpublished observations). This behavior has been identified as an ethanol withdrawal sign in mice (Freund, 1969). In Experiment 4, we noted that withdrawing WSR mice showed a trend towards walking backwards to a greater degree than did withdrawing WSP mice. Backward walking has been observed in mice following treatment with drugs which elevate central nervous system serotonin levels (Shimomura et al., 1980). Head twitches, which have been reported as a sign of ethanol withdrawal in mice (Collier et al., 1974), are also seen following elevation of CNS serotonin (Matthews and Smith, 1980). Thus there is preliminary evidence of a possible role for serotonin in ethanol withdrawal in WSP and WSR mice.

The WSP and WSR mice have been applied to questions of how membrane parameters are correlated with intoxication and withdrawal. Perlman and Goldstein (1984) studied the depressant action of ethanol on the WSP and WSR mice and on a second pair of selected lines, the LS and SS mice. LS (Long Sleep) and SS (Short Sleep) were selected for differing

duration of loss of righting reflex ("sleep time") following acute administration of ethanol. The WSP and WSR were tested for sensitivity to the ataxic effect of ethanol by determining brain ethanol concentrations at the time the mice lost their ability to maintain their balance on a stationary dowel. LS mice would be expected to be about twice as sensitive as SS mice on this test (Erwin et al., 1976). All four lines of mice were then tested for intrinsic membrane order, and sensitivity to membrane disordering using fluorescence polarization and EPR spectroscopy. No differences in the intrinsic membrane order of the four lines were detected with either technique. The sensitivities of the membranes from the four lines to ethanol-induced disordering were not significantly different when measured by fluorescence polarization, but EPR spectroscopy revealed line differences in disordering sensitivity that correlated with the relative sensitivity of the four lines to the CNS depressant action of ethanol. Specifically, SPM (synaptic plasma membranes) prepared from LS mice were about half again as sensitive as SS membranes to disordering by ethanol, while WSP and WSR mice showed no difference in their response to ethanol.

Harris et al. (1984b) looked at fluidity changes after chronic consumption of ethanol in a liquid diet. WSP and WSR mice showed similar baseline SPM fluidity. Following eight days of treatment, mice from both lines were killed for the preparation of brain membranes or scored for withdrawal. Ethanol-consuming WSP and WSR mice showed an increased membrane rigidity when compared to WSP and WSR mice fed a liquid diet in which sucrose was substituted for ethanol. This increase in rigidity might reflect the development of tolerance to the

acute disordering effects of ethanol, and thus tolerance appeared to develop at the same rate for WSP and WSR mice. Withdrawal seizure scores were about twice as severe for WSP and WSR mice. Thus severity of withdrawal appeared to be independent of membrane fluidity changes. However, it would be of interest to measure fluidity of WSP and WSR mice at time of peak withdrawal, or at a time when the animals are no longer intoxicated. It could be that the WSP mice are slower to recover initial membrane fluidity as blood ethanol levels fall.

CONCLUSIONS

Withdrawal from ethanol is a very complex syndrome, with components that appear to have multiple and independent genetic sources, as well as environmental influences. The WSP and WSR mice represent a sorting of these genetic influences, so that one particular aspect of the withdrawal syndrome can be dissected from the other components and studied independently. This approach has worked well in the study of sensitivity and tolerance to ethanol, which are in general studied in one system at a time, and should be a successful way to study ethanol dependence. Withdrawal will be understood when three questions have been answered: First, what are the behavioral symptoms which compose the withdrawal syndrome, and what is the relationship between dose and duration of ethanol treatment, and appearance, severity, and time course of those symptoms? Second, what are the physiologic correlates of the behavioral symptoms (and are there physiologic symptoms of ethanol withdrawal which have no behavioral manifestation)? And third, by what mechanisms does ethanol initiate the ethanol withdrawal syndrome? Or in other words, what are the direct actions of ethanol which lead to the eventual appearance of a withdrawal syndrome when ethanol is no longer present? Each of these questions depends to some degree on answering the previous question. Yet even the behavioral syndrome is not well characterized. Dose/response or time course relationships have been established for very few of the symptoms of withdrawal (Friedman, 1980). Many symptoms depend on time-consuming observations which are difficult to quantify. The WSP and WSR mice represent a model of a single withdrawal sign which has been

characterized for time course of appearance, and dose-dependency. Thus these mice can be used to study questions of the physiologic mechanisms underlying handling-induced convulsions during ethanol withdrawal. In this way one aspect of the ethanol dependence syndrome may be understood.

