SENSITIVITY TO CHEMICAL CONVULSANTS: GENETIC INFLUENCES AND RELATIONSHIP TO ETHANOL WITHDRAWAL SEVERITY IN MICE.

by

Ann Kosobud

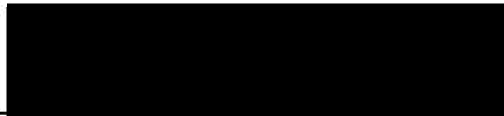
A DISSERTATION

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

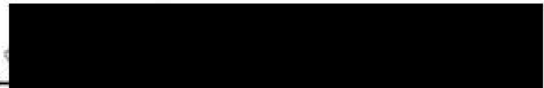
Doctor of Philosophy

October, 1988

Approved:



(Professor in charge of thesis)



(Chairman, Graduate Council)

TABLE OF CONTENTS

List of Figures v
List of Tables..... vi
List of Abbreviations vii
I. ABSTRACT 1
II. INTRODUCTION..... 4
A. Ethanol withdrawal. 4
1. Behavioral signs and symptoms in humans and animals. 4
2. Ethanol as a proconvulsant..... 6
3. Electrophysiological correlates of ethanol withdrawal. 7
4. Neurochemical correlates of ethanol withdrawal. 8
a. Monoamines. 9
b. Cholinergic systems. 10
c. GABA. 10
d. Glutamate. 12
B. Genetic techniques 12
1. Principles of genetic strategies..... 12
2. Inbred strains. 17
3 Genetically selected lines..... 18
4. Interpretations of statistical tests with replicated, selected lines. 20
5. Selection for EtOH withdrawal severity
The WSP and WSR mouse lines. 22
C. Genetic studies of EtOH dependence and withdrawal 23
D. Experimental studies of seizures and convulsions. 29
1. General aspects. 29
2. Studies with genetic models..... 31
III. EXPERIMENTS 33
A. Rationale..... 33
B. Methods..... 36
1. Subjects. 36
2. Experimental induction of convulsions..... 37
3. Convulsant drugs: Mechanisms of Action..... 38
a. Drugs acting at the GABA complex. 38
b. Strychnine. 41
c. Kainic acid..... 41
d. 4-aminopyridine. 42
e. CHEB. 43
4. Drug sources and preparation..... 45
5. Timed intravenous infusions. 46
6. Scoring of convulsions. 47
7. HIC as an index of withdrawal and hyperexcitability..... 49

8. Ethanol dependence and withdrawal 51
9. Statistical analyses 53
10. Correlational analyses; Special considerations 54
C. Results 56
1. Comparison of inbred strains: Within-drug 56
2. Comparison of inbred strains
Between-sign, between-drug 57
a. Picrotoxin 58
b. DMCM 58
c. TBPS 58
d. Bicuculline 58
e. Strychnine 59
f. Pentylenetetrazol 59
g. 4-aminopyridine 59
h. CHEB 59
i. Between-drug correlations 59
3. Comparisons of inbred Strains
Correlations between convulsant drug sensitivity, EtOH withdrawal severity, and HIC after saline or pyrazole treatment 60
4. Comparisons of WSP and WSR mice 61
a. Pentylenetetrazol: Effects of concentration 61
b. Pentylenetetrazol: Statistical analysis 62
c. DMCM 64
d. TBPS 65
e. Bicuculline 65
f. Picrotoxin 66
g. Strychnine 67
h. 4-Aminopyridine 68
i. CHEB 69
j. Kainic acid 70
IV. DISCUSSION 70
V. CONCLUSIONS 80
VI. REFERENCES 83

LIST OF FIGURES

Figure | Page

1 Mean HIC score for WSP, WSC and WSR mice following three days treatment with EtOH and pyrazole. Number of mice is indicated in parentheses. Female mice from Replicate 2 are pictured, but similar results were seen in Replicate 1 and using male mice. Mice were scored hourly for hours 2 through 15 post-withdrawal, and again at hours 24 and 25. .... 23a

2 A schematic diagram of the GABA complex, showing the chloride ionophore and four receptor sites. The site of action of relevant drugs and neurotransmitters is indicated. (after Lister and Nutt, 1987, and Olsen, 1981). 39a

3 A diagram of the between-drug correlations found in the present studies. A single line indicates that the connected variables were correlated. A double line indicates that the correlation between the variables was very strong. Variables not connected by any line were considered to be uncorrelated. 59a

4 Sensitivity to pentylenetetrazol-induced myoclonus in WSP and WSR mice. Latency to and ED for myoclonus were measured in both replicates at five concentrations of pentylenetetrazol. For discussion, see text. These data are also tabulated, with number of subjects included, in Tables 8B and 9B. 61f

5 Sensitivity to pentylenetetrazol-induced face and forelimb clonus in WSP and WSR mice. Latency to and ED for face and forelimb clonus were measured in both replicates at five concentrations of pentylenetetrazol. For discussion, see text. These data are also tabulated, with number of subjects included, in Tables 8B and 9B......... 61g

LIST OF TABLES

Table | Page

1 Interpretation of statistical tests using replicated, selected lines. 21a

2 Handling-induced convulsion scale. 50a

3A Effective dose (mg/kg) infused at time of appearance of convulsant sign in nine inbred mouse strains (mean and SE). 56a

3B Latency (sec) to appearance of convulsant sign in nine inbred mouse strains (mean and SE). 56b

4 Genetic correlation between ED (mg/ml) and latency (sec) for each drug and all convulsant signs (r_g, *n*, p). 57a

5 Intercorrelations among sensitivities to eight convulsant drugs (r_g). 57b

6 Genetic correlations among inbred mouse strains for sensitivity to convulsant drugs and HIC after saline, pyrazole or EtOH and pyrazole treatment (r_g (p)). 60a

7 Summary of results of tests of WSP and WSR mice 61a

8A Mean and SE (*n*) for ED for response to convulsant drugs for WSP and WSR mice of both replicates: Drugs showing evidence for a correlated response to selection 61b

8B Mean and SE (*n*) for ED for response to convulsant drugs for WSP and WSR mice of both replicates: Drugs showing little or no evidence of a correlated response to selection. 61c

9A Mean and SE for latency for response to convulsant drugs for WSP and WSR mice of both replicates 61d

9B Mean, SE and n for latency for response to convulsant drugs for WSP and WSR mice of both replicates. 61e

LIST OF ABBREVIATIONS

General

CNS	Central nervous system
CSF	Cerebrospinal fluid
ED	Effective dose
EEG	Electroencephalograph
EtOH	Ethanol
FF clonus	Face and forelimb clonus
GABA	Gamma-aminobutyric acid
HIC	Handling-induced convulsions
HS	Heterogeneous Stock (outbred mouse line)
RB clonus	Running/bouncing clonus
THE	Tonic hindlimb extensor seizure
WR clonus	Wild running clonus

Genetically selected mouse lines:

WSP	Withdrawal Seizure Prone
WSR	Withdrawal Seizure Resistant
WSC	Withdrawal Seizure Control
SEW	Severe Ethanol Withdrawal
MEW	Mild Ethanol Withdrawal
LS	Long Sleep
SS	Short sleep

Inbred mouse strains:

A
AKR
BALB
C3H
C57
C58
CBA
DBA
RIII
SWR

I. ABSTRACT

Withdrawal from ethanol (EtOH) is a complex syndrome involving multiple signs and symptoms. It is characterized by a general central nervous system hyperexcitability which can include seizures. EtOH can be viewed as a convulsant treatment in its production of a post-drug hyperexcitability. It is possible that the hyperexcitability seen during EtOH withdrawal is similar to that seen after acute administration of a convulsant drug. In the experiments described here, the genetic relationship between EtOH withdrawal severity and susceptibility to convulsant drugs was examined. Genetically selected mouse lines and inbred strains were tested to determine if severity of EtOH withdrawal was correlated with sensitivity to one or more convulsant drugs. The genetic correlations among sensitivity to different convulsant drugs were also measured, to determine if these drugs produce convulsions through related mechanisms.

The particular action or actions of EtOH responsible for the appearance of withdrawal remain unknown. Severity of withdrawal seizures has been shown to be influenced by genetic factors (Goldstein, 1973b). Pharmacogenetic techniques offer a strategy for identifying traits genetically correlated with EtOH withdrawal severity. In other words, additional effects of the genes which influence EtOH withdrawal severity can be identified. These additional effects (which could be alterations in neurotransmitter levels, receptors, brain proteins, or other genetic variations in brain function) may then indicate sites important in the etiology of EtOH physical dependence and withdrawal.

Two strategies were used to assess the degree of common genetic determination of responsiveness to convulsant drugs, and of sensitivity to EtOH withdrawal and convulsant drugs. First, a battery of 10 inbred strains was tested for convulsant sensitivity. Because all members of an inbred strain are genetically

identical, variation within a strain reflects the effects of environment. Given a number of inbred strains tested under similar environmental conditions, variation between strains reflects genetic effects. Thus, significant correlations between inbred strain mean values for EtOH withdrawal susceptibility and sensitivity to convulsant drugs imply common genes influencing the two responses (Hegmann and Possidente, 1980). In a similar way, a strong correlation between strain means for sensitivity to two different convulsant drugs is evidence that these drugs have similar mechanisms of action.

Withdrawal Seizure Prone (WSP) and Withdrawal Seizure Resistant (WSR) mice were also tested for sensitivity to the convulsant drugs. WSP and WSR mice have been selectively bred, respectively, for severe and mild handling-induced convulsions (HIC) following chronic exposure to EtOH. This is a replicated selection experiment, and two pairs of lines (WSP1 and WSR1, and WSP2 and WSR2) have been bred. Because these lines differ only in genes which affect EtOH withdrawal severity, systematic differences between these replicate pairs of WSP and WSR lines can be attributed to the effects of those genes. Screening WSP and WSR mice for sensitivity to convulsant drugs was performed to identify one or several drugs which produce a syndrome genetically related to EtOH withdrawal. In turn, this could indicate a site or sites which are important in EtOH withdrawal as modeled in the WSP and WSR lines.

Inbred strain mean sensitivities to the nine convulsant drugs were correlated with inbred strain means for EtOH withdrawal severity. Withdrawal severity was assessed by sensitivity to HIC after three days of continuous treatment with EtOH and pyrazole (an alcohol dehydrogenase inhibitor given to stabilize blood EtOH levels). No significant correlations were found. Thus, results from studies using inbred strains did not support a genetic correlation between EtOH withdrawal and sensitivity to any of the convulsant drugs tested.

The experiments with the selected lines yielded a different result. WSP mice were significantly more sensitive than WSR mice in response to the drugs picrotoxin, CHEB and 4-aminopyridine. To a lesser extent, WSP mice were also more sensitive than WSR mice to the drugs strychnine and kainic acid. For all other drugs, WSP1 mice were consistently more sensitive than WSR1 mice, while WSP2 mice were equal to, or occasionally less sensitive than, WSR2 mice. Thus, results from the WSP and WSR lines suggested a possible relationship between sensitivity to three drugs (CHEB, picrotoxin and 4-aminopyridine) and predisposition to severe EtOH withdrawal.

Strain sensitivities to the nine convulsant drugs showed a definite pattern of intercorrelation. Most drugs fell into one of two clusters. In other words, an inbred strain which was relatively sensitive to a drug in one cluster was also found to be sensitive to the other drugs in that cluster, but not to drugs in the other cluster. These clusters were defined by the type of convulsion produced by the drug, and may be a function of the anatomical structures involved in the motor expression of the seizure. One cluster (Group 1) was characterized by the convulsant sign, face and forelimb clonus. Four drugs (DMCM, pentylenetetrazol, TBPS and bicuculline) produced this type of convulsion. The second cluster (Group 2) was characterized by running/bouncing clonus and tonic hindlimb extensor (THE) convulsions. Four drugs (CHEB, strychnine, picrotoxin and bicuculline) produced this type of convulsion. Bicuculline was the only drug in which both types of convulsion were measured. Sensitivity to two drugs (4-aminopyridine and kainic acid) did not correlate strongly with either cluster.

In the inbred strains, sensitivities to the convulsant drugs were also compared with HIC in control animals treated with saline or pyrazole only. Severity of HIC after saline correlated significantly with sensitivity to several drugs in Group 1. This suggests that severity of HIC in inbred strains after saline treatment is genetically

related to sensitivity to drugs that produce face and forelimb clonus. Severity to HIC after pyrazole correlated significantly with sensitivity to several drugs, including drugs in both Group 1 and Group 2. This suggests that severity of HIC in inbred strains after pyrazole treatment may be genetically related to a generally increased susceptibility to convulsions.

In summary, the presence of a correlation between sensitivity to CHEB, 4-aminopyridine and picrotoxin and withdrawal severity in WSP and WSR lines suggests that the hyperexcitability produced by these drugs and EtOH withdrawal may have similar mechanisms. Results in the WSP and WSR lines did not support the pattern of distribution of the drugs into two groups seen in the inbred lines. Nor did they support a pattern based on the proposed site of action of these drugs. WSP1 mice seemed to be generally more sensitive to all convulsant drugs than WSR1 mice, while WSP2 and WSR2 mice showed a drug-specific pattern of sensitivity. Tests with inbred strains showed that correlations between sensitivity to the nine convulsant drugs tended to categorize the drugs into one or the other of two groups. These groups seemed to be characterized by the motor pattern of the convulsion caused by these drugs. Attributing differences in responsiveness to these drugs to differences at the site of initiation of convulsion appears unwarranted.

II. INTRODUCTION

II.A. Ethanol withdrawal.

II.A.1. *Behavioral signs and symptoms in humans and animals.*

EtOH withdrawal is a syndrome which has been extensively described but remains poorly understood. It is characterized by general hyperexcitability, beginning as EtOH levels in an organism are falling, and reaching maximum severity sometime after all EtOH has been eliminated. Victor and Adams (1953) studied the severe ethanol withdrawal syndrome in 266 patients in a Boston hospital. 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 consciousness. In some patients, delirium tremens (confusion, disorientation, severe hallucinations and agitation) began within 3 or 4 days, accompanied by severe autonomic overactivity; sweating, nausea, vomiting, diarrhea, and fever. Recovery from the acute phase of EtOH withdrawal appears to be complete after a week to 10 days, but full recovery may require a much longer time. Sleep disorders associated with chronic abuse have been observed 2 to 4 years after cessation of drinking in chronic alcoholics (Adamson and Budwiels, 1973; Wagman and Allen, 1975).

Animal models of the ethanol withdrawal syndrome have utilized measures similar to those seen in humans as well as some unique to animals. 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. Friedman (1980) summarized the withdrawal syndrome as observed in man, monkey, chimpanzee, dog, cat, mouse and rat. All species displayed tonic-clonic convulsions with recovery, fatal convulsions, and tremor. Other symptoms were seen in some species only. A variety of other motoric symptoms including muscle fasciculations and spastic rigidity have been reported. Behavioral symptoms reported included stereotypy, irritability, aggression, apparent 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. While the confusion and disorientation seen during delirium tremens cannot be verified in animals, hallucination-like behaviors have been observed in monkeys, dogs, and rats (Friedman, 1980; Barry, 1979). It is likely that these symptoms reflect multiple processes. EtOH withdrawal appears to

be the result of dysfunction in a number of distinct systems rather than a unitary phenomenon.

II.A.2. *Ethanol as a proconvulsant.*

Severity of withdrawal is related to dose and duration of treatment in humans (Ballenger and Post, 1978) and laboratory animals (Goldstein, 1972). Even after a single administration of EtOH, a period of increased sensitivity to induced convulsions can be demonstrated for several hours after EtOH has been eliminated. This effect can be seen whether convulsions are elicited using electrical kindling (Mucha and Pinel, 1979), pentylenetetrazol or electroconvulsive shock (McQuarrie and Fingl, 1958), flurothyl (Sanders, 1980), or handling (Kosobud and Crabbe, 1986).

Walker and Zornetzer (1974) subjected mice to two cycles of intoxication and withdrawal, and noted that the severity of the withdrawal syndrome appeared to be increased during the second episode. A similar phenomenon had also been observed in rats subjected to multiple episodes of intoxication and withdrawal (Branchey *et al.*, 1971; Baker and Cannon, 1979; Clemmesen and Hemmingsen, 1984; Poldrugo and Snead, 1984). Withdrawal in human alcoholics also appears to be exacerbated by repeated episodes (Ballenger and Post, 1978; Brown *et al.*, 1988). These investigators and others have suggested that this is due to a kindling-like phenomenon. Kindling was first identified by Goddard *et al.* (1967, 1969), who noted that repeated administration of subconvulsant doses of electrical stimulation led to a progressive and enduring increase in susceptibility to convulsions elicited by such treatments. A similar effect followed repeated administration of subconvulsant doses of convulsant drugs (Mason and Cooper, 1972; Pinel and Cheung, 1977) and can lead to the development of spontaneous convulsions.

The EtOH withdrawal state appears to share features with a variety of treatments which induce a state of hyperexcitability by direct means. Studies of the

discriminative stimulus properties of drugs have shown that animals identify withdrawal syndromes as similar to the state induced by an acute injection of pentylenetetrazol. This has been demonstrated during withdrawal from ethanol (Lal *et al.*, 1987), diazepam (Emmett-Oglesby *et al.*, 1983), triazolam (Lal *et al.*, 1984), morphine (Emmett-Oglesby *et al.*, 1984) and nicotine (Harris *et al.*, 1986). It may be useful to view EtOH administration as a proconvulsant treatment, and determine whether withdrawal resembles a specific convulsant treatment.

II.A.3. *Electrophysiological correlates of ethanol withdrawal.*

In humans, the EEG changes observed during EtOH withdrawal generally are interpreted as representing hyperexcitability (Kelley and Reilly, 1983). In unmedicated humans undergoing withdrawal, these changes range from a photomyoclonic response[1] (Lloyd-Smith and Gloor, 1961) to nonstimulated paroxysmal activity (Wikler *et al.*, 1956). Unstimulated epileptiform activity appears to be a relatively rare component of human EtOH withdrawal (Kelley and Reilly, 1983).

In animals, a number of electrophysiological abnormalities have been reported during withdrawal from EtOH. Bierley *et al.* (1979) found that visual evoked potentials were depressed in rats intoxicated with EtOH, but were enhanced during withdrawal. EEG recordings from the hippocampus, amygdala and mesencephalic reticular formation of cats during EtOH withdrawal showed an abnormal pattern of generalized slow waves, punctuated by spikes followed by short bursts of paroxysmal activity (Guerrero-Figueroa *et al.*, 1970). Walker and Zornetzer (1974) fed C57BL/6 mice a liquid diet containing alcohol for 6 days and measured both behavioral and EEG correlates of withdrawal. EEG recordings from the hippocampus showed a progression of abnormalities beginning with the

1 A photomyoclonic response is a spasm or jerk in response to light stimulus, which might be limited to ocular muscles but can be a generalized response involving the major skeletal muscles. This response has been shown to be a pathological enhancement of the normal subclinical photomotor response of Bichford (Meier-Ewert and Broughton, 1967).

appearance of single spikes resembling inter-ictal epileptiform events. These were not associated with any behavioral consequences. These single spikes increased in frequency of occurrence until a sustained epileptic seizure discharge developed. These were followed by varying periods of post-ictal depression interrupted by inter-ictal spikes. Sustained epileptic seizures were always associated with behavioral convulsions, but the onset of seizure activity preceded the appearance of the convulsion by as much as 20 seconds.

Hunter *et al.* (1978) used electrophysiological techniques to assess the anatomical localization of EtOH withdrawal seizures in rats. They found that limbic, mesencephalic and nonspecific thalamic structures appeared to be involved in primary generation of epileptic activity during withdrawal, with secondary involvement of specific thalamic, hypothalamic and cortical structures. The extrapyramidal motor system also was implicated (Hunter and Walker, 1978), with primary epileptiform activity observed in the red nucleus, substantia nigra, and caudate nucleus.

II.A.4. *Neurochemical correlates of ethanol withdrawal.*

Studies of neurochemical changes associated with physical dependence and withdrawal are complicated by a number of theoretical and methodological problems. First, when is the appropriate time to look for changes? If one looks during chronic treatment, the observed changes are confounded by the presence of ethanol, and the relationship between the changes seen and eventual withdrawal is difficult to determine. If one looks during withdrawal, it is difficult to separate underlying causes from the effects of the syndrome. For example, seizures cause the release of many neurotransmitters, so alterations in neurotransmitter levels in an animal undergoing seizures may reflect the underlying condition, withdrawal, that is responsible for the seizures, or it may be an effect of the seizures themselves. As noted by Hoffman and Tabakoff (1985), changes have been found in practically

every neurotransmitter system that has been studied, depend on brain area and are influenced by genetic constitution of animal being studied. Furthermore, changes in one neurotransmitter system influence others. Thus, the neurochemical changes following *in vivo* administration of EtOH occur both through direct effects and through indirect interactions among the various neurotransmitter systems. A brief discussion of some of the results is presented here; for a more detailed review, see Hoffman and Tabakoff (1985). For some neurotransmitters, further discussion will arise in the discussion of actions of convulsant drugs (Section III.B.3).

II.A.4.a. *Monoamines.*

Norepinephrine and dopamine levels do not appear to be altered during EtOH withdrawal when measured in whole rodent brain (Wallgren, 1973; Chopde *et al.*, 1977). However, mice fed a liquid diet containing EtOH showed a number of regional alterations during withdrawal. Forty-eight and 72 hours after withdrawal, striatal dopamine was elevated in mice treated for 10 or 30 days, while hypothalamic norepinephrine was elevated 24, 48 and 168 hours after withdrawal (10 days treatment) or 48, 72 and 168 hours after withdrawal (30 days treatment) (Dar and Wooles, 1984). Homovanillic acid and dihydrophenylacetic acid, metabolites of dopamine, were elevated in striatum on day 7 only (both 10 and 30 day treatments), associated with the return of dopamine levels to normal. Other investigators have reported that norepinephrine turnover increases at the time of withdrawal, and remains elevated throughout the course of EtOH withdrawal (Tabakoff and Hoffman, 1983; Pohorecky, 1974).

Dopamine turnover is usually found to be decreased during EtOH withdrawal (Hunt and Majchrowicz, 1974; Tabakoff and Hoffman, 1978), but Liljequist and Engel (1979) found increased dopamine turnover in animals undergoing severe withdrawal. Chronic treatment did not change number or affinity of striatal [^{3}H]spiroperidol or apomorphine binding sites in mice (Rabin *et al.*, 1980;

Tabakoff and Hoffman, 1979), but apparently increased such binding sites in rats (Barbaccia *et al.*, 1982).

Serotonin levels appeared normal during withdrawal (Wallgren, 1973; Chopde *et al.*, 1977). However, other investigators have found evidence of depressed turnover of serotonin (Tabakoff *et al.*, 1977). Little work has been done on the effect of chronic EtOH treatments on serotonin receptors (Hoffman and Tabakoff, 1985).

In summary, a consistent pattern of changes in catecholamines during EtOH withdrawal has not been found.

II.A.4.b. *Cholinergic systems.*

Binding of a cholinergic antagonist was significantly increased in hippocampus and cortex of mice at the time of withdrawal and at 8 hours after withdrawal (Rabin *et al.*, 1980; Tabakoff *et al.*, 1979), suggesting that receptor levels are elevated during chronic intoxication and remain elevated during expression of withdrawal. Rats that experienced seizures during withdrawal showed a smaller increase in striatal cholinergic receptors than rats which did not undergo seizures (Nordberg and Wahlstrom, 1982).