In summary, WSR and WSP mice given identical ethanol treatment show a marked difference in the severity of the withdrawal syndrome. This indicates that the action of ethanol on these mice differs in some fundamental way. The experiments reported here suggest strongly that the basis for these differences is not pharmacokinetic. Potentially, studies of the physiological differences between the WSP and WSR mice could reveal the mechanisms underlying physical dependence on ethanol, and yield information useful for both identification of those individuals genetically susceptible to alcoholism prior to their becoming dependent on alcohol, and for treatment of the alcohol abuser.

REFERENCES

Allen, D.L., Petersen, D.R., Wilson, J.R., McClearn, G.E. and Nishimoto, T.K. (1983) Selective breeding for a multivariate index of ethanol dependence in mice: Results from the first five generations. Alcoholism: Clinical and Experimental Research 7(4): 443-447.

Amir, S., Brown, Z. and Amit, Z. (1980) The role of acetaldehyde in the psychopharmacological effects of ethanol. In Rigter, H., and Crabbe, J.C., (Eds) Alcohol Tolerance and Dependence Amsterdam: Elsevier/North Holland Biomedical Press, 317-337.

Amit, Z., Brown, Z.W. and Rockman, G.E. (1977) Possible involvement of acetaldehye, norepinephrine and their tetrahydroisoquinoline derivatives in the regulation of ethanol self-administration. Drug and Alcohol Dependence 2: 495-500.

Asmussen, E., Hald, J. and Larsen, V. (1948) The pharmacological action of acetaldehyde on the human organism. Acta Pharmacologia 4: 311-320.

Barry, III, H. (1979) Behavioral manifestations of ethanol intoxication and physical dependence. In Majchrowicz, E. and Noble, E.P. (Eds) Biochemistry and Pharmacology of Ethanol, Volume 2 New York, Plenum Press, 511-531.

Belknap, J.K., Berg, J.H. and Coleman, R.R. (1977) Alcohol withdrawal and magnesium deficiency in mice. Pharmacology, Biochemistry and Behavior 9: 1-6.

Brown, Z.W., Amit, Z, Smith, B. and Rockman, G.E. (1978a) Differential effects on conditioned taste aversion learning with peripherally and centrally administered acetaldehyde. Neuropharmacology 17(11): 931-935.

Brown, F.C., Zavad, J. and Harralson, J.D. (1978b) Interactions of pyrazole and and ethanol on norepinephrine metabolism in rat brain. Journal of Pharmacology and Experimental Therapeutics 206: 75-80.

Carmichael, F.J., and Israel, Y. (1975) Effects of ethanol on neurotransmitter release by rat brain cortical slices. Journal of Pharmacology and Experimental Therapeutics 193: 824-834.

Chin, J.H. and Goldstein, D.B. (1977) Drug tolerance in biomembranes: A spin label study of the effects of ethanol. Science 196: 684-685.

Chin, J.H., Trudell, J.R., and Cohen, E.N. (1976) The compression-ordering and solubility-disordering effects of high pressure gases on lipid bilayers. Life Sciences 18: 489-498.

Chu, N.-S. (1983) Effects of ethanol on rat cerebellar purkinje cells. International Journal of Neuroscience 21: 265-278.

Cohen, G. (1976) Alkaloid products in the metabolism of alcohol and biogenic amines. Biochemical Pharmacology 25: 1123-1128.

Collier, H.O.J., Hammond, M.D. and Schneider, C. (1974) Biogenic amines and head twitches in mice during ethanol withdrawal. British Journal of Pharmacology 51: 310-311.