II.A.4.c. *GABA.*

Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the CNS. Recently, interest in the role of GABA in mediating EtOH sensitivity, tolerance, dependence and withdrawal has increased. Of particular interest is the $GABA_A$ receptor, which is associated with at least 3 other receptor sites (a benzodiazepine site, a barbiturate site, and a picrotoxin site) and a chloride ionophore. A detailed description of this receptor can be found in section III.B.3.

During ethanol withdrawal, GABA levels have been reported to be increased, or decreased in the brains of animals during EtOH withdrawal (Chopde

et al., 1977; Rawat, 1974; Sutton and Simmonds, 1973; Sytinsky *et al.*, 1975, Leitch *et al.*, 1977; Dar and Wooles, 1984). Activity of cortical GABAergic neurons appears to be reduced during EtOH withdrawal (Wixon and Hunt, 1980). Following chronic EtOH treatment, GABA binding tends to be altered in ways consistent with a decrease in efficacy of GABA turnover (Hoffman and Tabakoff, 1985). Decreases were noted in number (Ticku and Burch, 1980) and affinity (Unwin and Taberner, 1980) of low-affinity GABA binding sites in brains of mice during withdrawal following chronic ethanol treatment. Similar results were seen in rats, and appeared to be most pronounced at time of maximal withdrawal (Volicer, 1980; Ticku, 1980; Volicer and Biagoni, 1982).

Dar and Wooles (1985) found basal levels of GABA in the hypothalamus and corpus striatum to be normal 24 hours after withdrawal in mice, but they were unable to induce GABA accumulation by inhibiting GABA transaminase with aminooxyacetic acid. These investigators did not observe the mice for seizures during withdrawal. Their results suggested that GABA responsiveness was suppressed during EtOH withdrawal. Similarly, patients who experienced seizures during withdrawal showed normal GABA levels in CSF, while patients who did not experience seizures showed elevated levels of GABA (Volicer, 1980). This is consistent with the hypothesis that during EtOH withdrawal, a failure of GABA inhibition may contribute to the expression of seizure activity.

There is not much information available about the role of changes in sites of the GABA complex other than the $GABA_A$ receptor in withdrawal (Hoffman and Tabakoff, 1985). However, benzodiazepines show cross-dependence with EtOH, and continue to be the drugs of choice in the treatment of EtOH withdrawal (Jaffe and Ciraulo, 1985). Sensitivity to seizures induced by picrotoxin is increased during withdrawal (Szabo, *et al.*, 1984), suggesting a possible alteration at that site. EtOH and barbiturates show cross-dependence, suggesting that these two drugs cause

dependence through similar mechanisms, but it is not clear that the barbiturate site of the GABA complex is important for barbiturate dependence. For instance, some of the similarity of action of barbiturates and EtOH appears to relate to their fluidizing effect on neuronal membranes (Boisse and Okamoto, 1980).

II.A.4.d. *Glutamate.*

The role of glutamate in the EtOH withdrawal syndrome has recently received some attention. Chronic oral administration of EtOH caused an increase in number of high affinity glutamate binding sites in brain synaptosomal membranes. This increase was proportional to the duration of EtOH treatment (Michaelis *et al.*, 1978), and the time course of the changes appeared to correspond to the appearance of withdrawal seizures (Freed and Michaelis, 1978; Michaelis *et al.*, 1980).

II.B. Genetic techniques

II.B.1. Principles of genetic strategies.

The study of EtOH dependence and withdrawal can be confounded by the method used to chronically intoxicate animals. These methods are beset with a number of problems, including such things as compromise of the animal's health due to dehydration and inadequate nutrition, variability in the dosage each animal receives, and interactions with other drugs (as when pyrazole, an alcohol dehydrogenase inhibitor, is given to stabilize EtOH levels). If subjects are used whose genetic predisposition to EtOH withdrawal is known, they can be examined for differences that underlie that predisposition, and such studies may or may not require treatment of the animals with EtOH.

Genetic strategies also offer a means of identifying predisposing conditions. 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 the characteristic reaction of a given individual which is of interest.

Without necessarily understanding the mechanisms underlying individual differences, traits genetically correlated to the effect of interest can be identified, and used for prediction of a given individual's response to the drug. For instance, comparison of event-related potentials in the EEG of adult normal drinkers (Elmasian *et al.*, 1982) and boys with no prior exposure to EtOH (Begleiter *et al.*, 1984) revealed systematic differences between subjects with a positive family history for alcoholism compared to those subjects with a negative family history. This information could be used to identify individuals predisposed to alcoholism, and perhaps prevent their development of the disease.

The reaction of a given individual to a drug constitutes his or her phenotype with respect to that drug. A phenotype is simply the visible properties of an organism that are produced by the interaction of its genotype and the environment. All phenotypic variation in a trait measured in a population is attributable to genetic or environmental causes. In other words, differences between individuals can be completely described in terms of the effects of their genotype and the effects of their environment. In the paragraphs following, formal approaches for relating these two sources of variance are described.

Within a population, the variance due to phenotype can be partitioned as in the following equation,

$$V_P = V_G + V_E$$

where V_P is the phenotypic variance, V_G is the genotypic variance and V_E is the environmental variance. V_G can be broken down further as:

$$V_G = V_A + V_D + V_I$$

where V_A is variance in the additive genetic values, V_D is variance in dominance deviation, and V_I is variance in the interaction of V_A and V_D. V_A refers to the direct effect of genes acting independently, and is the basis for inherited resemblance between relatives. V_D refers to dominance effects (allelic interactions)

and V_I refers to epistasis (interactions between loci, or effects of one gene on another). In determining genetic correlations, we are most interested in the additive effects of genes. Effects of dominance and interaction are partitioned into environmental effects.

Given two phenotypes (red hair and blue eyes, for example) measured in each individual within a population, the phenotypic correlation of the two traits may be calculated. This could be calculated using any formula for the correlation coefficient (Pearson's r for instance). If blue eyes and red hair are always found together, the correlation coefficient (r) will approach 1. If they are rarely found together, r will approach -1. If their association is random, r will approach 0. The contribution of genetic and environmental correlations to this phenotypic correlation can be determined. The general strategy for calculating correlations involves calculating the variance of each trait separately and the covariance of the two traits. Phenotypic correlations can be partitioned, in a manner directly analogous to the partition of phenotypic variance just presented, into variance and covariance elements arising from genetic and environmental sources as follows (Falconer, 1983, Chapter 8),

$$r_p = h_x h_y r_a + e_x e_y r_e.$$

In this equation, r_p is the phenotypic correlation. The degree of genetic determination of trait x is represented by h_x, which, when squared, equals the *heritability* of trait x, calculated as V_{Ax}/V_{Px}. Thus, the heritability of trait x is simply the variance of trait x due to the additive effects of genes (V_{Ax}) as a proportion of the total phenotypic variance, and may range from 0 to 1. Similarly, h_y is the square root of the heritability of factor y (V_{Ay}/V_{Py}). The additive genetic correlation of the two traits (r_a) is the correlation of the additive genetic components of each phenotypic measure. The coefficients of environmental determination of x and y (e_x

and e_y), are derived in parallel to the heritabilities. The correlation of the environmental values of x and y is r_e.

The object of the experiments reported here was to estimate the genetic correlation between sensitivity to EtOH withdrawal and to convulsant drugs. The usefulness of estimating genetic correlations rather than phenotypic correlations derives from their implication of a physiological 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. For each such gene, this is an example of pleiotropy; one gene which influences more than one different character. 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 the context of an experiment, 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 act through a single mechanism, but have two apparently unrelated effects. For example, a single injection of EtOH (2.0 g/kg in rodents) results in reduction of body temperature and in ataxia. These effects may both be the result of a single action of EtOH (for instance, inhibition of excitable cells due to the fluidizing effect of EtOH of neuronal membranes). Alternatively, they may be due to two independent actions of EtOH (the former response might be mediated through an effect of EtOH on catecholamines in the hypothalamus, while the latter is mediated through the depression of synaptic activity in motor pathways). If it is possible to demonstrate a genetic correlation between the two effects, it implies that these effects are due to a single action of

EtOH (as in the first case), and manipulation of the relevant gene or genes should result in changes in both characters. If they are mediated through different actions of EtOH, 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. A genetic correlation could fail to be identified if it were masked by an environmental correlation in the opposite direction. For example, EtOH has effects on body temperature, usually hypothermia; it also has effects on activity, being activating at low doses, and depressant at high doses. At doses at which EtOH is activating, the hypothermic effect of EtOH may be masked by activity-induced heat generation. Also, the estimate of genetic correlation might be inaccurate if an environmental factor biases the measurement. For instance, the hypothermia observed following EtOH administration may be the result of a disruption of temperature regulation such that the animal fails to regulate its body temperature. Thus room temperature at which the measurement is taken will determine whether the response is identified as hypothermia, hyperthermia, or no response.

A number of limitations apply to the determination of genetic correlations. They are dependent upon the gene frequencies within the population in which they are measured, so they must be regarded as a property of that population (Falconer, 1983, p149). Furthermore, the model assumes two alleles per locus, Hardy-Weinberg-Castle equilibrium[2], linkage equilibrium, and only additive genetic effects (Carey, 1988). All methods of estimating genetic correlations violate these assumptions to some extent. Two different methods of estimating genetic

2 Hardy-Weinberg-Castle equilibrium requires a large population with random mating; no mutation, selection, or migration; constant gene and genotype frequencies across generations; and a simple relationship between gene and genotype frequencies (Falconer, 1983, p7).

correlations, one using inbred strains and the other using genetically selected lines, will be described below. Also, the general strategy and problems of interpretation of correlative data are discussed in Section III.B.9.

II.B.2. *Inbred strains.*

One technique useful for distinguishing genetic from phenotypic correlations is the study of inbred mice. An inbred strain is 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 for a given response, variability in that response reflects environmental effects. When tested under constant environmental conditions, differences between means for several inbred strains can be attributed to the effects of genes. 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 physiological factor that accounts for the observed difference. But two inbred strains will display any number of additional differences (for instance in body weight or brain size) arising from the random inbreeding of all genes 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. Statistically, the attempt to demonstrate a genetic correlation between a pair of variables in any two inbred strains has zero degrees of freedom. However, with the addition of several more inbred mouse strains which show a range of responses to the drug, genetic correlations can be estimated, as described below.

Inbred strains have been used to explore the genetic relationships between measures of initial sensitivity to, tolerance to and withdrawal from EtOH. In a study using 20 inbred strains, we (Crabbe *et al.*, 1983c) assessed strain sensitivities for eight measures of initial sensitivity to EtOH, tolerance to EtOH-induced hypothermia, and severity of withdrawal following chronic EtOH administration. The mean phenotypic values for each strain for each measure were then correlated to estimate the genetic correlation (r_g) (Blizard and Bailey, 1979). Of these measures, hypothermic tolerance showed a significant negative genetic correlation with withdrawal severity. Thus, by measuring hypothermic tolerance, one could, in theory, predict an animal's withdrawal score: animals that developed tolerance should show mild withdrawal, while little tolerance development would predict a severe degree of withdrawal. Since this analysis was based on genetic correlations, these traits presumably share some functional, physiological basis regulated by one or several genes.

II.B.3 *Genetically selected lines.*

Inbred strains constitute a resource available to be applied to many questions. But because correlational analyses require the use of a minimum of eight or ten different strains, they are not always practical. If a particular response is to be studied extensively, a subject population can be created in which true genetic correlations are minimally confounded by chance associations of genes. The technique of bidirectional 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 segregating gene loci.

A genetic selection begins with an outbred population of animals, with demonstrated genetic variability in the trait of interest. Genetically heterogeneous strains of mice have been developed by intercrossing mice from eight inbred strains with widely divergent origin. These mice constitute a particularly good starting

population for a genetic selection because the genetic composition of the parent population can be examined in detail, and reconstitution of the parent population is possible. The first step in establishing selected lines is to test a base population for the trait of interest. Breeding pairs are then selected at random to form a selection control line. From the remaining group, individuals are chosen on the basis of test scores for maximum expression of the trait of interest, and 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 within the minimum line are selected to continue the minimum line.

The effect of selection may be symmetrical with respect to the minimum and maximum effect lines: the difference between the control line and each of the two selected lines may be equivalent. However, a number of factors can result in asymmetrical selection:

1) Different genes may be involved in maximal as compared to minimal expression of the trait, so that heritability, speed of response, and total response achieved are independent qualities of each of the two lines.

2) Outside factors may influence maximum and minimum expression of the traits differently. For instance, natural selection may oppose selection in one direction (if, say,high expression of the trait is associated with reduced fitness).

3) The method of measurement may be more sensitive to responses in one direction than to responses in the other. For instance, if there is a ceiling or floor effect, selection will be asymmetrical.

The purpose of the control line is twofold: First, practical considerations limit the number of breeding pairs used to maintain a selection line. The individuals chosen to begin a selection line can only contain a subset of the genetic information

available in the genetically heterogeneous base population. Therefore if one wishes to assess how selection pressure has altered the minimum and maximum lines relative to their original genetic composition, one ideally should have a control line that represents the subset of the original population 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 can be assessed, if desired, 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 constitutes a second control 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 a true genetic correlation should appear in both replicates. Similarly, inbreeding or random processes during selection may lead to chance fixation of some alleles irrelevant to the selected trait. This would result in the appearance of a trait in one replicate which appears to be a correlated response to selection, but it is unlikely that the same alleles would also be fixed by chance in the other replicate. Thus, a difference between both replicates of the maximum and minimum lines in a trait in addition to the selected trait is strong evidence that a genetic correlation between the traits exists (Deitrich and Spuhler, 1984; DeFries, 1981).

II.B.4. *Interpretations of statistical tests with replicated, selected lines.*

Experiments utilizing bidirectionally selected lines should ideally involve a comparison of both replicates on a measure or measures of interest, under

environmental identical conditions. Consider a selection which has achieved a robust response, so that both replicates of the selected lines differ extremely in the selected trait. These lines are then tested on some other measure thought to be related to the selected trait. Since the selected trait is maximally different, tests using these animals are very likely to detect differences in correlated responses to selection.

For interpretive purposes, the critical result is the overall comparison of the lines, as could be obtained from an analysis of variance (ANOVA), followed by post-hoc tests. Table 1 summarizes the potential outcomes of a 2-way ANOVA, and the degree to which each outcome supports the hypothesis of a genetic correlation between the selected trait and the dependent measure. A significant difference between the high and low lines is strong evidence that the correlated measure plays a role in EtOH withdrawal, while a lack of difference is evidence that the measure is not related to withdrawal. The results from the replicates could result in interactions that modify the interpretation. A non-selected trait which differs significantly in both lines and both replicates (i.e., a significant main effect of Line with no interaction of Replicate, or an Line by Replicate interaction which arises from a difference in degree of line difference between the two replicates, who nonetheless agree in direction and are both significant in post-hoc tests) can safely be called a correlated response to selection. This is strong evidence that the response is genetically correlated to the selected trait. An analysis of a non selected trait yielding differences between the lines in one replicate but not the other (significant main effect of Line, Line by Replicate interaction due to a significant post-hoc difference in one replicate only) provides moderate evidence of a genetic correlation between the traits. Given a true correlated response to selections, a lack of difference in one replicate could arise for several reasons. First, the trait may be weakly correlated: The likelihood of detecting a significant difference in a

TABLE 1

Interpretation of statistical tests using replicated, selected lines.

Support for a correlated response to selection.	Main effect of Line	Line x Replicate interaction	Results of Post-hoc tests
Strong	MAX>MIN	ns	na
	MAX>MIN	significant	MAX1 > MIN1 (large) MAX2 > MIN2 (small)
Moderate	MAX>MIN	significant	MAX1 > MIN1 MAX2 = MIN2
Weak	ns	significant	MAX1 > MIN1 MAX2 = MIN2
Absent	ns	ns	
Evidence against a correlation	ns or significant	significant	MAX1 > MIN1 MAX2 < MIN2

Interpretation of the results of an ANOVA comparing lines selected for minimal (MIN1 and MIN2) and maximal (MAX1 and MAX2) expression of a trait. The ANOVA and subsequent post-hoc comparisons test the difference between the lines and replicates on a measure hypothesized to correlate with the selected trait. ns = not statistically significant, na = not applicable. MAX> MIN, for example, indicates that the minimum and maximum expression lines differed significantly.

correlated trait increases with the absolute value of the true genetic correlation between the traits. Second, there could be only a small degree of genetic variability in the correlated trait, so that between-group differences are difficult to detect. Third, the degree of error of measurement could be related to the magnitude of genetic variability. Fourth, some other difference between the replicates could serve either to enhance the difference in one replicate, or to obscure the difference in the other. Fifth, stochastic processes could lead to a smaller mean difference in the non-significant replicate, despite a true genetic correlation. Nonetheless, the significant main effect is the best statistical indicator of the existence of a genetic correlation (see Table 1).

If the main effect of Line effect is not significant, but the Line by Replicate interaction is, due to a significant post-hoc effect in one replicate, a potential relationship between the correlated measure and the selected trait is still indicated, but the evidence is weaker. This also indicates a possible difference in the genetic determination of the selected trait between the two replicates. A lack of difference in either replicate (nonsignificant main effects and interactions) suggests strongly that the measure is not correlated with the selected response. Finally, a line by replicate interaction due to a difference in one direction in one replicate, and in the other direction in the other, constitutes the strongest evidence that this measure cannot be genetically correlated with the selected trait.

II.B.5. *Selection for EtOH withdrawal severity: The WSP and WSR mouse lines.*

The Withdrawal Seizure Prone (WSP) and Withdrawal Seizure Resistant (WSR) mouse lines were developed by artificial selection as a model of differing genetic sensitivity to the development of EtOH withdrawal. Beginning from independent groups of outbred mice from the HS/ibg population, two sets of WSP and WSR lines were selected on the basis of handling-induced convulsions (HIC) after withdrawal from chronic EtOH treatment. Non-selected control lines (WSC)

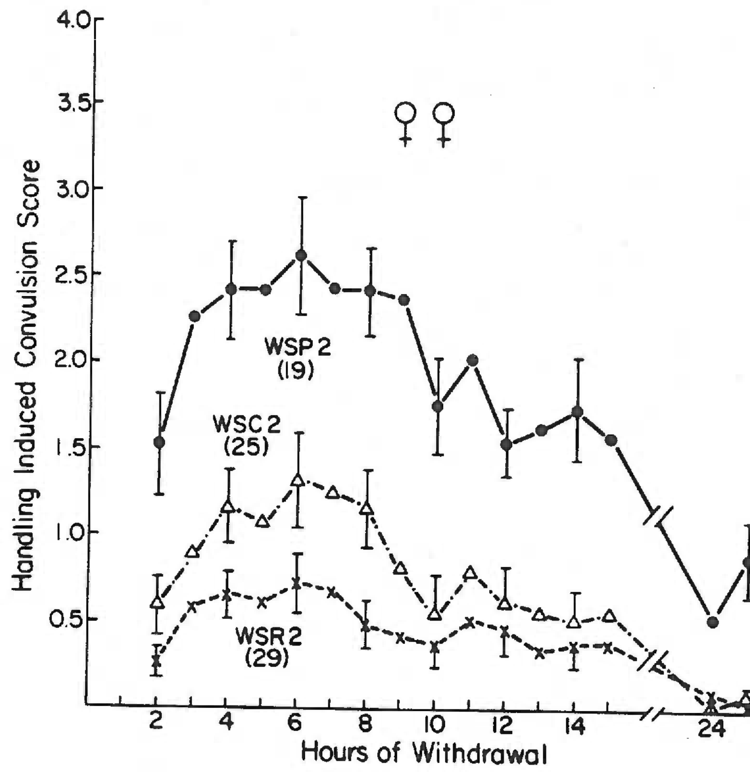
are also maintained for each replicate. Mice were made dependent by 72 hours of EtOH inhalation with daily injections of pyrazole, an alcohol dehydrogenase inhibitor. While there is no way to measure the development of dependence directly, the appearance of a withdrawal syndrome following cessation of chronic EtOH 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 EtOH.

Response to selection has been robust. 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). Figure 1 illustrates the typical pattern of waxing and waning HIC scores during 25 hours of withdrawal in female mice of the WSP, WSC and WSR lines (replicate 2). All mice were treated identically to induce dependence. Differences in the first replicate are of similar magnitude. A detailed discussion of HIC can be found in section III.B.7. This result confirms that there is a strong genetic influence on withdrawal severity as measured by HIC. These mice constitute an excellent population for studies of correlated responses to selection.

II.C. Genetic studies of EtOH dependence and withdrawal

The importance of genetic factors as a determinant of voluntary consumption of EtOH in mice (McClearn and Rodgers, 1959) and alcoholism (Kaij, 1960) was

FIGURE 1: Mean and SE HIC score for WSP, WSC and WSR mice following three days treatment in the same inhalation chamber with EtOH and pyrazole. Number of mice is indicated in parentheses. Female mice from Replicate 2 are pictured, but similar results were seen in Replicate 1 and using male mice. Mice were scored hourly for hours 2 through 15 post-withdrawal, and again at hours 24 and 25.



4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Handling Induced Convulsion Score
♀♀
WSP 2
(19)
WSC 2
(25)
WSR 2
(29)
2
4
6
8
10
12
14
24
Hours of Withdrawal

recognized almost 30 years ago, but active research into the nature of these genetic influences is relatively recent. Goldstein (1973b) selected mice for 3 generations and demonstrated a genetic influence on EtOH withdrawal susceptibility, using HIC as the measure of withdrawal. Subsequent studies with recombinant inbred and inbred mouse strains confirmed the importance of genetic differences (Goldstein and Kakihana, 1974; Grieve *et al.*, 1979; Crabbe *et al.*, 1983b, 1983c). Thus, lines selectively bred for the mild and severe EtOH withdrawal phenotype appeared to be a potentially useful tool in understanding EtOH withdrawal.

Belknap (1980) reviewed studies using genetic animal models and found substantial evidence for genetic determination of initial sensitivity and development of tolerance to EtOH, as well as susceptibility to EtOH withdrawal. Are the same genes responsible for all aspects of EtOH effects? If responses to EtOH are genetically correlated, it implies that EtOH has a single action that is a principal determinant of these different responses to the drug. Furthermore, it would be useful to know if a single gene is of primary importance in determining the action of EtOH. Using inbred mouse strains, we tested whether similar genes were responsible for the various effects of EtOH (Crabbe, 1983; Crabbe *et al.*, 1982, 1983c). Fifteen to 20 inbred strains were tested on measures of EtOH sensitivity, tolerance development, and withdrawal severity. The different mouse strains showed marked differences in response to each measure, confirming a high degree of genetic determination of responses to EtOH. EtOH withdrawal severity, as indexed by HIC, was negatively correlated with development of tolerance to the hypothermic effect of EtOH, and possibly to initial hypothermic sensitivity, but with no other tested variables. Thus, severity of withdrawal appeared to be modulated by genes that are different from those modulating many other effects of EtOH.

Among the inbred strains, C57BL/6 and related lines, and DBA/2 and related lines, were notably discrepant in their response to EtOH. These strains

tended to respond to EtOH in opposite ways. For instance, C57 mice showed mild withdrawal severity, drank EtOH willingly, and had a large initial hypothermic response to EtOH, while DBA mice showed severe withdrawal severity, did not voluntarily consume EtOH, and had a smaller initial hypothermic response to EtOH. Studies with recombinant inbred strains derived from crosses of C57BL/6 and DBA/2 mice confirmed heterogeneity in the genetic determination of responsiveness to EtOH. Each recombinant inbred strain did not consistently resemble one parent or the other, but could be DBA-like in one response, C57-like in another, and intermediate in yet another (Crabbe *et al.*, 1983b). This study also identified the influence of a potential major gene in determining EtOH withdrawal severity, since all but 1 of the 17 recombinant inbred strains tested closely resembled one or the other of the two parent strains.

Lines of mice have also been genetically selected for differences in initial sensitivity to EtOH. The LS (Long sleep) and SS (Short sleep) mice differ markedly in the length of time they lose the righting reflex following a single i.p. administration of EtOH. These lines were made dependent by three days exposure to EtOH vapor with pyrazole treatment, and then withdrawn (Goldstein and Kakihana, 1975). LS mice showed milder withdrawal than SS mice, suggesting a possible negative genetic correlation between loss of righting reflex sensitivity and EtOH withdrawal severity. This is in contrast to the results with inbred strains (Crabbe *et al.*, 1983c), where no correlation was indicated. Furthermore, the WSP and WSR mice do not differ in sensitivity to EtOH-induced loss of righting reflex (Crabbe and Kosobud, 1986). There are at least two possible explanations for this discrepancy. First, it may be that the correlation seen in the SS and LS lines is an accidental result of inbreeding: replicate LS and SS lines are not available to test this hypothesis. Second, many of the genes which are responsible for the difference between LS and SS mice might also affect EtOH withdrawal severity, but the

reverse might not be true: there may be many other genes which influence withdrawal, but have no effect on loss of righting reflex.

Two independent selection experiments have developed replicated lines of mice selected for differing severity of EtOH withdrawal. The WSR/WSP selection has been described. The Severe EtOH withdrawal (SEW) and Mild EtOH withdrawal (MEW) lines are made dependent by nine days of exposure to an EtOH adulterated liquid diet as their only food source, and are selected using a multivariate index of withdrawal severity. Hutchins *et al.* (1981) assessed multiple behavioral and physiological measures as indexes of withdrawal. On the basis of a principal component analysis, seven measures were selected (McClearn *et al.*, 1982). These measures included three measures of activity, two indices of seizure severity (HIC, and spontaneous seizures), body temperature, and EtOH consumption.

In the first 5 generations, SEW and MEW lines showed approximately 1.5-fold divergence in one replicate, but little divergence in the other. (Allen *et al.*, 1983). After 10 generations, divergence was present in both lines, although it remained small (Wilson *et al.*, 1984). The lines did not differ in loss of righting reflex or hypothermic response following an acute dose of EtOH. MEW mice appeared to metabolize EtOH slightly more slowly than SEW, but whether this affects the blood ethanol concentration the animals experience during the nine days of EtOH treatment is not known. One rather surprising finding is that consumption of the EtOH-containing liquid diet has increased dramatically in both replicates and all lines - MEW, SEW and the non-selected Control line. Apparently, selection is strongly favoring mice who can consume large amounts of the EtOH diet (Wilson *et al.*, 1984). SEW and MEW lines have been tested for sensitivity to withdrawal following treatment with phenobarbital, and were found not to differ (Cole-Harding *et al.*, 1986), although a difference could appear as selection progresses.

One potential flaw in the development of this model is that the development of the withdrawal severity index relied on phenotypic correlations from studies using outbred mice. Therefore, the correlations observed represented gene, environment, and gene x environment effects. This is particularly problematic because there is evidence that at least one measure in this index, EtOH consumption, may have opposing genetic and environmental correlation with other measures. Clearly, there is a positive environmental correlation between amount of EtOH consumed and severity of withdrawal. Experiments with the WSP and WSR mice suggest that there is a weak negative genetic correlation between voluntary EtOH consumption and withdrawal (Kosobud *et al.*, 1988). Thus, these lines are potentially being selected in the wrong direction on one trait of the index. Recently, studies by the group developing the SEW and MEW lines have reported a genetic analysis of the selection index confirming a negative genetic correlation between voluntary EtOH consumption and EtOH withdrawal (Corley and Allen, 1988). They report, in addition, that EtOH consumption has been eliminated from the selection index and that response to selection does appear to be increasing.

Similar to SEW and MEW lines, WSP and WSR mice do not differ in sensitivity to loss of righting reflex or hypothermic response following an acute dose of EtOH. With repeated EtOH administrations, both lines develop CNS tolerance to the loss of righting reflex and hypothermic effects of EtOH, but do not differ in degree of tolerance development (Crabbe and Kosobud, 1986). Thus, the correlation observed in the inbred strains between hypothermic tolerance and EtOH withdrawal has not been supported.

WSP and WSR mice have been tested for development of dependence on diazepam (Belknap *et al.*, in press), nitrous oxide (Belknap *et al.*, 1987), and phenobarbital (Belknap *et al.*, 1988). WSP mice showed more severe withdrawal following treatment with all three of these drugs. This suggests that dependence is

induced by similar mechanisms for all of these drugs. Phenobarbital, EtOH and nitrous oxide are thought to produce their anesthetic and sedative actions by similar mechanisms. One hypothesis explaining the similar action of these drugs was that they all act at the cell membrane of neurons, disordering them and thereby depressing their activity. It could be that chronic treatment with these drugs results in an increased membrane order to compensate for these fluidizing effects. When the drug is removed, a transient hyper-rigid state occurs in the membrane, and withdrawal excitability occurs. However, WSP and WSR mice did not differ in sensitivity to the acute membrane disordering properties of EtOH (Perlman and Goldstein, 1984), consistent with their lack of difference in acute sensitivity to EtOH. They also did not differ in baseline membrane order (Harris *et al.*, 1984). However, they also did not differ in degree of membrane disordering during chronic EtOH intoxication (Harris *et al.*, 1984). While it still may be worth seeing if membrane fluidity differs in these mice at time of peak withdrawal signs, or in a specific microenvironment of synaptosomal membranes, it currently does not appear that the membrane fluidizing effect is an important determinant of EtOH withdrawal, or of cross-dependence between EtOH and other drugs.

It is also possible that the difference between WSP and WSR mice reflects a general difference in sensitivity to convulsions. This could mean that the model is one of general sensitivity to any convulsant treatment, rather than specific to EtOH withdrawal hyperexcitability. One the other hand, EtOH itself may act very generally, so that a general difference is the appropriate response to selection. For instance, it has been proposed that glial cells regulate CNS excitability by maintaining appropriate potassium levels in the CNS (Grisar, 1986). Chronic EtOH treatment may disrupt this function, and withdrawal hyperexcitability could result from this aberration, which could simultaneously affect multiple areas in the CNS.

If the genetic alteration in WSP and WSR mice involves global changes of this sort (WSR mice could be much better at activating glia to maintain normal excitability levels in the face of challenges), then any convulsant treatment might affect WSP and WSR differently. This could be true even though to initiate convulsions, these drugs do not act by the same mechanisms, and even though the WSP and WSR mice do not differ in their sensitivity to initiation of convulsions by these drugs. Further, the difference could lie in propagation of the seizure once initiated. For instance, both the substantia nigra and the reticular formation have been implicated as important structures for the translation of a localized seizure to a generalized seizure.

McSwigan *et al.* (1984) studied sensitivity to convulsant treatments in WSP and WSR mice from the 8th to 10th selected generation. They determined ED_{50}s for maximal seizures elicited by electroconvulsive shock, bicuculline, flurothyl, strychnine, pentylenetetrazol, and picrotoxin. Naive WSP and WSR mice did not differ in sensitivity to these convulsant treatments. However, studies with later selected generations suggested that WSP mice were more sensitive to flurothyl and 4-aminopyridine than WSR mice, when latencies to convulse were measured (Crabbe and Feller, unpublished observations). This suggested that a method which assessed minimal seizure thresholds might be more sensitive to differences between the WSP and WSR mice.

II.D. Experimental studies of seizures and convulsions.

II.D.1. *General aspects.*

The categorization of different seizure disorders in humans is immensely complex, owing to the multiple etiologies and modes of expression of these disorders. For the purposes of this discussion, I will summarize some relevant information. A more thorough discussion is available in a number of recent reviews (Delgado-Escueta *et al.* 1986; Luders *et al.*, 1984; Seyfreid, 1982; Snead, 1983).

Jackson (1863, as reported in Delgado-Escueta *et al.*, 1986) described epilepsy as a disorder characterized by recurrent convulsive or non-convulsive seizures. He defined a seizure as excessive and disorderly activity in the central nervous system, usually the brain but in some cases limited to spinal cord. The term convulsion usually refers to the motor component of a seizure, but is also used in describing sustained paroxysmal, involuntary contractions of the skeletal musculature for which a central nervous system correlate has not been determined. Isolated seizures can occur in a normal individual for a variety of reasons, including infection, trauma, hypoglycemia, hyponatremia, renal or hepatic failure, or drug withdrawal. Recurrent seizures can be categorized according to etiology into three groups, those with a genetic origin, those with an environmental origin (symptomatic or secondary), and those of unknown or idiopathic origin. A number of genetic defects have seizures as part of the phenotype: a recent review listed 141 such genetic disorders (Anderson *et al.*, 1986). The known etiologies for secondary seizures include tumors, trauma, stroke and infection. It appears that seizures can be viewed as a potential outcome of any insult to the central nervous system.

The form seizures can take is also very heterogeneous. Seizures can be classified as generalized or focal. Generalized seizures are those which develop throughout the central nervous system more or less simultaneously, while focal or partial seizures are initiated in and sometimes limited to a particular central nervous system site. This classification represents the endpoints of a spectrum rather than a dichotomy: many apparently generalized seizures may be focal seizures which propagate extremely rapidly.

Jackson (1863, as reported in Delgado-Escueta, 1986) formulated what have remained the critical questions for understanding seizures. The first question is anatomic: Where is the defect? The second is physiological: What is the defect? The third is pathological: How does it arise? The third question is outside the

scope of this discussion, but the first two deserve attention. Three conditions are thought to be the major factors determining the presence of seizures (Benardo and Pedley, 1984). First, there must be a field of cells capable of firing in synchrony, although not normally doing so. Such fields are present in limbic areas, thalamus, and some areas of neocortex. Second, there must be a disruption in the balance of inhibitory and excitatory mechanisms in the brain such that excitatory forces predominate. Third, a focus or trigger is needed. A focus is an area of brain which because of a lesion or other pathology generates strong impulses capable of initiating synchronous activity. A trigger could be an external stimulus strong enough to act as a focus at some level within the brain.

II.D.2. *Studies with genetic models.*

The genetic influence on sensitivity to caffeine-induced seizures has been studied in a series of papers. In the first study (Seale *et al.*, 1984), seven inbred mouse strains were tested for susceptibility to a number of behavioral effects of caffeine. They found considerable genetic variation in all responses to caffeine, including spontaneous seizures and swim-stress-induced seizures. Sensitivity to the two types of seizure appeared to be similar within a strain. The least sensitive strain was the SWR strain, while the most sensitive strain was the CBA strain. Using these two divergent strains, subsequent studies explored the genetic structure of this sensitivity, and its relationship to sensitivity to 4 other convulsant drugs (picrotoxin, strychnine, Ro 5-4864, and DMCM). Sensitivity to caffeine/stress-induced seizures appeared to be determined by a single gene with 2 alleles, with susceptibility to seizures dominant to resistance to seizures (Seale *et al.*, 1985). SWR and CBA mice were equally sensitive to (nonstress-induced) seizures induced by picrotoxin, strychnine, and Ro 5-4864, but CBA mice were more sensitive than SWR mice to seizures induced by DMCM (Seale *et al.*, 1987a). Therefore, these investigators speculated that caffeine and DMCM might produce seizures by similar mechanisms.

However, when these strains were interbred in a Mendelian cross, in which F1 and F2 offspring are generated along with backcrosses to the parent strains, sensitivity to the two drugs did not cosegregate (Seale *et al.*, 1987b), indicating that the same gene or genes are not responsible for susceptibility to seizures produced by caffeine and DMCM.

In a series of studies by other investigators, designed to explore the mechanisms underlying genetic variation in seizure susceptibility, 3 inbred mouse strains were tested for sensitivity to convulsant drugs. In the first experiment, C57BL/6, C3H/2 and DBA/2 inbred mice, and outbred HS mice, were tested for sensitivity to i.p. administered flurothyl or 3-mercaptopropionic acid (Marley *et al.*, 1987). 3-mercaptopropionic acid is a potent inhibitor of glutamic acid decarboxylase, the synthetic enzyme for GABA formation, and causes a decrease in the amount of GABA released into the synaptic cleft (Loscher, 1979). The mechanism of action of flurothyl is not known. Latency to flurothyl-induced seizures is reduced by the GABA antagonist bicuculline (Greer and Alpern, 1977), but the mechanism of this interaction is not known. The four strains differed significantly in their sensitivity to seizures induced by these two drugs, confirming genetic heterogeneity in strain responsiveness to convulsants. Furthermore, rank order of strain sensitivity (from least to most) was different for each drug, (3-mercaptopropionic acid: C57BL<HS<DBA<C3H; flurothyl: C57BL<HS<C3H<DBA). This suggested that different genetic mechanisms controlled the response to each drug. GABA receptor binding and affinity were assessed in several brain regions of C57BL and DBA mice. These measures were found not to differ, suggesting that these parameters are not important determinants of seizure susceptibility in either strain of mouse.

Marley and Wehner (1987) then examined the genetic relationship between sensitivity to 3-mercaptopropionic acid-induced seizures and brain flunitrazepam

binding in four inbred mouse strains (C57BL, DBA, C3H and BALB) and two selected lines (LS and SS). Flunitrazepam binds to the benzodiazepine site of the GABA complex. A strong genetic correlation was found between latency to clonic or tonic seizure, and enhancement of 3H-flunitrazepam binding by GABA, suggesting that resistance to seizures is related to GABA-enhancement of benzodiazepine binding.

In a third study, C3H, DBA and C57BL mice were tested for sensitivity to bicuculline-induced seizures *in vivo*, and bicuculline-induced epileptiform activity was recorded in the CA1 pyramidal cell layer of hippocampal slices (Freund *et al.*, 1986). For latency to clonus, rank order of strains was (from least to most susceptible) C57BL<DBA=CBA. For latency to tonus and development of epileptiform activity, rank order of strains was C57BL=DBA<C3H. Thus, different convulsion phenotypes initiated by the same drug appeared to be under separate genetic control. Bicuculline-induced tonus may be strongly associated with the ability of bicuculline to activate the hippocampus, while clonus is associated with other mechanisms.

In summary, there appears to be considerable genetic diversity not only in responses to different convulsants, but also in different seizure signs caused by a single drug.

III. EXPERIMENTS

III.A. Rationale.

The purpose of these experiments was to test two hypotheses concerned with genetic factors. The first hypothesis was that there was a positive genetic correlation between sensitivity to convulsant drugs and ethanol withdrawal severity. In other words, the hyperexcitability seen during EtOH withdrawal may be due to similar mechanisms as the hyperexcitability caused directly by administration of convulsant drugs. This hypothesis was tested in two ways. First, a battery of 10 inbred mouse

strains was tested for sensitivity to nine convulsant drugs. Genetic variation in sensitivity to some of these convulsant treatments has been demonstrated in studies using 2 or 3 inbred strains, but systematic study of multiple inbred strains and multiple drugs has not been done. If the order of strain sensitivity remained the same regardless of drug tested, pharmacokinetic factors, or general CNS mechanisms affecting excitability might be implicated. Changes in the order of strain sensitivity to different drugs could be attributed to drug specific factors, which also could be pharmacokinetic, or could relate to the mechanism by which the drug produces convulsions.

As an additional test of the first hypothesis, the genetically selected WSP and WSR mouse lines were also tested for sensitivity to these convulsant drugs. WSP mice, genetically susceptible to EtOH withdrawal, were expected to be more sensitive to at least some convulsant treatments than WSR mice, genetically resistant to EtOH withdrawal. Differences between WSP and WSR mice in sensitivity to one or several of these drugs could reflect changes at the specific sites of action of these drugs, arising as correlated responses due to the changes in gene frequency imposed on these mice in the course of artificial selection. Thus, these sites would be implicated as having a role to play in ethanol withdrawal. Alternatively, a general difference to all drugs, consistent in the lines, could be predicted. For instance, WSP mice may be more sensitive to all convulsant treatments. This could reflect a difference between these mice that lies not at the site of initiation of seizures, but rather in some aspect of propagation or expression of seizures. Further, this would suggest that the genetic selection has resulted in these changes, and that they are responsible for the difference seen during withdrawal. A third possibility was that WSP and WSR mice would not differ in sensitivity to convulsant drugs, suggesting that 1) the site of the difference between these mice is not one where any of these drugs act, or 2) that differences between

WSP and WSR mice at these sites arise in the course of chronic EtOH treatment, and are not easily detectable, or present, prior to ethanol exposure.

The second hypothesis was that sensitivity to some convulsant drugs would be associated with that to other drugs, due to a similarity in their mechanisms of action. Three of the drugs tested (pentylenetetrazol, picrotoxin and TBPS) are thought to act, at least in part, at the same receptor, the picrotoxin receptor of the GABA complex. Bicuculline and DMCM cause convulsions through their actions at the GABA and benzodiazepine receptor, respectively. Thus, all five of these drugs operate through the same receptor complex, and influence a chloride ionophore (See Section III.B.3 and Figure 2). Strychnine also appears to work through a chloride channel, but at anatomically distinct sites. Thus, a strong genetic correlation among sensitivities to pentylenetetrazol, picrotoxin, and TBPS, and a weaker correlation between sensitivities to these three and bicuculline or DMCM was predicted. If a structural difference in the chloride channel were responsible for differences in sensitivity, there might also be a genetic correlation between sensitivities to these drugs and strychnine. The proposed mechanisms by which CHEB, 4-aminopyridine and kainic acid operate are quite different: the prediction was that sensitivity to these drugs would not be genetically correlated with that to the other drugs.

Results of tests using WSP and WSR mice were not formally applied to testing the second hypothesis, because detecting a pattern of genetic correlation using only 4 selected lines in this way would be nearly impossible. However, consistency of the WSP and WSR results with the results seen in the inbred strains was assessed.

Genetic differences in response to these drugs could be explained in a number of ways: 1) Genetic differences could be due to changes at the site of action of the drug. For instance, there may be differences in receptor number or

affinity, neurotransmitter release or activity of a particular ion pump; 2) The difference could arise because of factors other than the direct convulsant property of the drug. For instance there may be genetic differences in access of drug to brain or other pharmacokinetic properties; 3) There could be genetic differences in secondary responses of the central nervous system to excitation. For instance, there may be genetic variation in endogenous anticonvulsant mechanisms that may be activated generally by any increase in excitation.

III.B. Methods.

III.B.1. *Subjects.*

Inbred mouse strains were obtained from Jackson Laboratories (Bar Harbor, Maine). The following eight strains were included in all tests: A/J, AKR/J, BALB/cJ, C3H/HeJ, CBA/J, C57BL/6J, DBA/2J, SWR/J. In addition, RIIIS/J and C58/J were used as available. Withdrawal Seizure Prone (WSP) and Withdrawal Seizure Resistant (WSR) mice used in these experiments were bred at the Veterans Administration Medical Center or the Oregon Health Sciences University (both in Portland, Oregon). The genetic selection procedure used in developing these mice has been described in detail (Crabbe *et al.*, 1985). Two sets of independently selected, reproductively isolated lines exist. Lines in the first replicate are designated WSP1 and WSR1; lines in the second replicate are designated WSP2 and WSR2. WSP and WSR mice used in this study were from extra litters from the 26th and 27th selected generation. Withdrawal Seizure Control (WSC) mice were not tested. Naive male mice of similar ages (within 14 days) were used in all cases. The age at which the mice were tested was between 50-100 days old. Prior to the experiments, animals were housed 2-5 in polycarbonate boxes (28 x 17 x 11.5 cm) containing wood chips as bedding. Food (Rodent lab chow #5001, Ralston Purina Co.) and water were available ad libitum.

III.B.2. *Experimental induction of convulsions.*

A variety of models are available for studying sensitivity to seizures in animals. The possible preparations range from single cells to brain slice to whole animals. Chemical, electrical and sensory stimuli can be used to induce convulsions, and measurement can be made of EEG, evoked potentials, or convulsions. These experiments measured sensitivity to chemical induction of seizures in unanesthetized, unrestrained animals, assessed by convulsive behavior. Even within these limits, a number of techniques were possible. Drugs could be given directly to the brain or a brain area, or administered peripherally. Peripheral administration could be in a single bolus, spaced intervals, or in a timed infusion. The choice of a method of peripheral administration depends on how seizures are to be assessed. Seizures can be assessed by latencies between administration of drug and appearance of convulsion, or by the dose which produces a certain effect in some percentage of the animals. The timed-infusion procedure is a strategy which offers a number of advantages; a rapid response can be obtained, and a sensitivity measure can be obtained for each animal. Also, many drugs produce a proconvulsant state in the animal, but may take a long time (30 minutes to several hours) to produce a convulsion. When and whether the animals have convulsions may depend on the occurrence of environmental stimuli which trigger the seizure.

In theory, during a timed intravenous infusion procedure, drug accumulates in the brain until it reaches a critical level, at which time a convulsion occurs. The latency to appearance of a convulsant sign can be used as a measure of sensitivity to the drug. Differences in latency between two subject groups presumably reflect differences in neural sensitivity to seizure induction. However, accumulation of drug may occur more slowly in bigger mice. Thus, calculating the effective dose (ED) in terms of mg drug delivered/kg bodyweight might be a better estimate of sensitivity. Calculation of ED is analogous to the use of mg/kg doses in i.p.

injections. In both instances, the assumption is that bodyweight is an appropriate estimate of the size of the body compartment to which the drug is distributed. An obvious example of the potential error in this strategy is the case of a drug which distributes primarily to water, given to animals which differ in body fat content. Fatter animals will be given relatively larger doses of drug (in the case of i.p. injection), or will have lower EDs calculated, and be judged more sensitive to drugs given by timed i.v. infusion.

In addition to body compartment size, a number of other factors could affect the amount of drug reaching target. These include binding of the drug to plasma protein, elimination and metabolism, crossing the blood brain barrier (affected by lipid solubility, active transport mechanisms), and percentage of cardiac output which is distributed to brain. If there is systematic variation between WSP and WSR mice, or among the inbred strains, in the above variables, it could influence the results. Such differences can be controlled on a variety of levels. One could measure brain concentration of a highly lipid-soluble drug infused by tail vein; this would account for factors such as cardiac output, blood volume, and speed of diffusion across the blood brain barrier (which does not exclude lipid soluble drugs). However, this method would fail to account for drug-specific differences, such as plasma protein binding, and ability of less lipid-soluble drugs to cross the blood-brain barrier. In these studies, it was not possible to measure brain drug levels. To the extent that strain sensitivity order is drug specific, multiple factors are indicated, some of which may be distributional, and some of which may relate to CNS sensitivity. However, if the order of strain sensitivity were found to be similar for all drugs, it would suggest that some very general property, such as percent of cardiac output which is shunted to brain, was the major determinant of latency to convulsion.