Crabbe, J.C., Janowsky, J.S., Young, E.R. Kosobud, A., Stack, J. and Rigter, H. (1982) Tolerance to ethanol hypothermia in inbred mice: Genotypic correlations with behavioral responses. Alcoholism: Clinical and Experimental Research 6: 446-458.

Crabbe, J.C., Kosobud, A. and Young, E.R. (1983a) Genetic selection for ethanol withdrawal severity: differences in replicate mouse lines. Life Sciences 33: 955-962.

Crabbe, J.C., Kosobud, A., Young, E.R., and Janowsky, J. (1983b) Polygenic and single-gene determination of response to ethanol in BXD/Ty recombinant inbred mouse strains. Neurobehavioral Toxicology and Teratology 5: 181-187.

Crabbe, J.C, Kosobud, A., Young, E.R., Tam, B.R. and McSwigan, J.D. (1985) Bidirectional selection for susceptibility to ethanol withdrawal seizures in Mus musculus. Behavior Genetics 15(6): 521-536.

Crabbe, J.C., Young, E.R., Janowsky, J. and Rigter, H. (1981) Pyrazole exacerbates handling-induced convulsions in mice. Neuropharmacology 20: 605-609.

Crabbe, J.C., Young, E.R. and Kosobud, A. (1983c) Genetic correlations with ethanol withdrawal severity. Pharmacology, Biochemistry and Behavior 18: 541-547.

Crews, F.T., Majchrowicz, E. and Meeks, R. (1983) Changes in cortical synaptosomal plasma membrane fluidity and composition in ethanol-dependent rats. Psychopharmacology 81: 208-213.

Davis, V.E. and Walsh, M.J. (1970) Alcohol, amines and alkaloids: a possible biochemical basis for alcohol addiction. Science 167: 1005-1007.

Dawson, N.J. (1970) Body composition of inbred mice (Mus musculus) Comparative Biochemistry and Physiology 37: 589-593.

DeFries, J.C. (1981) Current perspectives on selective breeding: example and theory. In McClearn, G.E., Deitrich, R.A. and Erwin, V.G. (Eds.) Development of animal models as pharmacogenetic tools (USDHHS-NIAAA Research Monograph No. 6). Washington D.C.: U.S. Government Printing Office.

DeFries, J.C., Gervais, M.C. and Thomas, E.A. (1978) Response to 30 generations of selection for open-field behavior in laboratory mice. Behavior Genetics 8: 3-13.

DeFries, J.C., Wilson, J.R. and McClearn, G.E. (1970) Open-field behavior in mice: selection response and situational generality. Behavior Genetics 1: 195-211.

Deitrich, R.A. and Spuhler, K. (1984) Genetics of alcoholism and alcohol actions. In Research Advances in Alcohol and Drug Problems, Vol. 8 ed. by Smart, R.G., Cappell, H.C., Glaser, F.B., Israel, Y., Kalant, H., Popham, R.E., Schmidt, W., and Sellars, E.M. New York: Plenum Publishing Co., 47-98.

Duncan, C. and Deitrich, R.A. (1979) A critical evaluation of tetrahydroisoquinoline induced ethanol preference in rats. Pharmacology, Biochemistry and Behavior 13: 265-281.

Erwin, V.G., Heston, W.D.W., McClearn, G.E. and Deitrich, R.A. (1976) Effect of hypnotics on mice genetically selected for sensitivity to ethanol. Pharmacology, Biochemistry and Behavior 4: 679-683.

Eyring, H., Woodbury, J.W. and D'Arrigo, J.S. (1973) A molecular mechanism of general anesthesia. Anesthesiology 38: 415-424.

Falconer, D.S.: (1960) Introduction to Quantitative Genetics. Ronald Press, New York.

Friedman, H.J. (1980) Assessment of physical dependence on and withdrawal from ethanol in animals. In Rigter, H., and Crabbe, J.C., (Eds) Alcohol Tolerance and Dependence Amsterdam: Elsevier/North Holland Biomedical Press, 157-180.

Freund, G.: (1969) Alcohol withdrawal syndrome in mice. Archives of Neurology 21: 315-320.