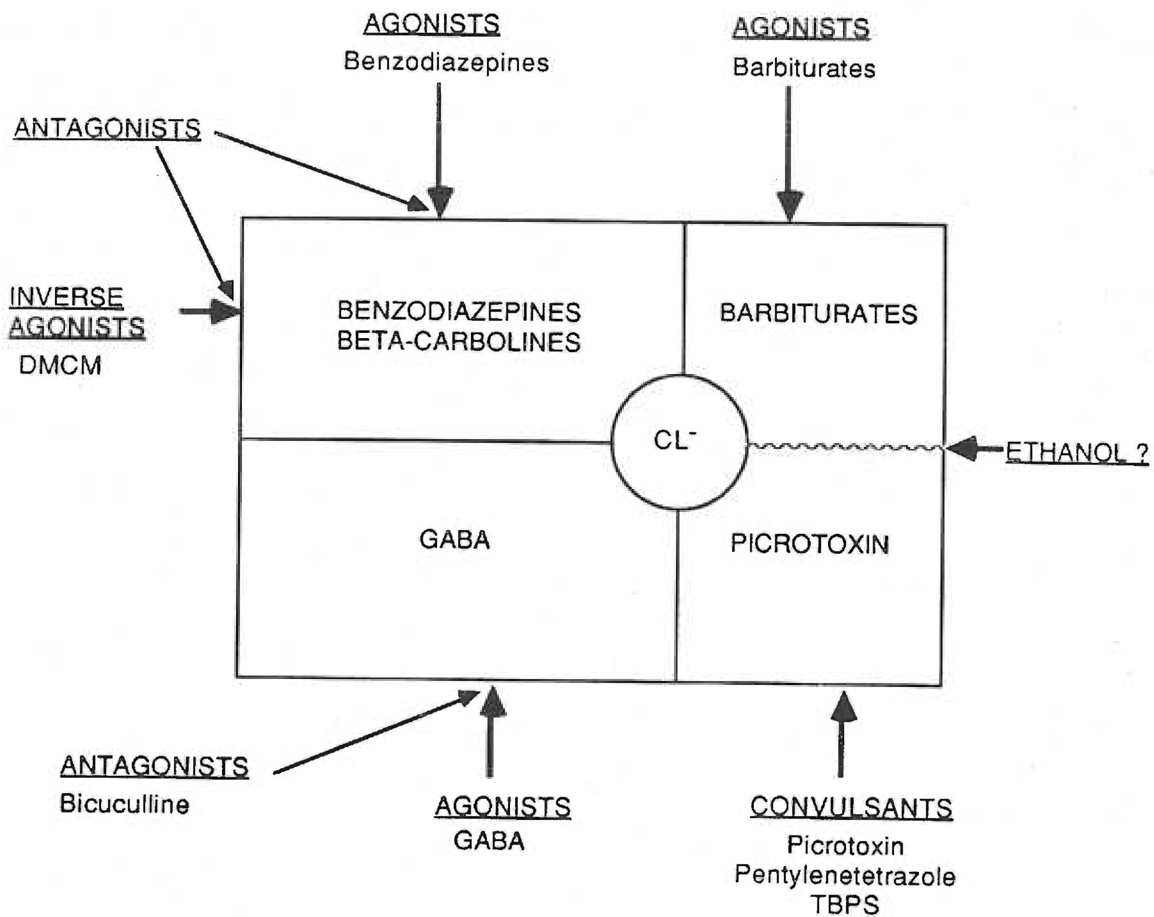
III.B.3. *Convulsant drugs: Mechanisms of Action.*

III.B.3.a. *Drugs acting at the GABA complex.*

The amino acid gamma-amino butyric acid (GABA) is a normal constituent of the mammalian CNS, but occurs in only trace amounts elsewhere (Cooper *et al.*, 1986). It is one of the most abundant neurotransmitters, present at approximately 30% of CNS synapses (Ticku and Maksay, 1983). In rhesus monkeys, GABA is distributed throughout brain, with highest concentrations in substantia nigra, globus pallidus, hypothalamus, superior and inferior colliculi, dentate nucleus and periaqueductal gray, while the lowest concentrations are found in lateral and anterior thalamus, and cortex (especially white matter) (Fahn and Cote, 1968). The distribution of GABA in humans shows a similar pattern (Perry *et al.*, 1971). In rats, GABA binding in synaptic membranes was greatest in cerebellum, intermediate in thalamus, hippocampus, hypothalamus, cerebral cortex and midbrain, less in corpus striatum and least in medulla oblongata-pons (Zukin *et al.*, 1974). It is found almost exclusively in interneurons rather than large tracts, consistent with a modulatory rather than an information-carrying role.

Two types of GABA receptor have been identified in the CNS, designated $GABA_A$ and $GABA_B$. The $GABA_B$ site is currently not well characterized and will not be discussed here. The $GABA_A$ site is associated with three or four distinct binding sites and a chloride ionophore, referred to collectively as the GABA complex. Currently, the GABA complex is thought to consist of three or four distinct binding sites, and a chloride channel (Figure 2, after Lister and Nutt, 1987, and Olsen, 1981). The GABA complex is a site of particular interest for both alcohol research and seizure research. GABA is the principal inhibitory neurotransmitter system in the brain, and is considered to play a role in EtOH withdrawal (Hunt, 1983; Allan and Harris, 1987). It is also implicated in some types of epilepsy (Wood *et al.*, 1979, 1980), although this remains controversial (Kresch *et*

FIGURE 2: A schematic diagram of the GABA complex, showing the chloride ionophore and four receptor sites. The site of action of relevant drugs and neurotransmitters is indicated. (after Lister and Nutt, 1987, and Olsen, 1981)


AGONISTS
Benzodiazepines
AGONISTS
Barbiturates
ANTAGONISTS
INVERSE AGONISTS
DMCM
BENZODIAZEPINES
BETA-CARBOLINES
BARBITURATES
CL$^-$
ETHANOL ?
GABA
PICROTOXIN
ANTAGONISTS
Bicuculline
AGONISTS
GABA
CONVULSANTS
Picrotoxin
Pentylenetetrazole
TBPS

al., 1987). Many anticonvulsant drugs currently in use are thought to work at least in part through the GABA complex (McDonald and McClean, 1986). The basis for the inhibitory effect of GABA is its action on neuronal membrane chloride (Cl^-) channels. Increasing Cl^- flux opposes depolarization and accelerates the rate of return to the resting potential of the membrane (Roberts, 1986). The GABA site directly influences Cl^- flux, by increasing both the frequency of channels opening, and the duration of opening. It is worth noting that the overall effect of GABA depends on the circuitry of the neuronal system in question. If the GABAergic neurons are inhibiting inhibitory systems, an increase in GABAergic activity could result in excitation.

The GABA complex also includes a benzodiazepine receptor. Three classes of compounds that act at this receptor have been identified. Agonists such as diazepam act directly on the Cl^- channel to increase frequency (but not duration) of opening (Study and Barker, 1981) but do not appear to affect GABA binding, although this point is controversial (Olsen *et al.*, 1986). However, GABA agonists increase benzodiazepine agonist binding (Chweh *et al.*, 1985). Inverse agonists inhibit GABA stimulated Cl^- influx (Obata and Yamamura, 1986) and decrease GABA binding (Braestrup *et al.*, 1982). They have been shown to be anxiogenic (Prado de Carvalho *et al.*, 1983; Dorow *et al.*, 1983) and proconvulsant to frankly convulsant (Tenen and Hirsch, 1980; Braestrup *et al.*, 1982). Benzodiazepine antagonists presumably have no effect on GABA binding or on the Cl^- channel; they bind to the receptor and prevent the action of either agonists or inverse agonists (Nutt *et al.*, 1982). It has been suggested that these drugs are actually weak inverse agonists or agonists (Jensen *et al.*, 1983).

A third site of the GABA complex is the picrotoxin site, which appears to be closely associated with the Cl^- channel. Picrotoxin, a potent convulsant, blocks Cl^- flux in a noncompetitive manner, possibly by direct blockade of the Cl^- ionophore.

A barbiturate binding site is also present near and possibly overlapping the picrotoxin site (Ticku and Olsen, 1978). Low concentrations of barbiturates increase the lifetime of activated Cl^- channels, and high concentrations produce a direct increase in conductance similar to that produced by GABA itself. The latter effect is blocked by GABA antagonists (Ticku and Maksay, 1983).

Convulsant drugs acting at each of these sites were included. Bicuculline competitively blocks GABA binding (Curtis *et al.*, 1970). DMCM is an inverse agonist at the benzodiazepine site (Braestrup *et al.*, 1982; Petersen, 1983). Picrotoxin , TBPS and pentylenetetrazol bind to the picrotoxin site (Ramanjaneyulu and Ticku, 1984), and presumably produce their convulsant action through that site (Squires *et al.*, 1984; Galindo, 1969). While pentylenetetrazol has been shown to block Cl^- conductance (Pellmar and Wilson, 1977), it also has a number of other actions which may contribute to its convulsant action. For instance, pentylenetetrazol has direct effects on membrane properties resulting in increases in excitability of isolated neurons (for review see Woodbury, 1980).

III.B.3.b. *Strychnine.*

Strychnine interacts with glycine receptors (Young and Snyder, 1973). The amino acid glycine is found throughout the CNS, but appears to act as an inhibitory neurotransmitter principally in brainstem and spinal cord (Werman *et al.*, 1968). Like GABA, the glycine receptor is associated with a Cl^- channel and is found principally in interneurons. The strychnine binding site appears to be closely associated with the Cl^- ionophore (Young and Snyder, 1974a). The binding sites for glycine and strychnine are distinct, but interact in a cooperative manner; binding of one compound inhibits the other noncompetitively (Young and Snyder, 1974b). Thus, strychnine appears to initiate convulsions by decreasing inhibition in a manner analogous to drugs acting at the picrotoxin site of the GABA complex, although acting at anatomically distinct levels of the CNS.

III.B.3.c. *Kainic acid.*

Kainic acid is an agonist at excitatory amino acid receptors (Olney *et al.*,, 1974). Systemic administration in rats induces a limbic seizure which has been proposed as a model for human temporal lobe epilepsy (Nadler, 1981, Ben-Ari, 1985). In rats, kainic acid preferentially affects limbic structures including the hippocampal formation, the septum, the amygdaloid complex, the cingulate cortex, the claustrum and related thalamic structures (Ben-Ari *et al.*, 1981; Lothman and Collins, 1981). The CA_3 region of the hippocampus appears to be particularly sensitive to kainic acid (Lothman and Collins, 1981). Kainic acid crosses the blood-brain barrier slowly, and doses necessary to produce convulsions when the drug is administered peripherally are far in excess of those necessary when it is administered centrally (Berger *et al.*, 1986).

III.B.3.d. *4-aminopyridine.*

4-aminopyridine and related aminopyridines facilitate synaptic transmission in a non-transmitter-specific manner (Thesleff, 1980). Peripherally, these compounds are able to reverse neuromuscular blockade produced by a variety of mechanisms including curare (Lundh, 1978), magnesium ions (Molgo *et al.*, 1980), aminoglycoside antibiotics (Molgo *et al.*, 1979) and by botulinum toxin (Lundh *et al.*, 1977). In the central nervous system, they have been shown to stimulate monosynaptic and polysynaptic reflexes in cats (Leimeignan, 1972, 1973) and frogs (Galindo and Rudomin, 1978), facilitating both excitatory and inhibitory pathways.

At the vertebrate skeletal muscle endplate, 4-aminopyridine appeared to act through a presynaptic mechanism that caused a massive increase in the number of transmitter quanta released (Molgo *et al.*, 1979; Lundh, 1978, 1979). 4-aminopyridine appears to mediate this transmitter release through an increase in Ca^{++} entry into the presynaptic terminal (Lundh, 1978). Katz and Miledi (1969) suggested that the aminopyridines block K^+ channels, prolonging the action

potential and thereby holding voltage sensitive Ca^{++} channels open longer, resulting in increased Ca^{++} influx. The ability of 4-aminopyridine to block K^+ channels was later confirmed (Meves and Pichon, 1977). It is also possible that the aminopyridines have a direct effect on the Ca^{++} channel (Lundh and Thesleff, 1977).

Given the apparent non-specific effect of 4-aminopyridine on all synapses, it is interesting that the result is excitatory. Roberts (1986) has proposed that the central nervous system is tonically active; controlled and regulated by inhibitory influences. This is consistent with the general tendency of central nervous system pathology to lead to disorders of excess.

The anatomical specificity of 4-aminopyridine or other aminopyridine seizures has not, to my knowledge, been investigated.

III.B.3.e. *CHEB.*

5-(2-cyclohexylidene-ethyl)-5-ethyl barbituric acid (CHEB) is a convulsant derivative of barbituric acid first synthesized and studied by Velluz *et al.* (1951). Although the barbiturates are generally noted for their depressant properties, many also have excitatory effects. These frequently precede, or are seen at lower doses, than the depressant actions (Downes and Williams, 1969; Downes *et al.*, 1970). The depressant property of barbiturates is a robust characteristic, changing only slightly as a function of molecular structure. The convulsant property, however, appears and disappears with the slightest variation of substituents (Velluz *et al.*, 1951, as reported in Downes *et al.*, 1970). A series of closely related barbiturates may contain one which has striking convulsant or excitatory properties, but the particular structural change necessary to reveal these properties varies greatly among convulsant barbiturates (Downes *et al.*, 1970).

The effects of CHEB have been studied in a variety of systems. CHEB caused contractions in vascular smooth muscle (Hupka *et al.*, 1969). The basis for the excitatory action of CHEB may be in its action on cation conductance,

particularly Ca^{++}. Skerritt and McDonald (1984) reported that CHEB potently activated a Ca^{++}-dependent cation conductance, which both produced the membrane depolarization (postsynaptic) and caused increased release of neurotransmitter (presynaptic). In brain synaptosomes, CHEB, but not the sedative barbiturate phenobarbital, reduced Ca^{++} uptake at the synaptosomal membrane, but both CHEB and phenobarbital reduced Ca^{++} in microsomes (Pincus and Hsiao, 1981). CHEB was more effective than PHB at decreasing uptake in microsomes. Since the microsomal system regulates intracellular Ca^{++} levels, inhibition of uptake by microsomes could result in an increase in intracellular Ca^{++} levels, even if Ca^{++} uptake across the neuronal membrane were reduced. Thus, the net effect of CHEB may be to increase intracellular Ca^{++}. Since increased intracellular Ca^{++} is associated with increased release of neurotransmitter, CHEB would tend to increase neurotransmitter release to a greater degree than PHB. We have already seen (for 4-aminopyridine) that the overall effect of increased neurotransmitter release appears to be excitatory.

However, when the effect of barbiturates on transmitter release was studied, CHEB, phenobarbital and secobarbital all inhibited K^{+}-stimulated release of ACh from mouse brain slices (Holtman and Richter, 1981). This inhibition has been attributed to the inhibition of Ca^{++} uptake at the cell membrane (Blaustein and Ector, 1975). On the other hand, in rat whole brain synaptosomes, CHEB and three depressant barbiturates inhibited adenosine reuptake, and enhanced release, resulting in elevated synaptic adenosine levels (Gonzales and Leslie, 1985).

CHEB also caused increased release of GABA from rat cortical minislices (Willow *et al.*, 1980). The interaction of CHEB with the GABA receptor appears to be similar to that of other barbiturates. Specifically, it enhances GABA-mediated inhibition, and could act as an anticonvulsant at this site, similar to other convulsant barbiturates (Andrews *et al.*, 1979, 1982; Allan and Harris, 1986). CHEB also binds

to the picrotoxin site (Ticku and Olsen, 1978) and may enhance [^{3}H] diazepam binding to benzodiazepine receptors (Leeb-Lundberg and Olsen, 1982). However, Ticku (1981) demonstrated that while depressant barbiturates enhanced [^{3}H] diazepam binding to benzodiazepine receptors, CHEB and other convulsant barbiturates did not.

In summary, CHEB appears to act in some respects in a similar manner to other barbiturates. It has similar actions at many sites regarded as important for the depressant properties of barbiturates. Depressant barbiturates, in turn, appear to act at many of the sites thought to be responsible for convulsant action. However, CHEB and the other convulsant barbiturates are more potent excitatory agents, particularly in actions such as the presynaptic increase in intracellular Ca^{++}. The depressant barbiturates appeared to be more potent in inhibitory actions, such as enhancement of benzodiazepine binding.

III.B.4. *Drug sources and preparation.*

Picrotoxin, pentylenetetrazol, kainic acid, 4-aminopyridine, bicuculline and strychnine SO4 were purchased from Sigma Chemical Co. Tert-butyl-bicyclo[2.2.2]phosphorothionate (TBPS) and methyl 6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM) were purchased from Research Biochemicals, Inc. 5-(2-cyclohexylidene-ethyl)-5-ethyl barbituric acid (CHEB) was a gift of Dr. Hall Downes, of the Oregon Health Sciences University.

WSP and WSR mice were tested using 2 concentrations of each drug, except that five concentrations of pentylenetetrazol were used. All drugs were administered at a single infusion rate, so the concentration of the drug (mg/ml) determined the rate of drug delivery (mg/min). Inbred strains were tested at one concentration only. All drugs were administered in solutions at a pH in the range of 5.0 to 9.0, and dissolved in vehicles which had no detectable effect on convulsant sensitivity. A single stock solution of each drug was prepared. Pentylenetetrazol, 4-

aminopyridine, DMCM, picrotoxin and strychnine were dissolved in saline and pH was measured. It was necessary to correct the pH of 4-aminopyridine using a small amount of 0.5M HCl. CHEB was solubilized in 0.5M NaOH, diluted with distilled H_2O, and pH was corrected with 0.5M HCl. TBPS was dissolved in 2 mls DMSO, diluted with H_2O, pH was corrected with 5M NaOH, sonicated, and administered as a suspension in 2% DMSO (the infusion syringe was shaken on a vortex between each infusion). Bicuculline was dissolved in concentrated HCl, diluted with saline, and pH corrected using 5M NaOH. Kainic acid was dissolved in distilled H_2O and pH corrected with 5M NaOH. Concentrations of drugs used in experiments were made by single dilutions of the stock solution with saline (except that distilled H_2O was used for CHEB and kainic acid). Stock solutions and dilutions were stored for 0-3 days before use.

III.B.5. *Timed intravenous infusions.*

Tests were performed in the animal colony or in a separate room (temperature 21-23 °C). In either case, home cages were removed from their normal location and moved to a location near the test apparatus. Animals were tested between 12:00 pm and 6:00 pm. Each mouse was taken from its home cage, weighed, and placed in a Plexiglas chamber, 8 x 8 x 19 cm, with numerous 0.5 cm holes drilled in all walls for ventilation. The tail of the mouse was drawn through a hole, and warmed for 1 minute under a tensor lamp. The immediate test area was illuminated by a 100 watt spotlight, which brought the temperature to 25-26 °C[3]. A

3 It is possible that the body temperature of the mice was elevated during the test procedure, due to the tail warming, stress, or because the test area was 4-5 degrees warmer than colony temperatures (although since the mouse was no longer in an enclosed cage, with bedding and other animals, may have tended to counteract these factors). Penfield and Erikson (1941) noted that some epileptics show an increased frequency of seizures during fever, while others show a decreased frequency of seizures. Swinyard and Toman (1948) studied the relationship between body temperature and threshold to convulsion elicited by electroshock, pentylenetetrazol and picrotoxin. They found a negative linear relationship, with reductions in body temperature correlated with increased sensitivity to convulsions regardless of method of induction. They also noted that at either extreme (body temperatures below 27 °C or above 43 °C) spontaneous convulsions occurred, confirming the clinical observations, and demonstrating that factors in addition to seizure threshold are important in the expression of seizures.

27x3/8 butterfly infusion needle was inserted in the lateral tail vein of the mouse, and correct placement was verified by the appearance of blood in the infusion tubing, or by whitening of the vein during the infusion. A footswitch was operated to start both the infusion pump (Harvard) and a digital timer (Gralab). The mouse was observed throughout the infusion, and latency to initial and intermediate convulsion signs recorded. When the final endpoint was seen, the footswitch was operated to stop the timer and pump. Latencies were recorded to the nearest second. If the seizure was not terminal, the mouse was immediately euthanized by cervical dislocation. Infusion rate was 0.246 (most drugs) or 0.486 (CHEB and kainic acid) mls/min. Tests in our laboratory showed that these two infusion rates resulted in essentially identical latencies when rate of drug delivery was held constant. Concentrations of drug were selected that yielded total infusion times of not more than three minutes, as it was difficult to maintain the infusion longer due to movement of the mouse. Thus, the time between initially picking up the animal and finishing the procedure was usually not more than 5 minutes.

III.B.6. *Scoring of convulsions.*

In these experiments, five convulsant signs were seen following the administration of convulsant drugs. The terms "clonic" and "clonus" indicate rhythmic movements due to alternating contraction and relaxation of muscles. The terms "tonic" and "tonus" indicate rigidity due to contraction of muscles. A list of all signs measured for each drug can be found in Tables 3a and 3b.

Clonus: Three types of clonus were observed:

Face and forelimb clonus (FF clonus): A brief episode of clonus, characterized by rapid writhing movements of the head and neck, and clonic forelimb movements. This type of convulsion resembled stage 5 kindling in rats, as

---

Heat would have to affect WSP and WSR mice, and inbred strains, differentially, to interfere with the interpretation.

described by Racine (1972). It could also be elicited in rats by electroshock administered through corneal electrodes (Browning, 1987).

Running/bouncing clonus (RB clonus): Again, this type of clonus was described by Browning (1987). It consisted of a very dramatic and violent whole-body clonus, usually terminating in THE.

Wild running clonus (WR clonus): A typical convulsion of this type began as treadle movements limited to one forelimb. The second forelimb then became involved, and eventually the movements spread to the hindlimbs. The animal did not maintain posture, but fell over on its side. The running movements continued to increase in intensity, and in some cases developed into a running/bouncing clonus. This seizure terminated in tonic hindlimb extension, which was briefer than that seen after running/bouncing clonus.

Myoclonus: A sudden involuntary muscle jerk. Occasionally this was accompanied by a head twitch, a squeak, or a brief (approximately 3 seconds or less) episode of clonus.

Tonic hindlimb extensor convulsion (THE): This convulsion was characterized by extreme rigidity. Forelimbs and hindlimbs extended caudally, and the head of the animal was perpendicular to the body. Ears were flattened and eyes were usually closed. This seizure was frequently terminal. For most drugs, a brief flexor seizure, in which the limbs draw toward the body, preceded the extensor seizure. The flexor response was rapidly overpowered by the stronger extensors. In contrast to the other drugs, strychnine does not cause the flexor portion of the seizure, only extension (Esplin and Woodbury, 1961).

Pentylenetetrazol, bicuculline and TBPS caused myoclonus, followed within 5-20 seconds by face and forelimb clonus. The animal was then quiet, until it displayed running/bouncing clonus followed rapidly by THE. DMCM also caused myoclonus followed by face and forelimb clonus. However, myoclonus did not

always occur, and unlike the other three drugs, the seizure did not always proceed to running/bouncing clonus and THE. Latency to onset of myoclonus, face and forelimb clonus, RB clonus and THE were measured for bicuculline. Latency to myoclonus and face and forelimb clonus only were measured for DMCM, pentylenetetrazol, and TBPS.

Picrotoxin and 4-aminopyridine caused RB clonus, followed by THE. 4-aminopyridine caused considerably more agitation than picrotoxin in the intervals before clonus and between clonus and THE, suggesting that the pattern of CNS excitation was different although the pattern of the convulsions was similar. Latency to onset of running/bouncing clonus and THE were measured for picrotoxin and 4-aminopyridine.

CHEB and strychnine caused myoclonus, running/bouncing clonus and THE. Strychnine, in addition, caused several small bouts of clonus, marked agitation of the mouse, and very abbreviated tonus (with death occurring almost simultaneous to THE). Latency to onset of myoclonus, running/bouncing clonus (first instance, for strychnine) and THE were measured for CHEB and strychnine.

Kainic acid caused wild running clonus followed rapidly by THE. Some animals displayed tonic forelimb extension without hindlimb extension, and occasionally an animal died without displaying any tonus at all, or displaying only a mild facial tonus. Latency to onset of wild running and the appearance of THE or death were measured.

In addition, sensitivity to each convulsant sign was expressed both as latency to sign (sec), and effective dose (ED) of drug given at onset of a given sign (mg drug delivered/kg bodyweight).

III.B.7. *HIC as an index of withdrawal and hyperexcitability.*

Chance (1953) described a postural reflex seen in the falling mouse. In this reflex, the mouse's head was thrown back, pinnae erect and jaws partly open. Its

limbs were held rigid and extended. The hindlimbs were splayed, but its forelimbs were held close together or overlapping. Chance demonstrated that anesthetized mice suffered more severe injuries than unanesthetized mice when dropped from a height of 10 feet. He proposed that this postural reflex had a protective effect.

Chance noted that this posture could be elicited by holding a mouse by the tail and jerking the hand downwards. He also noted that a certain strain of mice, when stimulated by this procedure, developed a convulsion which perpetuated the posture for up to 10 seconds. During the convulsion the mouse was tense and trembling. Picrotoxin and pentylenetetrazol also evoked this posture in the early stages of a convulsion.

The term handling-induced convulsion (HIC) was first applied by Goldstein. She demonstrated that this reflex and its exaggeration could be used as a quantitative index of excitability produced by EtOH withdrawal (Goldstein and Pal, 1971) and by administration of reserpine or of convulsant drugs (Goldstein, 1973a). She found that the reflex could be evoked by holding a mouse by the tail and gently twirling it 180°. In some mice, merely lifting was an adequate stimulus. She developed a scale based on the stimulus necessary to evoke the convulsion (upon lifting, or after twirling) and the severity of convulsion evoked (tonic or clonic convulsion). A tonic convulsion was the milder form, consisting of the posture, adopted and sustained for several seconds. A clonic convulsion was the more severe form, consisting of the tonic phase accompanied by vigorous trembling. Table 2 shows a modified version of this scale, used by our laboratory for scoring EtOH withdrawal.

Goldstein demonstrated that HIC severity was dose dependent, both for convulsant drugs (1973b) and EtOH withdrawal (1972). HIC has also been used as an index of withdrawal severity for other drugs, including diazepam (Belknap *et al.*, in press), nitrous oxide (Belknap *et al.*, 1987) and pentobarbital (Belknap *et al.*,

TABLE 2

Handling-induced convulsion scale

Score	Description
4:	Severe tonic-clonic convulsion when lifted by the tail, with rapid onset and long duration, often continuing for several seconds after the mouse is released.
3:	Tonic-clonic convulsion when lifted by the tail, often with onset delayed by 1 to 2 sec.
2:	Tonic convulsion when lifted by the tail or tonic-clonic convulsion after gentle spin.
1:	No convulsion when lifted by tail, but tonic convulsion elicited by spin.
0.5:	Only facial grimace after spin.
0:	No convulsion.

1988). A number of additional features make HIC a very attractive measure of central nervous system excitation. It is present in at least some normal mice, allowing determination of a baseline measurement. It is a very sensitive index, elevated by low doses of convulsant drugs. Using HIC, withdrawal from EtOH can be detected in mice given a single ip administration of EtOH (Kosobud and Crabbe, 1986). It has a broad range, and can also be used to monitor very severe EtOH withdrawal. Repeated measurements are possible, so that the time course of excitation can be mapped. The stages are very easy to identify, so that there is high inter-rater reliability. No complicated or expensive equipment is necessary for the determinations, and mice can be tested extremely rapidly (an experienced observer can score 3 mice per minute).

However, a number of cautions apply to the use of HIC as an index of excitability. The anatomical and physiological basis for HIC is unknown. EEG measurements during these convulsions have not been made. The scale used to quantify HIC is currently ordinal rather than parametric; a score of 4 is demonstrably more severe than a score of 2, but not necessarily twice as severe. Lyon (1951) studied a strain of mice that do not show this reflex, and attributed this to a lack of otoliths (glass-like particles that float in the inner ear), so that these mice were unable to detect the absence of gravitational attraction during falling. Thus, it appears that the vestibular system may be important for the initiation of this reflex.

III.B.8. *Ethanol dependence and withdrawal.*

HIC were scored using a procedure modified from that developed by Goldstein and Pal (1971). Table 2 shows the scale used for HIC scoring. Mean values for each of the inbred strains for measures of EtOH withdrawal severity and appropriate controls were obtained from published data (Crabbe *et al.*, 1983c). Inbred mice were made dependent by 3 days of inhalation of EtOH vapor. On the

first day, one group of mice was given a priming dose of EtOH (1.5g/kg) and pyrazole (1mM/kg), an alcohol dehydrogenase inhibitor given to stabilize blood EtOH levels. These mice were then placed in the EtOH inhalation chamber. On days 2 and 3, these mice received additional doses of pyrazole. An independent group of mice received 3 daily injections of pyrazole, but were not exposed to EtOH vapor. Twenty-four hours after the last pyrazole administration, the EtOH-treated animals were removed from the EtOH inhalation chamber and blood samples were collected for determination of blood EtOH concentration. Severity of EtOH withdrawal was assessed using HIC. HIC were scored hourly for 15 hours, and again at 24 and 25 hours post-withdrawal. The area under the HIC curve plotted over time was calculated for each animal. Inbred strains differed from one another in severity of HIC seen after pyrazole treatment. Therefore, inbred strain mean values for EtOH withdrawal HIC were corrected for HIC following pyrazole-only treatment, by subtracting the strain values for the pyrazole only group. Typical HIC data can be found in Figure 1 (section II.B.5). Inbred strains also differed from one another in blood ethanol concentration during EtOH inhalation. The corrected HIC scores were further corrected for blood EtOH concentration at time of withdrawal, since severely-withdrawing strains might have simply received higher EtOH doses. A predicted HIC value for each strain was estimated, given that strains blood alcohol concentration, using the linear regression of HIC on blood alcohol concentration (Crabbe *et al.*, 1983c).

In a second experiment, genetic variability in HIC after saline or pyrazole treatment was assessed. Mice from inbred strains were treated for 3 days with saline or pyrazole. Twenty-four hours after the last treatment, these mice were scored hourly for HIC for nine hours. HIC area under the curve was determined for each strain.

Four variables representing inbred strain sensitivity to HIC were used for correlations with strain means for convulsant drug sensitivity:

EtOH $Area_{25}$ represents inbred strain mean values for HIC during EtOH withdrawal corrected for pyrazole-stimulated HIC.

Corrected $Area_{25}$ represents EtOH $Area_{25}$ adjusted for blood ethanol concentration at time of withdrawal.

Saline $Area_{9}$ represents inbred strain means for HIC after treatment with saline.

Pyrazole $Area_{9}$ represents inbred strain means for HIC after treatment with pyrazole.

III.B.9. *Statistical analyses.*

Analyses were performed on an IBM computer using the CRUNCH statistical package (Version 3, 1987). Differences between inbred strains in response to each drug were analyzed using one-way ANOVAs (unweighted means). Comparisons between strain means were made using a Bonferonni test (Keppel, 1982; Winer, 1971). Correlations between strain means for sensitivity to pairs of drugs were assessed using Pearson's product moment correlation. Because the HIC scale is ordinal rather than interval, correlations between convulsant drug and HIC data were assessed using Spearman's rank correlation. For the most part, the HIC scale approximates an interval scale. Analysis of these data using the raw scores or their ranks produced identical results. Significance of the correlations was based on a two-tailed t-test of the hypothesis that the population correlation was equal to zero. WSP and WSR data were analyzed using 2x2x2 ANOVAs for the factors Line, Replicate and Concentration, using an unweighted means solution for unequal n's (Keppel, 1982; Winer, 1971). Significant interactions were assessed using two-way and one-way ANOVAs, and Student's t-test, as appropriate. For pentylenetetrazol,

a 2x2x5 (Line x Replicate x Concentration) ANOVA was used, and concentration effects were analyzed using a Bonferroni test.

III.B.10. *Correlational analysis; special considerations.*

In these experiments, correlation coefficients were used to estimate the genetic correlation between responsiveness of the strains to convulsant drugs and EtOH withdrawal, and among different convulsant drugs. Correlation coefficients reflect the degree of association between two variables. Associations can arise for a number of reasons. First, a causal relationship may exist between two factors; one variable might be responsible for the second (A-->B, or B-->A). Second, a third factor may simultaneously control both variables (A<--C-->B). Third, a complicated network may result in the correlation between the two factors, but their relationship might be trivial. An example of the third case (from Minium, 1970) is the correlation between length of big toe and reading comprehension in children between the age of 6 and 12. These variables are most likely highly correlated, because they both relate to maturational status, but the mechanism underlying the correlation is extremely complex. The correlation coefficient, r_{xy}, can be calculated a number of ways. The Deviation Score Formula (Minium, 1970) has the advantage of clarity:

$$r_{xy} = \mathrm{Sum}(xy)/nS_xS_y$$

The numerator is the sum of the products of the paired deviation scores ($x = \mathrm{x} - \overline{\mathrm{X}}$, $y = \mathrm{y} - \overline{\mathrm{Y}}$). Individual values within each sample are represented by x and y, and $\overline{\mathrm{X}}$ and $\overline{\mathrm{Y}}$ are the means from each sample. The denominator is the product of n (the number of pairs of scores) and the standard deviations of the two distributions. The range of r_{xy} is from 1 to -1, with 0 representing no correlation, 1 representing a positive correlation (increases in one variable associated with increases in the other) and -1 representing a negative correlation (increases in one variable associated with decreases in the other).

Estimating the correlation of two variables within a population is considerably less reliable than estimating the mean of either variable. Correlation coefficients obtained using samples drawn randomly from a population can easily vary over a third of the range of possible correlations. The accuracy of a given estimate is, unsuprisingly, dependent on the sample size, but it is also dependent on the true value of the correlation: The better a correlation within the population, the more accurate the estimates will be (Minium, 1970). This arises because the random sampling distribution of r_{xy} is approximately normal only when p (the population correlation coefficient) = 0, and becomes increasingly skewed as the absolute value of p approaches 1, and the possible variation in value of p becomes increasingly restricted in one direction. Thus, a shift in correlation from 0.2 to 0.3 does not constitute as much of an increase in reliability of an estimate as a shift from 0.8 to 0.9. The p value associated with a correlation coefficient, which can be calculated a number of ways, indicates the probability that r differs from 0. The validity of a correlation, however, is not necessarily related to its size. In a given circumstance a correlation of 0.4 or 0.5 may be meaningful and important. However, correlations of low absolute value are more difficult to demonstrate because of the increased unreliability in the calculation of the coefficient, and they are more difficult to prove significantly different from 0 (an n of 16 pairs is required to demonstrate that a correlation coefficient of 0.5 is significantly different from 0 at the 0.05 level, versus 7 pairs for a correlation coefficient of 0.75). Therefore, the hypothesis that one is testing is an important determinant of what numerical value of the correlation coefficient is important.

Correlations can be biased by a number of factors not assessed in the calculation of the coefficient or the probability. Bias can be introduced by clusters and outliers, which tend to lead to overestimation of the (absolute) value of the correlation coefficient (because they tend to emulate a two point determination of a

line). Also, if the relationship between the two variables is non-linear, the correlation may be underestimated. Visual examination of the scatterplots for presence of such problems is very important.

III.C. Results.

In these experiments, nine drugs were tested, with 1-4 convulsant signs measured for each drug. Values for each convulsive sign were expressed either as latency to appearance of sign, or effective dose given to produce sign. Thus, the comparison of one drug to another potentially involved as many as 64 separate correlation coefficients, and over all drugs, approximately 766 correlation coefficients were generated. Since the variation of r is known to be high, the potential for both type I and type II error was enormous, rendering a meaningful pattern of results difficult to extract. Thus, the first step in analysis of these data was to determine systematic and logical ways to reduce the data set.

To begin, within-drug correlations were examined with two questions in mind. First, data were expressed as ED and latency. If these two variables were highly correlated, only one of them would be necessary, and a 50% reduction of the data set could be accomplished. Correlations between ED and latency included those within a single sign (for instance, latency to myoclonus - ED for myoclonus, or latency to THE - ED for THE) and those between signs (for instance, latency to myoclonus - ED for THE). Since no two convulsant signs measured for a given drug were perfectly correlated, between-sign correlations contained an extra element of variability. Thus, those correlations which assessed the association of ED and latency within a single convulsant sign were considered preferentially in determining whether ED or latency were to be used.

III.C.1. *Comparison of inbred strains: Within-drug.*

Table 3A shows inbred strain means and SEs for ED (mg/kg) for all nine convulsant drugs for all signs. Table 3B shows the same data for latency to each

TABLE 3A

Effective dose (mg/kg) infused at time of appearance of convulsant sign in nine inbred mouse strains (mean and SE).

DRUG SIGN	INBRED STRAINS									
	C57	CBA	AKR	BALB	A	C3H	SWR	DBA	RIII	C58
PICROTOXIN										
RB CLONUS	19.4± 0.4	14.3± 0.4	14.7± 0.6	12.0± 0.2	14.3± 0.5	13.8± 0.4	15.9± 0.4	16.3± 0.3	12.9± 0.3	15.8± 0.4
THE	24.3± 0.5	18.6± 0.4	18.0± 1.1	19.8± 0.9	25.8± 0.8	19.2± 1.0	20.2± 1.4	27.0± 0.7	18.6± 0.7	24.1± 0.1
DMCM										
FF CLONUS	2.20± .18	1.34± .10	1.18± .10	0.96± .04	1.31± .05	1.86± .10	1.36± .05	1.20± .08	1.96± .07	1.47± .09
TBPS										
MYOCLONUS	.282± .006	.178± .009	.234± .011	.162± .004	.192± .009	.183± .007	.204± .011	.213± .006	.212± .011	-
FF CLONUS	.342± .010	.198± .005	.243± .010	.174± .007	.225± .012	.256± .006	.215± .009	.230± .005	.223± .011	-
BICUCULLINE										
MYOCLONUS	.624± .017	.273± .015	.465± .036	.294± .011	.284± .015	.376± .016	.477± .025	.399± .012	.471± .054	-
FF CLONUS	.712± .016	.412± .046	.481± .033	.316± .013	.341± .020	.515± .030	.504± .019	.481± .015	.547± .058	-
RB CLONUS	1.16± .09	0.76± .09	1.25± .02	0.56± .09	0.69± .02	0.88± .05	1.08± .02	1.09± .07	0.59± .05	-
THE	1.29± .08	0.90± .09	1.24± .04	0.60± .09	0.75± .03	0.90± .05	1.13± .02	1.17± .07	0.62± .06	-
STRYCHNINE										
MYOCLONUS	.609± .048	.601± .033	.578± .036	.470± .035	.391± .028	.454± .045	.614± .037	.626± .026	.550± .019	-
RB CLONUS	.811± .044	.660± .043	.670± .022	.638± .042	.481± .043	.599± .022	.891± .062	.768± .024	.604± .032	-
THE	.929± .033	.755± .023	.773± .012	.733± .039	.626± .053	.684± .014	1.04± .04	.870± .025	.683± .062	-
PENTYLENETETRAZOL										
MYOCLONUS	36.0± 1.7	20.5± 0.9	25.0± 0.9	18.5± 0.6	22.1± 1.8	20.7± 0.9	27.9± 1.4	17.8± 0.8	31.4± 1.3	34.4± 4.4
FF CLONUS	58.1± 5.1	34.0± 3.7	30.6± 4.1	21.9± 0.8	26.5± 2.7	43.3± 3.5	31.9± 1.6	22.1± 1.2	38.2± 1.7	38.4± 5.5
4-AMINOPYRIDINE										
RB CLONUS	29.0± 0.5	25.7± 0.6	21.3± 1.2	22.8± 1.3	21.0± 0.9	20.2± 0.9	29.9± 1.4	19.6± 0.9	26.0± 2.0	-
THE	30.6± 0.5	26.6± 0.6	24.1± 1.1	26.3± 1.3	35.5± 3.5	24.5± 2.3	31.6± 1.5	20.7± 0.9	27.3± 1.8	-
CHEB										
MYOCLONUS	4.41± .26	3.24± .14	3.11± .07	2.52± .10	2.66± .14	3.81± .07	4.32± .24	4.22± .19	3.07± .38	-
RB CLONUS	5.70± .24	5.32± .21	4.64± .27	4.12± .14	3.80± .33	5.09± .08	5.25± .10	5.02± .18	3.58± .06	
THE	7.06± .46	5.58± .19	5.22± .22	4.38± .15	4.76± .30	5.43± .11	5.46± .09	5.42± .15	4.14± .18	
KAINIC ACID										
WR CLONUS	97.4± 5.9	103± 7	104± 6	81.0± 5.1	87.5± 4.9	97.4± 4.1	125± 6	75.1± 5.3	189± 12	-
THE	109± 6	121± 9	119± 7	87.9± 5.2	101± 5	113± 5	134± 5	87.8± 7.9	201± 10	

For each drug and convulsant sign, inbred strains differed significantly (all $F>6$, $p<.01$).

RB clonus = running/bouncing clonus
FF clonus = face and forelimb clonus
WR clonus = wild running clonus
THE = tonic hindlimb extensor seizure

TABLE 3B

Latency (sec) to appearance of convulsant sign in nine inbred mouse strains (mean and SE).

DRUG SIGN	INBRED STRAINS C57	CBA	AKR	BALB	A	C3H	SWR	DBA	RIII	C58
PICROTOXIN										
RB CLONUS	140± 2	119± 3	142± 4	97± 1	107± 2	108± 2	112± 3	129± 2	106± 1	122± 7
THE	176± 3	154± 4	174± 8	159± 5	193± 4	150± 4	142± 7	214± 6	153± 4	186± 5
DMCM										
FF CLONUS	121± 10	84± 7	86± 7	58± 2	70± 2	118± 8	73± 1	70± 5	112± 2	89± 8
TBPS										
MYOCLONUS	168± 7	115± 4	177± 7	107± 4	119± 6	122± 7	113± 5	136± 4	129± 9	-
FF CLONUS	203± 6	128± 2	184± 7	115± 6	140± 9	171± 6	118± 5	146± 3	136± 8	-
BICUCULLINE										
MYOCLONUS	61.2± 1.4	30.4± 2.3	58.3± 4.8	32.3± 1.3	27.4± 1.7	41.3± 2.1	43.1± 1.7	40.4± 1.2	46.5± 2.8	-
FF CLONUS	70.1± 2.2	45.6± 4.9	60.3± 4.5	34.6± 1.3	33.0± 2.4	56.6± 3.5	45.7± 1.6	48.7± 1.5	54.0± 2.5	-
RB CLONUS	114± 10	85± 12	157± 2	62± 10	66± 3	97± 7	98± 4	110± 6	59± 4	-
THE	127± 10	101± 12	155± 5	66± 10	72± 4	99± 7	102± 3	118± 7	62± 2	-
STRYCHNINE										
MYOCLONUS	70.9± 5.9	80.4± 3.6	86.3± 5.9	57.9± 4.9	45.5± 2.4	63.2± 4.0	69.4± 4.9	77.7± 3.2	72.7± 1.2	-
RB CLONUS	96.0± 5.1	89.7± 4.2	100.2± 3.9	78.1± 4.9	55.7± 4.9	82.0± 2.5	97.2± 6.3	95.4± 3.3	72.7± 1.2	-
THE	110± 3	103± 2	116± 2	90± 5	73± 6	94± 3	114± 3	108± 3	77± 2	-
PENTYLENETETRAZOL										
MYOCLONUS	51.6± 2.1	33.0± 1.8	44.6± 1.8	28.3± 1.4	33.1± 2.4	34.7± 1.8	38.6± 1.7	27.1± 1.2	41.9± 3.2	50.6± 4.5
FF CLONUS	83.4± 6.8	55.1± 6.7	55.1± 7.5	33.3± 1.0	39.5± 3.6	72.1± 5.2	44.1± 2.3	33.6± 2.0	50.7± 3.45	56.4± 5.4
4-AMINOPYRIDINE										
RB CLONUS	88.0± 2.7	83.1± 2.3	72.3± 2.7	68.0± 3.0	63.6± 3.2	66.3± 3.4	84.6± 4.3	55.6± 2.1	70.3± 3.2	-
THE	92.8± 2.6	86.0± 2.3	82.0± 2.4	78.6± 2.7	107± 10	80.4± 7.6	89.6± 4.7	58.7± 2.0	74.0± 2.5	-
CHEB										
MYOCLONUS	51.2± 3.2	42.3± 2.1	46.1± 0.9	29.8± 1.4	32.3± 1.5	50.7± 1.7	49.7± 2.8	50.9± 2.5	38.5± 4.5	
RB CLONUS	65.9± 3.0	67.7± 2.6	66.5± 4.3	48.1± 2.0	47.1± 4.2	67.7± 1.8	61.4± 1.9	60.3± 2.0	45.0± 1.0	-
THE	82.0± 6.7	71.0± 2.2	74.5± 2.8	51.1± 2.2	58.0± 3.6	72.3± 1.6	63.9± 1.9	65.5± 1.6	52.0± 2.0	-
KAINIC ACID										
WR CLONUS	115± 7	134± 6	151± 11	90± 4	108± 7	122± 5	143± 5	88± 6	218± 6	-
THE	129± 7	157± 8	174± 13	98± 4	124± 8	141± 6	154± 5	103± 9	232± 4	-

For each drug and convulsant sign, inbred strains differed significantly (all $F>6$, $p<.01$).

RB clonus = running/bouncing clonus
FF clonus = face and forelimb clonus
WR clonus = wild running clonus
THE = tonic hindlimb extensor seizure

convulsant sign. All one-way ANOVA's for each sign and drug tested yielded a significant effect of strain (all F's>6.0, p<.01), confirming that there was genetic heterogeneity in response to convulsant drugs in this group of inbred strains. Therefore, these data were further examined for genetic correlations between drugs.

Table 4 shows, for each drug, the convulsant signs recorded and the correlation between mean strain latencies and EDs for each sign. For almost all signs measured, and for all drugs, ED and latency were highly correlated. ED was chosen as the unit for further analysis.

III.C.2. *Comparison of inbred strains: Between-sign, between-drug.*

Correlation coefficients were initially calculated for all nine drugs, and all convulsant signs were measured. However, four variables did not significantly correlate with any other variables. These included picrotoxin (THE), 4-aminopyridine (THE) and kainic acid (WR clonus, THE). These variables were omitted from further between-drug analyses. Table 5 shows the correlation coefficients (r) for 17 remaining convulsant variables. Note that for those drugs which had more than one sign measured, the final group of entries represents correlations between signs within one drug. For all comparisons, 8-10 inbred strains were used. For $n = 9$, an r value of .65 or greater was significantly different from 0 (p<.05), while an r value of .58 tended to be significantly different from 0 (p<.10). Decisions concerning which correlations were regarded as representing true correlations were made considering both the numerical value of the correlation coefficient, and the pattern of all comparisons between each pair of drugs. In Table 5, boldface type indicates groups of correlations judged to represent meaningful relationships. Groups of correlations judged not to represent meaningful relationships are indicated by italic type. The results, both between and within drug, are discussed below, drug by drug. These descriptions contain some redundancy, since they describe the pattern of correlations found for each drug.

TABLE 4

Genetic correlation between ED (mg/ml) and latency (sec) for each drug and all convulsant signs (r_g, n, p).

DRUG: Concentrations SIGN	r_g	n	p
Picrotoxin: 0.75 mg/ml			
RB clonus	.74	10	.01
THE	.85	10	.002
DMCM: 0.1 mg/ml			
FF clonus	.94	10	.0002
TBPS: 0.01 mg/ml			
myoclonus	.86	9	.003
FF clonus	.89	9	.001
Bicuculline: 0.06 mg/ml			
myoclonus	.92	9	.0005
FF clonus	.91	9	.0006
RB clonus	.93	9	.0003
THE	.93	9	.0004
Strychnine: 0.05 mg/ml			
myoclonus	.83	9	.006
RB clonus	.83	9	.006
THE	.78	9	.01
Pentylenetetrazol: 4 mg/ml			
myoclonus	.94	10	.0001
FF clonus	.95	10	.0001
4-aminopyridine: 2 mg/ml			
RB clonus	.89	9	.002
THE	.90	9	.0009
CHEB: 0.25 mg/ml			
myoclonus	.92	9	.0006
RB clonus	.89	9	.001
THE	.90	9	.001
Kainic acid: 5 mg/ml			
WR clonus	.88	8	.004
THE	.89	8	.003

Infusion rate was .246 ml/min for all drugs except CHEB, which was infused at .486 ml/min.