Gage, P.W. (1967) The effect of methyl, ethyl and n-propyl alcohol on neuromuscular transmission in the rat. Journal of Pharmacology and Experimental Therapeutics. 150: 236-243.

Gilliam, D.M., Bloedow, D.C. and Collins, A.C.: Nonlinear pharmacokinetics of ethanol elimination in long sleep and short sleep mice. Alcoholism: Clinical and Experimental Research 7: 95-99, 1983.

Goldman, P.S. and Docter, R.F. (1966) Facilitation of bar-pressing and "suppression" of a conditioned suppression in cats as a function of alcohol. Psychopharmacologia 9: 64-72.

Goldstein, D.B. (1972) Relationship of alcohol dose to intensity of withdrawal signs in mice. Journal of Pharmacology and Experimental Therapeutics 180: 203-215.

Goldstein, D.B. (1983) The Pharmacology of Alcohol New York: Oxford Press.

Goldstein, D.B. and Pal, N. (1971) Alcohol dependence produced in mice by inhalation of alcohol: Grading the withdrawal reaction. Science 172: 288-290.

Halsey, M.J. and Wardley-Smith, B. (1975) Pressure reversal of narcosis produced by anaesthetics, narcotics and tranquilizers. Nature 257: 811-813.

Harris, R.A., Baxter, D.M., Mitchell, M.A. and Hitzemann, R.J. (1984a) Physical properties and lipid composition of brain membranes from ethanol tolerant-dependent mice. Molecular Pharmacology 25: 401-409.

Harris, R.A., Crabbe, J.C. and McSwigan, J.D.: (1984b) Relationship of membrane physical properties to alcohol dependence in mice selected for genetic differences in alcohol withdrawal. Life Sciences 35, 2601-2608.

Heron, D.S., Shinitzky, M., Hershkowitz, M., and Samuel, D. (1980) Lipid fluidity markedly modulates the binding of serotonin to mouse brain membranes. Proceedings of the National Acadamy of Science 77(12): 7463-7467.

Hill, M.W. and Bangham, A.D. (1975) General depressant drug dependency: A biophysical hypothesis. Advances in Experimental Medicine and Biology 59: 1-9.

Himwich, H.E. and Callison, D.A. (1972) The effects of alcohol on evoked potentials of various parts of the central nervous system of the cat. In Kissin, B and Begleiter, H. (Eds) The Biology of Alcoholism. Volume 2: Physiology and Behavior. New York: Plenum Press, 67-84.

Holloway, F.A. and Wansley, R.A. (1973) Factors governing the vulnerability of DRL performance to the effects of ethanol. Psychopharmacology 28: 351-362.

Hutchins, J.B., Allen, D.L., Cole-Harding, S. and Wilson, J.R. (1981) Behavioral and physiologic measures for studying ethanol dependence in mice. Pharmacology, Biochemistry and Behavior 15: 55-59.

Israel, Y., Khanna, J.M. and Lin, R. (1970) Effect of 2,4-dinitrophenol on the rate of ethanol elimination in the rat in vivo. Biochemistry Journal 120: 447

Jones, A.W. (1984) Interindividual variations in the disposition and metabolism of ethanol in healthy men. Alcohol 1: 385-391.

Jörnvall, H. (1985) Alcohol dehydrogenases, aldehyde dehydrogenases, and related enzymes. Alcohol 2: 61-66.

Kalant, H. (1975) Direct effects of ethanol on the nervous system. Federation Proceedings 34: 1930-1941.

Keppel, G. (1973) Design and Analysis: A Researcher's Handbook New Jersey: Prentice-Hall, Inc.

Kopun, M. and Propping, P. (1977) The kinetics of ethanol absorption and elimination in twins and supplementary repetitive experiments in singleton subjects. European Journal of Clinical Pharmacology 11: 337.

Lee, A.G. (1976) Model for action of local anesthetics. Nature 262: 545-548

Lieber, C.S., Rubin, E., DeCarli, L.M., Misra, P. and Gang, H. (1970) Effects of pyrazole on hepatic function and structure. Laboratory Investigation 22: 615-621.