RB clonus = running/bouncing clonus
FF clonus = face and forelimb clonus
WR clonus = wild running clonus
THE = tonic hindlimb extensor seizure

TABLE 5

Intercorrelations among sensitivities to eight convulsant drugs (r_g)

DRUG SIGN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Picrotoxin																
1 clonus																
DMCM																
2 FF clonus	*.42*															
TBPS																
3 myoclonus	*.54*	*.57*														
4 FF clonus	*.41*	*.53*	**.87**													
Bicuculline																
5 myoclonus	**.71**	**.65**	**.80**	**.49**												
6 FF clonus	**.73**	**.83**	**.63**	**.65**	**.92**											
7 clonus	**.75**	*.09*	**.70**	**.55**	**.64**	**.57**										
8 THE	**.84**	*.14*	**.72**	**.49**	**.63**	**.69**	**.98**									
Strychnine																
9 myoclonus	**.57**	*-.04*	*.11*	*.51*	**.55**	**.55**	**.61**	**.67**								
10 clonus	**.66**	*.09*	*.47*	*.44*	**.62**	**.54**	**.66**	**.70**	**.82**							
11 THE	**.69**	*.05*	*.46*	*.29*	**.60**	**.49**	**.66**	**.71**	**.76**	**.98**						
Pentylenetetrazol																
12 myoclonus	*.53*	**.65**	**.78**	**.20**	**.85**	**.58**	*.28*	*.30*	*.34*	*.36*	*.37*					
13 FF clonus	*.57*	**.91**	**.06**	**.51**	**.30**	**.64**	*.28*	*.34*	*.20*	*.23*	*.20*	**.72**				
4-aminopyridine																
14 clonus	*.41*	*.42*	*.37*	*.24*	*.54*	*.50*	*.10*	*.18*	**.49**	**.60**	**.62**	**.74**	**.51**			
CHEB																
15 myoclonus	**.80**	*.44*	*.56*	*.60*	**.66**	**.75**	**.70**	**.76**	**.65**	**.81**	**.79**	*.36*	*.49*	*.38*		
16 clonus	**.72**	*.23*	*.38*	*.50*	**.38**	**.52**	**.68**	**.68**	**.61**	**.71**	**.68**	*.14*	*.48*	*.34*	**.79**	
17 THE	**.74**	*.45*	*.12*	*.35*	**.02**	**.69**	**.69**	**.80**	**.49**	**.59**	**.59**	*.41*	*.15*	*.38*	**.75**	**.89**

Numbers along the top indicate variables as defined in the leftmost column. Seven to 10 inbred strains were used to calculate each correlation coefficient. In most cases, correlation coefficients of 0.70 and above were significantly different from 0 at the .05 level. For $r > 0.60$, $p \leq .10$. Numbers in boldface indicate groups of correlations judged to represent meaningful relationships. in italic indicate correlations judged to be unimportant.

RB clonus = running/bouncing clonus
FF clonus = face and forelimb clonus
WR clonus = wild running clonus
THE = tonic hindlimb extensor seizure

III.C.2.a. *Picrotoxin.*

Correlations between picrotoxin (clonus) and other variables are found in the first column of numbers in Table 5. Picrotoxin (clonus) was significantly correlated with bicuculline (all signs), strychnine (all signs) and CHEB (all signs).

III.C.2.b. *DMCM.*

Correlations between sensitivity to DMCM (FF clonus) and other variables are found in the first (DMCM/picrotoxin only) and second columns of Table 5. DMCM (FF clonus) correlated significantly with bicuculline (myoclonus and FF clonus) and pentylenetetrazol (myoclonus and FF clonus).

III.C.2.c. *TBPS.*

The two signs measured for TBPS, myoclonus and FF clonus, were strongly correlated. This was reflected both in the correlation between these two signs ($r = .87$) and in the pattern of between-drug correlations. TBPS (both signs) correlated with bicuculline (all signs) and pentylenetetrazol (myoclonus and FF clonus).

III.C.2.d. *Bicuculline.*

Correlations between the various convulsant signs elicited by bicuculline revealed an interesting pattern. Myoclonus correlated very strongly with FF clonus ($r = .92$) and RB clonus correlated very strongly with THE ($r = .98$), but the correlations across these pairs of variables were not as strong (range of $r = .57$-$.69$). Myoclonus/FF clonus, and RB clonus/THE appear to reflect somewhat independent effects of bicuculline. Consistent with this notion, bicuculline (myoclonus/FF clonus) correlated significantly with DMCM and pentylenetetrazol, while bicuculline (RB clonus/THE) did not. On the other hand, picrotoxin (clonus), CHEB (all signs) and strychnine (all signs) correlated with all four signs elicited by bicuculline.

III.C.2.e. *Strychnine.*

All three signs measured for strychnine were significantly intercorrelated; this pattern was maintained in intercorrelations between sensitivity to strychnine and other drugs. Strychnine correlated significantly with CHEB (all signs), 4-aminopyridine (clonus), bicuculline (all signs) and picrotoxin (clonus).

III.C.2.f. *Pentylenetetrazol.*

Sensitivity to myoclonus and FF clonus were significantly correlated for pentylenetetrazol. Both signs correlated significantly with DMCM (FF clonus), TBPS (myoclonus, FF clonus), bicuculline (myoclonus, FF clonus) and 4-aminopyridine (clonus).

IIII.C.2.g. *4-aminopyridine.*

Sensitivity to 4-aminopyridine (clonus) correlated significantly with strychnine (all signs) and pentylenetetrazol (myoclonus, FF clonus).

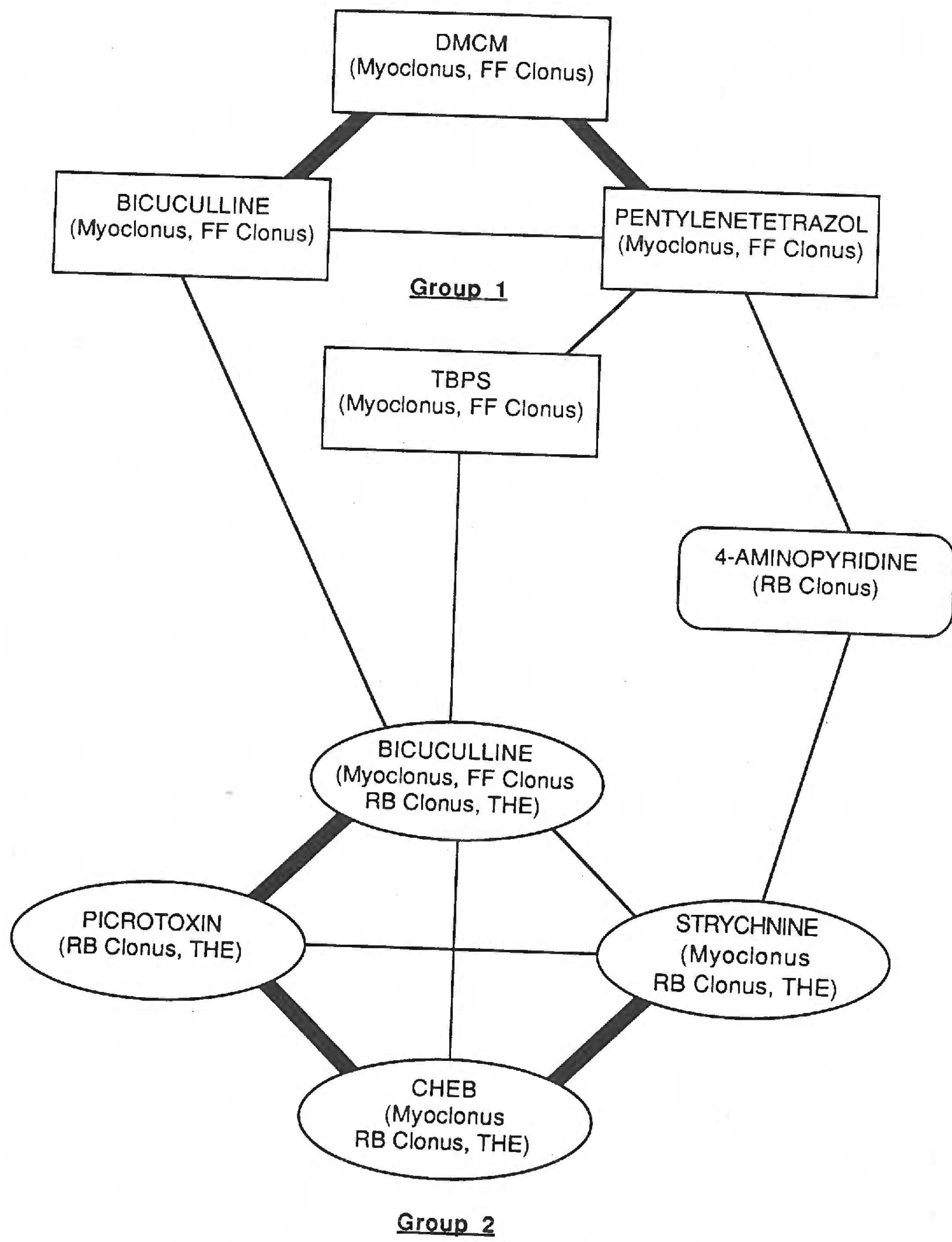
III.C.2.h. *CHEB*

The three signs measured for CHEB sensitivity were significantly intercorrelated. This pattern was reflected in the between-drug correlations. CHEB (all signs) was correlated with picrotoxin (clonus), bicuculline (all signs) and strychnine (all signs).

III.C.2.i. *Between-drug correlations*

Figure 3 summarizes the pattern of correlations. Variables connected by two solid lines are considered to be strongly correlated. A single solid line represents a slightly less robust correlation. Variables which are not connected by any line are considered uncorrelated. This figure indicates that convulsant sensitivity variables generally fell into one of two groups. Group 1 comprised those drugs which caused face and forelimb clonus (pentylenetetrazol, TBPS, DMCM and bicuculline). Group 2 comprised those drugs which caused running/bouncing clonus and THE (picrotoxin, strychnine, CHEB and bicuculline). 4-aminopyridine (RB clonus)

FIGURE 3: A diagram of the between-drug correlations found in the present studies. A single line indicates that the connected variables were correlated. A double line indicates that the correlation between the variables was very strong. Variables not connected by any line were considered to be uncorrelated.


DMCM
(Myoclonus, FF Clonus)
BICUCULLINE
(Myoclonus, FF Clonus)
PENTYLENETETRAZOL
(Myoclonus, FF Clonus)
Group 1
TBPS
(Myoclonus, FF Clonus)
4-AMINOPYRIDINE
(RB Clonus)
BICUCULLINE
(Myoclonus, FF Clonus
RB Clonus, THE)
PICROTOXIN
(RB Clonus, THE)
STRYCHNINE
(Myoclonus
RB Clonus, THE)
CHEB
(Myoclonus
RB Clonus, THE)
Group 2

correlated with variables in both groups. As reported, bicuculline was represented by two patterns of correlations which correlated with Group 1 (myoclonus, FF clonus) and Group 2 (myoclonus, FF clonus, RB clonus, THE), respectively. Picrotoxin (THE), 4-aminopyridine (THE) and kainic acid (wild running clonus, THE) did not correlate with any other variables.

III.C.3. *Comparisons of Inbred Strains: Correlations between convulsant drug sensitivity, EtOH withdrawal severity, and HIC after saline or pyrazole treatment.*

Table 6 shows Spearman correlation coefficients, p values and *n*s for all convulsant signs, and HIC variables. EtOH withdrawal severity is expressed as EtOH $Area_{25}$, and as Corrected $Area_{25}$.

In columns 1 and 2, no correlations between the EtOH withdrawal variables and convulsant sensitivity were significant. For Saline $Area_9$, strong positive correlations between HIC severity and sensitivity to Group 1 variables were found (Table 6, column 2). In other words, HIC severity after saline appeared to predict sensitivity to any drug which caused convulsions of the myoclonus-face and forelimb clonus type. Following three days of pyrazole treatment, a slightly different pattern was seen, shown in the last column of Table 6. Significant positive correlations were found between pyrazole $Area_9$ and the Group 1 variables; pentylenetetrazol (myoclonus/FF clonus), and TBPS (myoclonus/FF clonus), but not DMCM. Significant positive correlations were also present with the Group 2 variables, bicuculline (all signs) and picrotoxin (clonus). For strychnine, an ambiguous relationship existed, with one of the three variables significant (myoclonus), but not the other two. Although HIC severity after pyrazole treatment was clearly associated with sensitivity to some drugs, a clear relationship with Group 1 or Group 2 variables was not indicated.

TABLE 6

Genetic correlations among inbred mouse strains for sensitivity to convulsant drugs and HIC after saline, pyrazole or EtOH and pyrazole treatment (r_g (p))

DRUG SIGN	HIC variables: EtOH $Area_{25}$	Corrected $Area_{25}$	Saline $Area_9$	Pyrazole $Area_9$
Pentylenetetrazol				
myoclonus	*.39 (.29)*	*.30 (.44)*	**.70 (.02)**	**.65 (.04)**
FF clonus	*.17 (.66)*	*.11 (.77)*	**.75 (.01)**	**.49 (.15)**
DMCM				
FF clonus	*-.08 (.84)*	*-.12 (.75)*	**.75 (.01)**	*.43 (.22)*
TBPS				
myoclonus	*.19 (.64)*	*.05 (.91)*	**.71 (.03)**	**.79 (.01)**
FF clonus	*.07 (.87)*	*-.05 (.90)*	**.78 (.01)**	**.61 (.08)**
Bicuculline				
myoclonus	*.09 (.83)*	*-.15 (.72)*	**.73 (.03)**	**.75 (.02)**
FF clonus	*-.06 (.90)*	*-.17 (.68)*	**.90 (.001)**	**.80 (.01)**
RB clonus	*.01 (.97)*	*-.10 (.80)*	*.37 (.33)*	**.55 (.13)**
THE	*-.04 (.93)*	*-.11 (.80)*	*.49 (.29)*	**.60 (.09)**
Picrotoxin				
RB clonus	*-.12 (.77)*	*-.14 (.71)*	*.52 (.12)*	**.65 (.04)**
THE	*-.33 (.39)*	*-.25 (.52)*	*.17 (.64)*	*.27 (.45)*
Strychnine				
myoclonus	*-.09 (.83)*	*-.04 (.93)*	*.40 (.28)*	**.80 (.01)**
RB clonus	*-.24 (.56)*	*-.37 (.37)*	*.23 (.55)*	*.56 (.12)*
THE	*-.28 (.50)*	*-.43 (.29)*	*.14 (.72)*	*.47 (.21)*
CHEB				
myoclonus	*-.54 (.17)*	*-.60 (.12)*	*.56 (.12)*	*.60 (.09)*
RB clonus	*-.22 (.59)*	*-.17 (.68)*	*.33 (.38)*	*.43 (.25)*
THE	*-.05 (.90)*	*-.07 (.88)*	*.50 (.17)*	*.54 (.13)*
4-aminopyridine				
RB clonus	*.02 (.97)*	*-.10 (.81)*	*.19 (.62)*	*.32 (.40)*
THE	*0 (1)*	*-.14 (.75)*	*-.26 (.51)*	*-.36 (.34)*
Kainic acid				
WR clonus	*.02 (.97)*	*-.09 (.83)*	*.03 (.94)*	*.003 (.99)*
THE	*.02 (.95)*	*-.02 (.97)*	*.10 (.80)*	*.03 (.94)*

Inbred strain mean HIC scores for all four HIC variables were converted to rank scores for correlation with means for convulsant drug sensitivity. Eight to 10 inbred strain were used in each correlation. Numbers in boldface indicate correlations judged to be meaningful. Numbers in italic indicate correlations judged to be unimportant.

EtOH $Area_{25}$: Inbred strain mean values for HIC during EtOH withdrawal corrected for pyrazole-stimulated HIC.
Corrected $Area_{25}$: EtOH $Area_{25}$ adjusted for BEC at time of withdrawal.
Saline $Area_9$: Inbred strain means for HIC after treatment with saline.
Pyrazole $Area_9$: Inbred strain means for HIC after treament with pyrazole

III.C.4. *Comparisons of WSP and WSR mice.*

WSP and WSR mice were tested using 2 concentrations of each drug (except for pentylenetetrazol, which was tested at 5 concentrations). The arguments used to simplify the data set for the inbred strains were not applicable to the comparison of WSP and WSR mice. Thus, both ED and latency, and all convulsant signs measured, were considered in the WSP and WSR mice. A summary of the results can be found in Table 7. Tables 8A and 8B show mean, SE and *n* for ED for response to convulsant drugs for WSP and WSR mice of both replicates. Tables 9A and 9B show mean, SE and *n* for latency to response to convulsant drugs for WSP and WSR mice of both replicates. A narrative description of the analysis for each drug is provided in the following section. Drugs are discussed in the order in which they appear in Tables 7, 8 and 9, except that pentylenetetrazol is discussed first.

III.C.4.a. *Pentylenetetrazol: Effects of concentration.*

Two convulsant signs were measured for pentylenetetrazol: myoclonus and writhe. In addition, pentylenetetrazol was tested at five concentrations. The main effect of concentration was significant for all measures (values reported below), so the data were further analyzed using the Bonferonni test. As expected, lower concentrations of drug resulted in longer latencies, while higher concentrations resulted in shorter latencies (Figures 4, 5). At the highest concentration tested (10 mg/ml), some animals responded within 16 seconds, and the responses were not much quicker than those to 6 mg/ml. If the drug is administered more rapidly than the animal can respond, the effective dose calculated will be artificially increased. This was probably occurring, to some degree, reflected in the slightly elevated ED for myoclonus and writhe at 10 mg/ml. Although this elevation was not significant, it is clear that at higher doses ED must become elevated, as the animals are responding to 10 mg/ml in about 20 seconds, and cannot respond much faster.

TABLE 7

SUMMARY OF RESULTS OF TESTS OF WSP AND WSR MICE

Drug: convulsant sign	Strength	Relative sensitivity
PICROTOXIN: RB clonus and THE	STRONG:	WSP > WSR
CHEB: Myoclonus, RB clonus, THE	STRONG:	WSP > WSR
4-AMINOPYRIDINE: RB clonus, THE	STRONG:	WSP > WSR
STRYCHNINE: Myoclonus, RB clonus, THE	MODERATE	
KAINIC ACID: WR clonus, THE	MODERATE	
DMCM: Myoclonus, FF clonus	WEAK:	WSP1 > WSR1
		WSP2 = WSR2
TBPS: Myoclonus, FF clonus	WEAK:	WSP1 > WSR1
		WSP2 = WSR2
BICUCULLINE: Myoclonus, FF and WR clonus, THE	ABSENT:	WSP1 > WSR1
		WSP2 $\leq$ WSR2
PENTYLENETETRAZOL: Myoclonus, FF clonus	ABSENT:	WSP1 > WSR1
		WSP2 $\leq$ WSR2

Relative sensitivity of WSP and WSR mice to drug and convulsant sign is indicated.

TABLE 8A

Mean and SE (n) for ED for response to convulsant drugs for WSP and WSR mice of both replicates: Drugs showing evidence for a correlated response to selection

DRUG CONCENTRATION SIGN	Replicate 1 WSP	 WSR	Replicate 2 WSP	 WSR
Picrotoxin				
RB clonus				
0.75 mg/ml	12.9± .7(8)	14.2± .4(8)	12.5± 1.1(8)	14.1± .4(8)
1.5 mg/ml	19.4± .6(8)	21.4± .6(8)	20.4± .5(8)	19.5± .5(8)
THE				
0.75 mg/ml	18.3± 1.3(7)	19.7± 1.3(6)	14.7± 1.3(8)	17.6± .6(8)
1.5 mg/ml	25.5± .6(8)	30.4± 1.6(8)	24.1± .5(8)	24.1± .8(8)
CHEB				
myoclonus				
.25 mg/ml	4.8± .3(11)	5.6± .2(10)	4.2± .3(8)	4.4± .3(5)
.5 mg/ml	4.8± .4(8)	5.7± .5(7)	5.2± .2(7)	5.1± .4(7)
RB clonus				
.25 mg/ml	7.3± .3(11)	8.0± .3(10)	6.3± .3(8)	8.5± .3(8)
.5 mg/ml	7.4± .6(8)	8.0± .6(8)	7.9± .4(7)	7.3± .3(11)
THE				
.25 mg/ml	7.7± .2(8)	8.6± .3(8)	6.7± .3(8)	7.5± .4(7)
.5 mg/ml	8.9± .9(8)	8.9± .8(8)	8.8± .3(7)	9.1± .3(8)
4-aminopyridine				
RB clonus				
1 mg/ml	20.7± .8(10)	22.8± 1.0(10)	19.2± .7(8)	19.0± .6(8)
2 mg/ml	25.0± .5(10)	32.1± 1.6(11)	22.1± .6(8)	24.0± 1.6(9)
THE				
1 mg/ml	21.3± .8(10)	23.9± 1.0(10)	19.2± .7(8)	19.6± .6(8)
2 mg/ml	26.2± .6(10)	35.2± 2.1(11)	22.1± .6(8)	26.1± 1.7(9)
Strychnine				
myoclonus				
.05 mg/ml	.47± .04(10)	.55± .03(11)	.55± .03(7)	.55± .03(8)
.1 mg/ml	.67± .03(5)	.72± .04(7)	.79± .03(8)	.74± .04(12)
RB clonus				
.05 mg/ml	.53± .04(10)	.65± .03(11)	.70± .02(9)	.67± .03(10)
.1 mg/ml	.67± .03(5)	.80± .03(11)	.87± .03(10)	.84± .04(12)
THE				
.05 mg/ml	.71± .03(10)	.84± .03(11)	.82± .02(9)	.79± .03(10)
.1 mg/ml	.96± .05(10)	.98± .03(11)	1.03± .03(10)	.93± .05(12)
Kainic acid				
WR clonus				
5 mg/ml	125± 9(8)	127± 5(8)	183± 14(9)	182± 10(10)
10 mg/ml	139± 9(8)	186± 14(9)	251± 15(9)	271± 23(12)
THE				
5 mg/ml	146± 11(8)	152± 7(8)	156± 10(8)	186± 14(9)
10 mg/ml	198± 16(9)	197± 11(10)	251± 15(9)	271± 23(12)

TABLE 8B

Mean and SE (*n*) for ED for response to convulsant drugs for WSP and WSR mice of both replicates: Drugs showing little or no evidence of a correlated response to selection

DRUG CONCENTRATION SIGN	Replicate 1		Replicate 2	
	WSP	WSR	WSP	WSR
DMCM				
FF clonus				
.1 mg/ml	7.7± .3(8)	9.4± .2(8)	11.0± .3(9)	12.0± .2(10)
.2 mg/ml	9.7± .3(8)	11.8± .3(8)	11.0± .1(7)	13.1± .3(9)
TBPS				
myoclonus				
.01 mg/ml	.251± .005(8)	.269± .014(8)	.240± .020(5)	.236± .015(7)
.02 mg/ml	.392± .011(8)	.437± .025(8)	.351± .018(8)	.330± .021(7)
FF clonus				
.01 mg/ml	.270± .006(8)	.298± .012(8)	.240± .014(5)	.234± .016(8)
.02 mg/ml	.428± .011(8)	.488± .024(8)	.367± .018(8)	.354± .022(8)
Bicuculline				
myoclonus				
.03 mg/ml	.45± .03(8)	.48± .02(8)	.49± .04(7)	.46± .02(8)
.06 mg/ml	.51± .03(8)	.48± .03(8)	.56± .03(8)	.48± .02(8)
FF clonus				
.03 mg/ml	.48± .03(8)	.55± .05(7)	.57± .03(8)	.47± .03(7)
.06 mg/ml	.55± .03(8)	.58± .05(8)	.54± .04(8)	.51± .03(8)
RB clonus				
.03 mg/ml	1.22± .06(5)	1.28± .12(5)	1.17± .06(5)	.98± .12(5)
.06 mg/ml	1.13± .06(7)	1.24± .11(8)	.91± .09(8)	1.13± .03(8)
THE				
.03 mg/ml	1.24± .06(5)	1.30± .12(5)	1.18± .06(5)	.99± .12(5)
.06 mg/ml	1.18± .07(7)	1.28± .10(8)	.96± .09(8)	1.15± .03(8)
Pentylenetetrazol				
myoclonus				
2 mg/ml	29.4± 4.0(6)	32.3± 1.1(8)	39.6± 2.4(8)	35.3± 1.9(10)
4 mg/ml	22.3± 2.1(8)	29.6± 2.3(10)	33.7± 2.3(8)	33.7± 4.1(8)
5 mg/ml	23.0± 1.7(7)	28.7± 1.8(9)	36.0± 3.1(7)	31.5± 1.7(8)
6 mg/ml	22.3± 1.3(8)	26.9± 1.3(10)	29.6± 2.3(8)	31.4± 1.3(9)
10 mg/ml	27.5± 1.7(7)	32.4± 3.0(9)	33.2± 1.2(8)	34.9± 2.0(8)
FF clonus				
2 mg/ml	34.3± 4.4(6)	42.2± 2.2(7)	44.9± 3.2(8)	39.2± 2.0(9)
4 mg/ml	32.7± 3.7(8)	36.1± 2.4(9)	37.1± 3.4(8)	40.7± 3.3(8)
5 mg/ml	26.6± 2.1(6)	31.0± 1.8(9)	44.4± 3.1(8)	33.7± 1.9(8)
6 mg/ml	24.1± 1.6(8)	30.1± 0.6(10)	34.3± 2.8(9)	28.1± 1.5(9)
10 mg/ml	31.1± 1.9(7)	34.4± 3.2(9)	38.6± 1.6(8)	31.5± 1.1(8)

TABLE 9A

Mean and SE for latency for response to convulsant drugs for WSP and WSR mice of both replicates

DRUG SIGN CONCENTRATION	Replicate 1		Replicate 2	
	WSP	WSR	WSP	WSR
Picrotoxin				
RB clonus				
0.75 mg/ml	110± 7	123± 4	111± 8	140± 6
1.5 mg/ml	82± 3	98± 1	87± 3	100± 3
THE				
0.75 mg/ml	155± 11	174± 10	130± 8	175± 8
1.5 mg/ml	107± 2	140± 7	123± 4	123± 4
CHEB				
myoclonus				
.25 mg/ml	62± 4	73± 2	61± 4	70± 6
.5 mg/ml	31± 3	38± 3	36± 1	38± 3
RB clonus				
.25 mg/ml	101± 2	103± 4	92± 3	115± 6
.5 mg/ml	47± 3	53± 3	55± 1	63± 3
THE				
.25 mg/ml	101± 2	111± 5	98± 2	116± 6
.5 mg/ml	57± 6	58± 4	61± 2	68± 3
4-aminopyridine				
RB clonus				
1 mg/ml	132± 6	139± 5	125± 3	125± 3
2 mg/ml	77± 2	94± 3		80± 6
THE				
1 mg/ml	136± 6	145± 4	129± 3	129± 3
2 mg/ml	81± 3	103± 4	125± 3	87± 6
Strychnine				
myoclonus				
.05 mg/ml	59± 5	73± 3	72± 5	76± 5
.1 mg/ml	42± 2	47± 4	48± 3	53± 2
RB clonus				
.05 mg/ml	66± 5	86± 4	90± 3	96± 5
.1 mg/ml	51± 3	52± 2	53± 3	60± 2
THE				
.05 mg/ml	90± 4	111± 4	106± 2	112± 6
.1 mg/ml	60± 2	63± 3	62± 3	67± 3
Kainic acid				
WR clonus				
5 mg/ml	81± 7	98± 5	97± 7	125± 8
10 mg/ml	62± 5	65± 2	83± 5	91± 7
THE				
5 mg/ml	95± 9	117± 7	108± 8	137± 11
10 mg/ml	68± 6	71± 2	89± 5	100± 7

Number of subjects can be found in Tables 8A and 8B.

TABLE 9B

Mean, SE and n for latency for response to convulsant drugs for WSP and WSR mice of both replicates

DRUG CONCENTRATION SIGN	Replicate 1 WSP	Replicate 1 WSR	Replicate 2 WSP	Replicate 2 WSR
DMCM				
FF clonus				
.1 mg/ml	64± 5	75± 3	75± 6	86± 5
.2 mg/ml	38± 3	45± 10	51± 3	52± 3
TBPS				
myoclonus				
.01 mg/ml	156± 5	181± 8	168± 12	157± 9
.02 mg/ml	124± 5	145± 8	110± 4	117± 7
FF clonus				
.01 mg/ml	168± 5	201± 8	164± 9	159± 9
.02 mg/ml	135± 5	163± 10	115± 4	123± 7
Bicuculline				
myoclonus				
.03 mg/ml	95± 9	112± 6	119± 12	121± 4
.06 mg/ml	56± 2	56± 4	68± 4	64± 5
FF clonus				
.03 mg/ml	100± 9	129± 11	139± 9	125± 6
.06 mg/ml	60± 2	66± 5	66± 5	69± 5
RB clonus				
.03 mg/ml	261± 24	302± 32	282± 21	251± 28
.06 mg/ml	125± 10	142± 12	114± 12	153± 8
THE				
.03 mg/ml	266± 24	307± 32	285± 21	253± 29
.06 mg/ml	130± 10	147± 12	119± 13	155± 8
Pentylenetetrazol				
myoclonus				
2 mg/ml	97± 13	115± 2	128± 6	114± 7
4 mg/ml	40± 3	52± 4	51± 4	56± 6
5 mg/ml	33± 2	41± 3	44± 4	41± 1
6 mg/ml	25± 2	33± 2	30± 3	31± 2
10 mg/ml	19± 1	22± 1	21± 1	20± 1
FF clonus				
2 mg/ml	113± 15	151± 11	147± 12	126± 9
4 mg/ml	59± 5	64± 4	56± 5	68± 5
5 mg/ml	38± 3	44± 3	54± 4	44± 2
6 mg/ml	27± 2	37± 1	35± 3	34± 1
10 mg/ml	22± 1	23± 2	24± 1	23± 1

Number of subjects can be found in Tables 8A and 8B.

FIGURE 4: Sensitivity to pentylenetetrazol-induced myoclonus in WSP and WSR mice. Latency to and ED for myoclonus were measured in both replicates at five concentrations of pentylenetetrazol. For discussion, see text. These data are also tabulated, with number of subjects included, in Tables 8B and 9B.

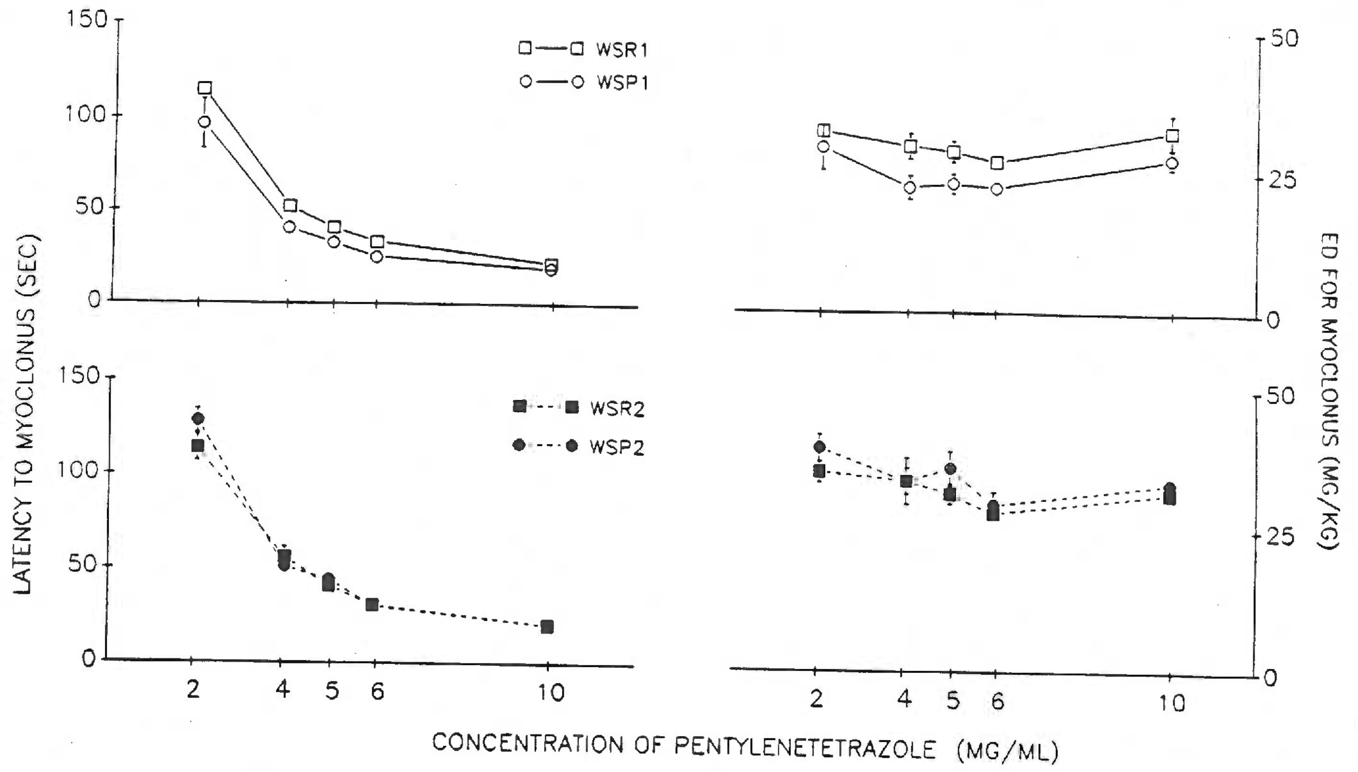
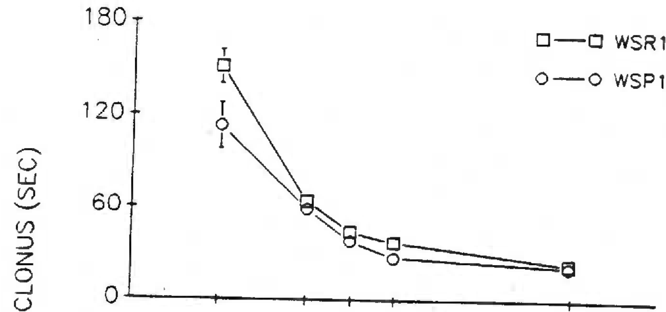
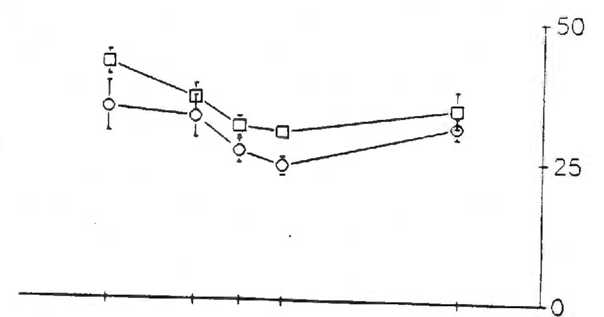

150
100
50
0
WSR1
WSP1
50
25
0
ED FOR MYOCLONUS (MG/KG)
LATENCY TO MYOCLONUS (SEC)
150
100
50
0
WSR2
WSP2
50
25
0
2
4
5
6
10
2
4
5
6
10
CONCENTRATION OF PENTYLENETETRAZOLE (MG/ML)

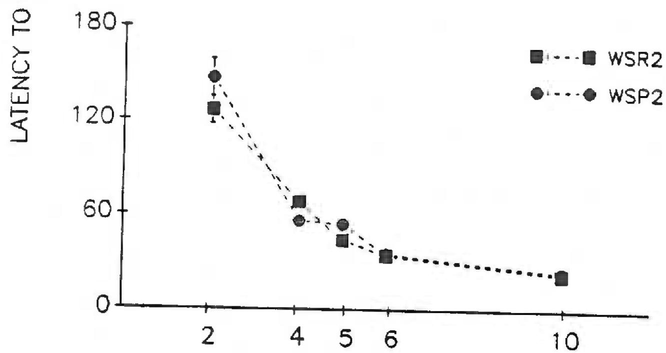
FIGURE 5: Sensitivity to pentylenetetrazol-induced face and forelimb clonus in WSP and WSR mice. Latency to and ED for face and forelimb clonus were measured in both replicates at five concentrations of pentylenetetrazol. For discussion, see text. These data are also tabulated, with number of subjects included, in Tables 8B and 9B.



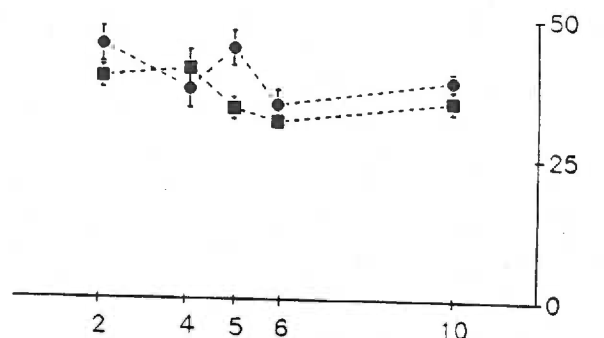
180
120
60
0
WSR1
WSP1



50
25
0



180
120
60
0
WSR2
WSP2
2
4
5
6
10



50
25
0
2
4
5
6
10

LATENCY TO FF CLONUS (SEC)
ED FOR FF CLONUS (MG/KG)
CONCENTRATION OF PENTYLENETETRAZOL (MG/ML)

At the lowest concentrations, other factors serve to raise the effective dose artificially, including drug distribution and metabolism, activation of compensatory systems, such as endogenous anticonvulsant systems (increased release of GABA, etc.), and volume effects - the effective volume of distribution can be considerably enlarged during a long infusion. This is reflected in an elevation of ED at 2 mg/ml relative to 5 mg/ml ($p < .09$) for latency to myoclonus, and at 2mg/ml relative to 5 mg/ml ($p < .005$) and 6 mg/ml ($p < .07$) for writhe. ED at 4, 5 and 6 mg/ml were very similar despite 20-30 second differences in latency. The dose-response curve for timed infusions was, therefore, an inverted U-shape, with a middle range in which concentration did not strongly influence effective dose. In theory, a similar concentration-neutral range should exist for each drug, and I attempted, through pilot testing, to find concentrations in this range for all drugs. Difficulties occasionally arose because infusions longer than 2 or 3 minutes were in some cases impossible to maintain, especially if the drug caused excessive excitation and movement in the animal.

III.C.4.b. *Pentylenetetrazol: Statistical analysis.*

The two replicate pairs of WSP and WSR lines were tested at different times of year, in different generations of the lines, and in a different room and building. However, responsiveness to pentylenetetrazol appears to be very stable, with similar values found at different times of day, in different studies, and in different laboratories. Additionally, the between-replicate comparison is the one most likely to be affected by the difference in time, and this is the least interesting comparison. For these reasons, data were analyzed together for the two replicates.

Latency and ED for myoclonus may be found in Tables 8A and 9A. For latency, the main effect of Line approached significance ($F(1, 145) = 3.62$, $p < 0.06$), Replicate was significant ($F(1, 145) = 9.64$, $p < 0.002$) and Concentration was significant ($F(4, 145) = 296$, $p < .0001$). The Line x Replicate interaction was also

significant ($F(1,145) = 10.3$, $p < 0.002$), and was analyzed independently using a two-way ANOVA (Line x Concentration) for each replicate. WSP1 mice were significantly more sensitive to pentylenetetrazol than WSR1 mice ($F(1,72) = 33.6$, $p < .0001$). There was no significant difference between WSP2 and WSR2 mice. For ED for myoclonus, significant main effects of Replicate ($F(1,145) = 35.5$, $p < 0.0001$) and Concentration ($F(4,145) = 5.94$, $p < 0.0002$) were found, as well as a Line x Replicate interaction ($F(1,145) = 14.6$, $p < 0.0002$). Two-way ANOVA's done independently for each replicate showed that WSP1 mice were significantly more sensitive to pentylenetetrazol than WSR1 mice ($F(1,72) = 14.1$, $p < .0004$). WSR2 mice tended to be more sensitive than WSP2 mice ($F(1,73) = 2.79$, $p < .10$).

For latency to face and forelimb clonus, the main effect of Line tended towards significance ($F(1,145) = 3.34$, $p < 0.07$). The main effect of concentration was significant ($F(4,145) = 329$, $p < 0.0001$), and significant two-way (Line x Replicate: $F(1,145) = 13.9$, $p < 0.0003$) and three-way (Line x Replicate x Concentration: $F(4,145) = 6.65$, $p < 0.0001$) interactions were present. The three-way interaction was tested by two-way ANOVA's independently for each replicate. WSP1 mice were more sensitive than WSR1 mice ($F(1,69) = 31.3$, $p < .0001$). There was no significant Line effect in replicate 2. For both replicates, a Line x Concentration interaction was present. T-tests were used to compare the lines at each concentration independently for each replicate. WSP1 mice were more sensitive than WSR1 mice at 6 mg/ml ($t(16) = 4.1$, $p < .001$). However, WSR2 mice were more sensitive than WSP2 mice at 5 mg/ml ($t(14) = 2.52$, $p < .02$). For ED for face and forelimb clonus, significant main effects of Replicate ($F(1,145) = 25.3$, $p < 0.0001$) and Concentration ($F(4,145) = 8.80$, $p < 0.0001$), and a Line x Replicate interaction ($F(1,145) = 15.6$, $p < 0.0001$) were present. Two-way ANOVA's (Line x Concentration) showed that WSP1 mice were significantly more sensitive to pentylenetetrazol than WSR1 mice ($F(1, 69) = 10.1$, $p < .002$). WSR2 mice were

more sensitive to pentylenetetrazol than WSP2 mice as assessed by ED for face and forelimb clonus ($F(1,73) = 5.86$, $p < .02$).

In summary, for measures of latency to seizure, WSP mice appeared to be more sensitive to pentylenetetrazol than WSR mice, reflected in the trend toward a significant main effect of Line. The difference was significant in replicate 1 only. However, for ED, WSR2 mice appear to be more sensitive than WSP2 mice. Thus, the evidence appears to be against a genetic correlation between pentylenetetrazol sensitivity and EtOH withdrawal severity.

III.C.4.c. *DMCM.*

Two convulsion signs were measured for DMCM: myoclonus and face and forelimb clonus. WSP mice tended to be more sensitive than WSR mice in latency to myoclonus ($F(1,59) = 3.39$, $p < 0.07$). For ED, the main effect of Line was not significant, but a Line x Replicate interaction was found ($F(1,59) = 6.12$, $p < 0.02$). Subsequent analysis with a 2-way ANOVA for each replicate revealed that WSP1 mice were more sensitive to DMCM than WSR1 mice ($F(1,28) = 6.45$, $p < 0.02$), while WSP2 and WSR2 mice did not differ.

WSP mice were more sensitive to DMCM than WSR mice in latency to face and forelimb clonus ($F(1,59) = 6.13$, $p < 0.02$, main effect of Line). In ED, the main effect of Line tended to be significant ($F(1,59) = 3.18$, $p < .08$), and the Line x Replicate interaction was significant ($F(1,59) = 4.92$, $p < 0.03$). Subsequent analysis with a 2-way ANOVA for each replicate revealed that WSP1 mice were more sensitive to DMCM than WSR1 mice ($F(1,31) = 6.64$, $p < 0.02$), while WSP2 and WSR2 mice did not differ.

These results are consistent with a correlated response to selection, although a weak one. WSP1 mice in particular appeared to be more sensitive than WSR1 mice, while no difference between WSP2 and WSR2 mice was seen.

III.C.4.d. *TBPS.*

Two convulsion signs were measured for TBPS: myoclonus and face and forelimb clonus. For latency to myoclonus, no significant line effect was observed. A significant Line x Replicate interaction was present ($F(1,51) = 4.971$, $p<0.03$). A 2-way ANOVA for each replicate showed that WSP1 mice were significantly more sensitive than WSR1 mice ($F(1,23) = 11.1$, $p<0.003$), while WSR2 and WSP2 mice did not differ. ED for myoclonus showed no Line effect, and a trend toward a Line x Replicate interaction ($F(1,51) = 3.01$, $p<.09$)

For latency to face and forelimb clonus, a significant main effect of Line (WSP<WSR: $F(1,56) = 9.01$, $p<.004$) and a Line x Replicate interaction ($F(1,56) = 7.37$, $p<0.009$) were present. A 2-way ANOVA for each replicate showed that WSP1 mice were significantly more sensitive than WSR1 mice ($F(1,28) = 24.8$, $p<0.0003$), while WSR2 and WSP2 mice did not differ ($F(1,28) = .038$). For ED for face and forelimb clonus, the Line effect was not significant, but a Line x Replicate interaction ($F(1,56) = 5.42$, $p<0.02$) was found, due to significantly greater sensitivity to TBPS in WSP1 mice compared to WSR1 mice ($F(1,28)$, $p<0.006$), while WSP2 and WSR2 mice did not differ.

Overall, WSP1 mice appeared to be more sensitive to TBPS than WSR1 mice, but WSP2 and WSR2 mice did not differ in sensitivity to TBPS. The lack of a significant Line effect denotes this as a relatively weak correlated response to selection.

III.C.4.e. *Bicuculline.*

Four convulsion signs were recorded for bicuculline: myoclonus, face-and-forelimb clonus, clonus and THE. No significant main effects or interactions involving Line were found for the variables myoclonus, clonus or THE. A significant Line x Replicate x Concentration interaction was present for latency to face and forelimb clonus ($F(1,54) = 3.89$, $p<0.05$). A two-way ANOVA was

performed for each replicate, and showed that WSP1 mice were significantly more sensitive to bicuculline than WSR1 mice ($F(1,27) = 5.99$, $p < 0.02$), but WSP2 and WSR2 mice did not differ. For ED for face and forelimb clonus, a Line x Replicate interaction was significant ($F(1,54) = 4.99$, $p < 0.03$). However, two-way ANOVA's (Line x Concentration) performed for each replicate revealed that WSR2 mice were significantly more sensitive than WSP2 mice ($F(1,27) = 4.10$, $p < 0.05$), no significant interactions. WSP1 and WSR1 mice did not differ.

Overall, WSP and WSR mice did not differ markedly in response to bicuculline.

III.C.4.f. Picrotoxin.

Two convulsion signs were measured for picrotoxin: running/bouncing clonus and THE. WSP mice were significantly more sensitive to picrotoxin than WSR mice, as reflected by significant main effects of Line for latency to clonus ($F(1,56) = 29.3$, $p < 0.0001$) and ED for clonus ($F(1,56) = 4.40$, $p < 0.04$), and no interactions.