Lieber, C.S., Teschke, R., Hasamura, Y., and DeCarli, L.M. (1975) Differences in hepatic and metabolic changes after acute and chronic alcohol consumption. Federation Proceedings 34(11): 2060-2074.

Lindros, K.O. (1978) Acetaldehyde - Its metabolism and role in the actions of alcohol. In: Y. Israel, B. Glaser, H. Kalant, R.E. Popham, W. Schmidt and R.G. Smart (Eds), Research Advances in Alcohol and Drug Problems, New York, Plenum Publishing Co., 111-176.

Lyon, R.C. and Goldstein, D.B. (1982) Increased membrane order after chronic ethanol treatment. Alcoholism: Clinical and Experimental Research 6: 148 (abstract).

Macdonald, E. (1976) Effect of pyrazole, 4-methylpyrazole, 4-bromopyrazole and 4-iodopyrazole on brain noradrenaline levels of mice and rats. Acta Pharmacologia 39: 513-524.

Matthews, W.D. and Smith, C.D. (1980) Pharmacological profile of a model for central serotonin receptor activation. Life Sciences 26: 1397-1403.

McComb, J.A. and Goldstein, D.B. (1979) Quantitative comparison of physical dependence on tertiary butanol and ethanol in mice: Correlation with lipid solubility. Journal of Pharmacology and Experimental Therapeutics 208: 113-117.

McNamee, J.E., Tong, J.E. and Piggins, D.J. (1980) Effects of alcohol on velocity perception: I. Stimulus velocity and change in performance over time. Perceptual and Motor Skills 51: 779-785.

McQuarrie, D.G. amd Fingl, E. (1958) Effects of single doses and chronic administration of ethanol on experimental seizures in mice. Journal of Pharmacology and Experimental Therapeutics 124: 264-271.

Meyer, K.H. (1937) Contributions to a theory of narcosis. Transactions of the Faraday Society 33: 1062-1068.

Mitchell, M.A., Peris, J. and Harris, R.A. (1985) Barbiturate tolerance and dependence: Effects on synaptosomal sodium transport and membrane fluidity. Pharmacology, Biochemistry and Behavior 22: 955-960.

Mucha, R.F. and Pinel, J.P.J. (1979) Increased susceptibility to kindled seizures in rats following a single injection of alcohol. Journal of Studies of Alcohol 40(3): 258-271.

Mullins, L.J. (1954) Some physical mechanisms in narcosis. Chemical Review 54: 289-323.

Murphree, H.B. (1973) Electroencephalographic and other evidence for mixed depressant and stimulant actions of alcoholic beverages. Annals of the New York Acadamy of Science 215: 325-331.

Myers, R.D. and Critcher, E.C. (1982) Naloxone alters alcohol drinking induced in the rat by tetrahydropapaveroline (THP) infused ICV. Pharmacology, Biochemistry and Behavior 16: 827-836.

Myers, R.D. and Melchior, C.L. (1975) Alcohol drinking in the rat: Abnormal intake caused by tetrahydropapaveroline in brain. Science 196: 554-556.

Okada, K. (1967) Effects of alcohols and acetone on the neuromuscular junction of the frog. Japanese Journal of Physiology 17: 245-261.

Ortiz, A., Griffiths, P. and Littleton, J.M. (1974) A comparison of the effects of chronic administration of ethanol and acetaldehyde to mice: evidence for a role of acetaldehyde in ethanol dependence. Journal of Pharmarmacy and Pharmacology 26: 249-260.

Perlman, B.J. and Goldstein, D.B. (1984) Genetic influences on the central nervous system depressant and membrane-disordering actions of ethanol and sodium valproate. Molecular Pharmacology 26: 547-552.

Phillips, T.J., Gilliam, D.M. and Dudek, B.C.: An evaluation of the role of ethanol clearance rate in the differential response of long-sleep and short-sleep mice to ethanol. Alcohol 1: 373-378, 1984.

Pohorecky, L.A. (1977) Biphasic action of ethanol. Biobehavioral Reviews 1: 231-270.

Raby, K. (1954) Relation of blood acetaldehyde levels to clinical symptoms in the disulfiram-alcohol reaction. Quarterly Journal of Studies on Alcohol 15: 21-32.

Sanders, B. (1980) Withdrawal-like signs induced by a single administration of ethanol in mice that differ in ethanol sensitivity. Psychopharmacology 68: 109-113.

Schlesinger, K., Kakihana, R. and Bennett, E.L. (1966) Effects of tetraethylthiuram-disulfide (Antabuse) on the metabolism and consumption of ethanol in mice. Psychosomatic Medicine 28: 514-520.

Scholz, R. and Nohl, H. (1976) Mechanism of the stimulating effect of fructose on ethanol oxidation in perfused rat liver. European Journal of Biochemistry 63: 449-458.

Seeman, P. (1974) The membrane expansion theory of anesthesia: Direct evidence using ethanol and a high-density precision density meter. Experentia 30: 759-760.

Seeman, P. (1972) The membrane actions of anesthetics and tranquilizers. Pharmacological Reviews 24(4): 583-655.

Shimomura, K., Mori, J. and Honda, F. (1981) Backward walking induced by L-5-Hydroxytryptophan in mice. Japanese Journal of Pharmacology 31: 39-46.

Sinclair, J.G. and Lo, G.F. (1978) Acute tolerance to ethanol on the release of acetylcholine from the cat cerebral cortex. Canadian Journal of Physiology and Pharmacology 56: 668-670.

Stubbs, C.D. and Smith, A.D. (1984) The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochimica et Biophysica Acta 779: 89-137.

Takagi, T., Alderman, J., and Lieber, C.S. (1985) In vivo roles of alcohol dehydrogenase (ADH), catalase and the microsomal ethanol oxidizing system (MEOS) in deermice. Alcohol 2: 9-12.

Thieden, H.I.D., Jansen, J.A. and Münster, K. (1983) The extracellular space and the compartmentation of injected ethanol. Acta Pharmacologia et Toxicologia 52: 395-397.

Thurman, R.G., McKenna, W.R. and McCaffrey, T.B. (1976) Pathways responsible for the adaptive increase in ethanol utilization following chronic treatment with ethanol: inhibitor studies with the hemoglobin-free perfused rat liver. Molecular Pharmacology 12: 156-166.

Trudell, J.R. (1977) A unitary theory of anesthesia based on lateral phase seperations in nerve membranes. Anesthesiology 46: 5-10.

Victor, M. and Adams, R.D. (1953) The effect of alcohol on the nervous system. Research Publications - Assocociation for Research in Nervous and Mental Disease 32: 526-573.

von Wartburg, J.P. and Bühler, R. (1984) Biology of disease. Alcoholism and aldehydism: New biomedical concepts. Laboratory Investigation 50(1): 5-15.

Wallgren, H., Kosunen, A.L. and Ahtee, L. (1973) Technique for producing an alcohol withdrawal syndrome in rats. Israeli Journal of Medical Science 9(suppl): 63-71.

Wendell, G.D. and Thurman, R.G. (1979) Effect of ethanol concentration on rates of ethanol elimination in normal and alcohol-treated rats in vivo. Biochemical Pharmacology 28: 273-279.

Widmark, E.M.P. (1933) Verteilung und unwandltung des athyl alkohols in organimus des hundes. Biochemica Zeitschrift 267: 128-134, 1933.

Wilkinson, P.K. (1980) Pharmacokinetics of ethanol: A review. Alcoholism: Clinical and Experimental Research 4: 6-21.

Wilson, J.R., Erwin, V.G., DeFries, J.C., Petersen, D.R. and Cole-Harding, S. (1984) Ethanol dependence in mice: Direct and correlated responses to ten generations of selective breeding. Behavior Genetics 14(3): 235-256.

York, J.L.: Body water content, ethanol pharmacokinetics, and the responsiveness to ethanol in young and old rats. Developmental Pharmacology and Therapeutics 4: 106-116, 1982.