For THE, the main effect of Line was significant for latency ($F(1,53) = 36.5$, $p < .0001$) and ED ($F(1,53) = 8.56$, $p < .005$). In addition, a significant 3-way interaction (Line x Replicate x Concentration) was seen for both measures (Latency: $F(1,53) = 3.86$, $p < .05$; ED: $F(1,53) = 4.12$, $p < .05$). Therefore, these measures were further assessed using 2-way ANOVAs (Line x Replicate) at each concentration. For latency to THE, WSP mice were more sensitive than WSR mice at both concentrations (significant effect of Line, no interactions: 0.75 mg/ml: $F(1,25) = 11.6$, $p < .002$; 1.5 mg/ml: $F(1,28) = 36.6$, $p < .0001$). The 3-way interaction arose because at 0.75 mg/ml, replicate 1 = replicate 2, while at 1.5 mg/ml, replicate 2 was more sensitive than replicate 1 ($F(1,28) = 5.96$, $p < 0.02$). For ED for THE, at the lower dose, 0.75 mg/ml, WSP mice of both replicates tended to be more sensitive than WSR mice ($F(1,25) = 8.53$, $p < 0.06$, no

interactions), but at the higher dose, a significant Line x Replicate interaction occurred ($F(1,28) = 6.30$, $p < .02$). Lines were compared independently for each replicate using a t-test, which revealed that WSP1 mice were more sensitive than WSR1 mice ($t(14) = 2.83$, $p < .01$), but WSP2 and WSR2 mice were equally sensitive.

In summary, the data supported a strong genetic correlation between sensitivity to picrotoxin and EtOH withdrawal severity, with a slightly stronger effect present in replicate 1 than in replicate 2.

III.C.4.g. *Strychnine.*

Three convulsion signs were measured for strychnine: myoclonus, clonus and THE. For latency to myoclonus, a significant effect of Line was seen ($F(1,60) = 7.35$, $p < 0.009$), and no interactions. No main effect or interaction involving Line was present for ED for myoclonus.

For latency to clonus, a significant effect of Line ($F(1,75) = 12.78$, $p < .0006$), and a Line x Replicate x Concentration interaction ($F(1,75) = 5.13$, $p < 0.03$) were seen. The three-way interaction was analyzed using two-way ANOVAs independently for each replicate, and each concentration. WSP1 mice were more sensitive than WSR1 mice ($F(1,38) = 8.61$, $p < 0.006$), and WSP2 mice tended to be more sensitive than WSR2 mice ($F(1,37) = 3.65$, $p < 0.06$). WSP mice were more sensitive than WSR mice at 0.05 mg/ml ($F(1,36) = 9.52$, $p < 0.004$), but not at 0.1 mg/ml. For ED for clonus, no main effect of Line, but a trend toward a Line x Replicate interaction ($F(1,75) = 3.58$, $p < .06$) was present.

For latency to THE, the main effect of Line ($F(1,75) = 11.82$, $p < .001$), and a Line x Concentration interaction ($F(1,75) = 4.18$, $p < 0.04$) were present. The 2-way interaction was evaluated using 2-way ANOVA's (Line x Replicate) at each concentration. A significant effect of Line (WSP more sensitive than WSR) was found at 0.05 mg/ml ($F(1,36) = 9.98$, $p < 0.003$) but not at 0.1 mg/ml. For ED, no

significant effect of line was seen, but a significant Line x Replicate interaction was present ($F(1,75) = 8.06$, $p<.006$). WSP2 and WSR2 mice did not differ. Two-way ANOVA's (Line x Concentration) done independently for each replicate showed that WSP1 mice were significantly more sensitive than WSR1 mice ($F(1,38) = .03$, $p<0.03$), and WSP2 mice tended to be more sensitive than WSR2 mice ($F(1,37) = 3.37$, $p<0.07$).

WSP mice appeared to be moderately more sensitive to strychnine than WSR mice, but in most cases this was limited to replicate 1.

III.C.4.h. *4-Aminopyridine.*

Two convulsion signs were measured for 4-AP: clonus and THE. For latency to clonus, a significant effect of Line was present (WSP more sensitive than WSR: $F(1,66) = 8.34$, $p<0.005$). For ED, the main effect of Line ($F(1,66) = 11.61$, $p<.001$), the Line x Replicate interaction ($F(1,66) = 5.42$, $p<.02$) and Line x Concentration interaction ($F(1,66) = 4.84$, $p<.03$) were significant. The Line x Replicate interaction was assessed using 2-way ANOVA's (Line x Concentration) for each replicate, which showed that WSP1 mice were more sensitive than WSR1 mice ($F(1,37) = 17.58$, $p<0.0002$) but WSP2 and WSR2 mice did not differ. The Line x Concentration interaction was assessed using 2-way ANOVA's for each concentration, which showed a significant main effect of Line at 2 mg/ml ($F(1,34) = 12.37$, $p<0.001$), and an significant Line x Replicate interaction ($F(1,34) = 4.18$, $p<.05$). The interaction was tested using a t-test, and it was found that WSP1 mice were significantly more sensitive than WSR1 mice ($t(19) = 4.17$, $p<.0005$), but WSP2 mice equalled WSR2 mice. No significant effect of Line or interaction was present at 1 mg/ml.

For latency to THE, a significant effect of Line ($F(1,66) = 12.6$, $p<.0007$) and a Line x Concentration interaction ($F(1,66) = 4.46$, $p<0.04$) were present. This interaction was tested using a 2-way ANOVA (Line x Replicate) for each

concentration. WSP mice were more sensitive than WSR mice at 2 mg/ml ($F(1,34) = 16.3$, $p < 0.0003$, main effect of Line) but not at 1 mg/ml.

For ED for THE, a significant main effect of Line ($F(1,66) = 13.5$, p,.0005), and Line x Replicate ($F(1,66) = 5.71$, $p < 0.02$) and Line x Concentration ($F(1,66) = 5.87$, $p < 0.02$) interactions were present for THE. Two-way ANOVA's for each replicate (Line x Concentration) and each concentration (Line x Replicate) revealed further interactions. Therefore, the data were further analyzed by t-tests For replicate 1, WSP mice were significantly more sensitive than WSR mice at 2 mg/ml ($t(19) = 3.99$, $p < .0008$) and tended to be more sensitive at 1 mg/ml ($t(18) = 2.04$, $p < .06$). WSP2 and WSR2 did not differ at either concentration.

In general, WSP mice were more sensitive to 4-AP than WSR mice. A strong genetic correlation between sensitivity to 4-aminopyridine and EtOH withdrawal was indicated.

III.C.4.i. *CHEB.*

The convulsion signs measured for CHEB were myoclonus, running/bouncing clonus and THE. A significant effect of Line (WSP more sensitive than WSR) was found in latency to myoclonus ($F(1,55) = 7.47$, $p < 0.008$), and effective dose for myoclonus showed a trend in that direction ($F(1,55) = 3.476$, $p < 0.07$). No significant interactions were present for either measure.

A significant effect of Line (WSP more sensitive than WSR) was found in latency to running/bouncing clonus ($F(1,60) = 20.69$, $p < 0.0001$) and effective dose ($F(1,60) = 7.364$, $p < 0.009$). No significant interaction of Line was present.

A significant Line effect was present for THE latency ($F(1,54) = 9.44$, $p < ..003$) but not for ED. No significant interactions were present.

Concentration had a strong, significant effect on latency, specifically longer latencies for the lower concentrations (myoclonus: $F(1,55) = 142$, $p < 0.0001$; clonus: $F(1,60) = 352$, $p < 0.0001$; THE: $F(1,54) = 230$, $p < 0.0001$). Concentration

had a weaker effect on ED, with a significant effect on clonus and THE, but not myoclonus (myoclonus: $F(1,55) = 3.34$, $p<0.07$; clonus: $F(1,60) = 6.79$, $p<0.01$; THE: $F(1,54) = 12.7$, $p<0.0008$). The difference between EDs for the two concentrations is relatively small, compared to the difference in latencies, suggesting that these two doses were close to the concentration-neutral point of the dose response curve.

In general, WSP mice were more sensitive to CHEB than WSR mice. A strong genetic correlation between sensitivity to CHEB and EtOH withdrawal was indicated.

III.C.4.j. *Kainic acid.*

Two convulsion signs were measured for kainic acid: clonus and tonus. For latency to clonus, a significant main effect of Line (WSP<WSR, $F(1,65) = 11.1$, $p<.001$) and a Line x Concentration interaction ($F(1,65) = 3.88$, $p<0.05$) were seen. This was assessed using a 2-way ANOVA for each concentration. WSP mice were more sensitive than WSR mice at 5 mg/ml ($F(1,29) = 10.5$, $p<0.003$), but not at 10 mg/ml. WSP mice and WSR mice did not differ in ED for clonus.

For latency to THE, WSP mice were significantly more sensitive than WSR mice ($F(1,65) = 10.3$, $p<0.002$). For ED for THE, WSR and WSP mice did not differ.

In summary, latency measures indicated a difference between WSP and WSR mice in responsiveness to kainic acid, but ED variables did not. Overall, the data supported a moderate correlation between sensitivity to kainic acid and EtOH withdrawal.

IV. DISCUSSION.

The first hypothesis, that the excitability seen during EtOH withdrawal might be similar to that seen after acute convulsant treatments, was not supported by data from the inbred mouse strains. No correlation between sensitivity to the various

convulsant drugs and ethanol withdrawal severity was seen. The strain means for drug sensitivity variables and for EtOH withdrawal severity appeared to reflect a broad range of responses. However, using only 8-10 observations, the probability of detecting a weak correlation is very low. Thus, it is possible that a genetic correlation could be detected using a larger number of strains.

For WSP and WSR lines, reasonably strong evidence was found for a genetic correlation between EtOH withdrawal and sensitivity to three convulsant drugs, CHEB, 4-aminopyridine and picrotoxin. Moderate evidence was found for a genetic correlation between EtOH withdrawal and sensitivity to two more convulsant drugs; strychnine and kainic acid. For the other four drugs, WSP1 mice were more sensitive than WSR1 mice, but WSP2 were equally or less sensitive than WSR2 mice. These results differ from those reported by McSwigan *et al.* (1984), who found no difference in sensitivity to convulsant drugs in WSP and WSR mice. This may be a function of the method used in each study (timed infusion as compared to ED_{50}). Alternatively, it could be because the mice used in the earlier test had only been selected for 8-10 generations, while the mice used in the present studies were from the 26th and 27th selected generation. The withdrawal severity difference in WSP and WSR mice has not become any larger since the 11th selected generation, but this does not necessarily indicate that selection has ceased. Between the studies of McSwigan *et al.* and the present studies, selection may have acted on genes which had a relatively small effect on the withdrawal severity phenotype, but a significant sensitivity to convulsant drugs.

The different pattern of results seen in replicate 1 versus replicate 2 of the WSP and WSR selection could be interpreted a number of ways. Given the multivariate nature of EtOH withdrawal, the selected genes responsible for the dissimilarity in EtOH withdrawal in the two replicates are likely to be somewhat distinct. Thus, the genes which make a WSP1 mouse different from a WSR1 mouse

may also promote the general difference in convulsant sensitivity seen in this replicate. The genes which make a WSP2 mouse different from a WSR2 mouse may, instead, promote the specific differences in sensitivity to only certain convulsant drugs seen in this replicate.

Alternatively, the difference in sensitivity to all convulsant drugs seen in replicate 1 may reflect selection for general sensitivity to any treatment that affects CNS hyperexcitability, EtOH included. If this is the case, replicate 2, which displays selective differences to convulsant treatments, becomes the more useful model for exploring the relationship between convulsant sensitivity and EtOH withdrawal.

Another factor which could cause a non-parallel response in the two replicates is the different gene pools from which each replicate was selected. This could happen in two ways: First, different genes were available for selection to act upon. Although the replicate sets of lines were selected from a common genetically heterogeneous (HS) mouse stock, only nine mating pairs were chosen to initiate the selected lines. These mice necessarily retained only a portion of the total genetic variability in HS mice. Second, the genetic background of each replicate could differentially influence expression of correlated responses. For instance, the general difference between WSP1 and WSR1 mice in response to all convulsant drugs tested makes it difficult to assess their relative sensitivity to any specific drug.

For all drugs tested, the differences in drug sensitivity between WSP and WSR mice of both replicates was very small relative to the available genetic variation as expressed in the inbred strains. The inbred strains tested included 6 of the 8 parent strains used in founding HS mice. Since WSP and WSR mice were selected from the HS stock, it is likely that the genetic potential for larger variations in convulsant sensitivity in WSP and WSR mice was present. The differences in EtOH withdrawal between WSP and WSR mice were very large, generally exceeding the range of variability expressed in inbred mouse strains. This suggests

that the genetic correlation between sensitivity to convulsant drugs and EtOH withdrawal severity is not large. The differences between WSP and WSR mice in sensitivity to some convulsants were clearly genetic, but the genes involved were not necessarily those also influencing EtOH withdrawal

In sum, the results using inbred strains and WSP and WSR lines were consistent in failing to support a strong correlation between EtOH withdrawal severity and sensitivity to one or more convulsant drugs. If the results using WSP and WSR mice are considered in terms of the variables identified using inbred strains, a somewhat consistent pattern was seen. The convulsant sensitivity variables to which both WSP mouse lines were more sensitive than both WSR mouse lines included most of the Group 2 variables (characterized by running/bouncing clonus), but also included those variables that did not correlate with either Group 1 or Group 2 [Picrotoxin (THE), 4-aminopyridine (THE) and kainic acid (WR clonus and THE)]. In general, WSP1 mice were more sensitive than WSR1 mice to Group 1 variables (characterized by myoclonus and face and forelimb clonus). WSP2 and WSR2 mice were equally sensitive on these measures. Note that the results for bicuculline were somewhat inconsistent with this interpretation. To be consistent, WSP2 mice should have been more sensitive than WSR2 mice to bicuculline-induced running/bouncing clonus and THE.

In the inbred strains, drug sensitivities expressed as ED or latency were highly correlated. Furthermore, use of one or the other unit did not substantially alter the interpretation of correlations between sensitivity to different drugs, or between drug sensitivity and EtOH withdrawal severity. For the WSP and WSR mice, latency and ED did not always agree. This may have occurred because among inbred strains, differences in convulsant drug sensitivity were large while differences in bodyweight were, for the most part, small. For WSP and WSR mice, however, differences in bodyweight were relatively large, while differences in convulsant

sensitivity were small. Also, individual variability within-group was largely eliminated as factor in the inbred strains, because strain mean values were used in the correlations. In the view of this investigator, it is not possible to decide, *a priori*, that ED or latency is necessarily the better measure. The answer to this question depends largely on pharmacokinetic factors influencing the accumulation of drug in brain. Experiments are currently underway to determine brain concentrations of pentylenetetrazol at time of convulsion in the battery of inbred strains and in the WSP and WSR lines. With these data, it will be possible to better assess the relative merits of latency and ED as indices of neural sensitivity to pentylenetetrazol.

In these experiments, brain concentrations of drugs were not measured. The results did not support a major contribution for distributional factors. If, for instance, the proportion of cardiac output which was shunted to brain varied systematically in the inbred strains, and constituted a major determinant of convulsant sensitivity, it should have affected the response of the strains to all drugs to some degree, and thus, the rank order of strain sensitivity to all drugs would have been similar. Nonetheless, some distributional factor may systematically influence the seizure sensitivity variables forming one or both of the two clusters identified. The pattern of convulsant sensitivity in replicate 1 of the WSP and WSR mice is consistent with a potential general difference in access of drug to the CNS.

The second hypothesis addressed in these experiments was that strain sensitivity to convulsant drugs might show a pattern of genetic correlation consistent with drug sites of action. Inbred mouse strains were found to display significant heterogeneity in responsiveness to nine convulsant drugs. In other words, inbred strains were not consistently sensitive or insensitive to convulsants, but showed drug-specific sensitivity. Thus, genetic differences modified sensitivity to convulsant drugs. For some drugs, sensitivity of each strain depended upon which convulsant sign was used as an index. These results suggested that different genes, and

potentially, different neurophysiological processes, underlie sensitivity to different drugs and expression of different convulsant signs.

Evidence for genetic heterogeneity in sensitivity to different convulsant signs has been noted previously. Greer and Alpern (1977) treated mice with the convulsant drug flurothyl and found that SS mice had shorter latencies to myoclonus than LS, but longer latencies to clonus. Within each line, latencies to myoclonus and clonus did not correlate, supporting the idea that these two convulsant signs are mediated by different substrates. Sanders (1980) confirmed that SS mice were more sensitive to flurothyl-induced myoclonus than LS mice, but found the lines equally sensitive to clonus. Furthermore, different convulsant signs produced by the same drug show different patterns of sensitivity to anticonvulsant treatments. For instance, diazepam is effective against THE produced by pentylenetetrazol, but not against pentylenetetrazol-induced face and forelimb clonus (File *et al.*, 1984).

The source of genetic differences in sensitivity to convulsions elicited different drugs, or in sensitivity to particular convulsion types, could be in a number of processes. Production of convulsions is the result of a complex set of events. The drug must have access to the brain, and must initiate excitability. Endogenous inhibitory mechanisms must be overcome, and spread of the excitation into brain areas important in the propagation and expression of seizures must occur. Genetic differences in sensitivity to these drugs could be due to actions at one or several of these levels. There could be differences in pharmacokinetic factors such that drug accumulates in brain at different rates. There could be genetic variations in cellular responsiveness at the site at which a drug initiates excitability. Other potential sites of genetic variation include anatomical sites important for propagation of the seizure, and general inhibitory and excitatory processes.

When sensitivity of the strains to different convulsant drugs was correlated between drugs, two patterns of responsiveness were found, suggesting that there are

two predominant mechanisms determining sensitivity to convulsions in this set of drugs. The defining feature of the two groups was the type of convulsant sign the drug produced, rather than the drug itself. Group 1 included all drugs which produced myoclonus and face and forelimb clonus: DMCM, TBPS, bicuculline and pentylenetetrazol. Group 2 included drugs which produced running/bouncing clonus and THE: CHEB, picrotoxin, strychnine and bicuculline. Both categories of convulsant sign were measured in bicuculline, and it was found to be correlated with members of both groups. 4-aminopyridine (running/bouncing clonus) was correlated with one variable in each group.

It is possible that these different convulsions are based in different anatomical locations. Browning (1987) hypothesized that face and forelimb clonus was due to forebrain mechanisms. This type of seizure, and minimal electroshock seizures, have a very different pharmacological profile from other seizure types, being very sensitive to ethosuximide (which is not effective in other types of seizure), and insensitive to a variety of other anticonvulsants. This type of seizure has been used as a model of human absence attacks (Woodbury, 1972). Face and forelimb clonus cannot be produced if electrical stimulation is applied through earclip electrodes rather than ocular electrodes (Browning and Nelson, 1985), further implicating structures above the midbrain in these convulsions.

Clonic seizures of the explosive, or running-bouncing type, and tonic seizures, are thought to reflect brainstem and spinal cord mechanisms (Burnham and Browning, 1987). Esplin and Freston (1960) demonstrated that clonic and tonic seizures could be driven by direct electrical stimulation of the spinal cord, and that the form of seizure seen was directly related to the intensity of stimulation. As stimulation was increased, clonus developed into tonus. Seizures of this form are seen in a variety of animal models of epilepsy, and share a pharmacological profile with human tonic-clonic seizures (Burnham and Browning, 1987). However, Freund

et al. (1986) found that sensitivity to THE elicited by bicuculline was genetically correlated with sensitivity of hippocampal slices to bicuculline. Thus, genetic differences in sensitivity might be the result of alterations at the hippocampus. The principal site important for expression of a particular convulsion, and the site of genetic variation in sensitivity to a drug which elicits that convulsion, may not be the same site.

4-aminopyridine (THE), picrotoxin (THE) and kainic acid (wild running clonus) did not correlate with any other variables. Kainic acid appears to act as a convulsant through a very different mechanism than other convulsant drugs studied. The limbic system, particularly the hippocampus and the lateral septum, are implicated in the initial convulsant actions of kainic acid (Ben-Ari *et al.*, 1981). It is not clear why sensitivity to picrotoxin (THE) and 4-aminopyridine (THE) did not correlate with sensitivity to other drugs which cause THE. The within-drug correlations between running/bouncing clonus and THE for 4-aminopyridine and picrotoxin were not as large as those seen for bicuculline, CHEB or strychnine. Perhaps a single mechanism is responsible for the appearance of convulsions after bicuculline, CHEB and strychnine, while 4-aminopyridine and picrotoxin have two or more actions that influence convulsions. The expression of running/bouncing clonus could be due to a single mechanism in all five of these drugs, while expression of THE is altered by some independent effect of picrotoxin, and of 4-aminopyridine.

Drugs in Group 1 invariably produced myoclonus as well as face and forelimb clonus, but myoclonus was also produced by the Group 2 drugs CHEB and strychnine. Myoclonus in humans is defined as involuntary muscle jerks of central origin (Pranzatella and Snodgrass, 1985). Yet, there is clearly heterogeneity in myoclonic disorders in terms of etiology, anatomy, pathology and physiology. It occurs occasionally in almost all normal individuals under restricted circumstances

such as falling asleep (Coleman *et al.*, 1980), and pathologically in some individuals. In its pathologic form, it is most prevalent in association with idiopathic generalized epilepsy. Its appearance in epileptic patients corresponds to increases in potential for convulsions, appearing prior to convulsions or as a convulsive feature. Brainstem, spinal cord and cortex are implicated (Marsden *et al.*, 1982). Animal models do not exactly duplicate the human disorder, but the diversity of myoclonic phenomenon seen in animals reflects the varied human myoclonic syndrome. In general, experimental myoclonus is caused by various neurotransmitter manipulations and lesions, and frequently is found to precede convulsions. It appears likely that myoclonus observed in these experiments was a heterogeneous phenomenon. When observed preceding face and forelimb clonus, it may have been due to processes underlying face and forelimb clonus, and when it was observed preceding running/bouncing clonus, it may have been due to processes underlying running/bouncing clonus.

The three drugs which act at the picrotoxin receptor (TBPS, pentylenetetrazol and picrotoxin) did not consistently correlate with each other, or with drugs from either group. Similarly, the five drugs which act at the GABA complex (DMCM, bicuculline, TBPS, pentylenetetrazol and picrotoxin) correlated with drugs from both groups. Group membership was therefore not consistent with the hypothesized sites at which a drug initiates convulsions. Rather, it appeared that the type of convulsion is the determinant of inclusion in one or the other group.

The results of correlations with HIC variables were quite interesting. It appeared that basal (i.e. post-saline) severity of HIC may be genetically related to Group 1 convulsion signs, which may be based in forebrain mechanisms. Thus, forebrain mechanisms might have a modulating role in this reflex, accounting for the apparent genetic correlation between basal HIC and Group 1 convulsions.

HIC scores are elevated when mice are treated for three days with pyrazole. Sensitivity of different inbred strains to this enhancement is known to differ (Crabbe *et al.*, 1980, 1981, 1983c). In this experiment, significant correlations between sensitivity to various convulsant drugs and HIC after pyrazole administration were found. Severity of HIC after pyrazole treatment correlated with variables in both groups. This implies that genes affecting sensitivity to HIC after pyrazole treatment also influence sensitivity to a broad range of convulsant treatments.

Mice treated with saline only rarely show HIC scores of 2, and almost never show scores above 2 (see Table 2). Mice treated with pyrazole may show occasional scores above 2, but mostly, the elevation of HIC score consists of more frequent scores of 1 or 2. Only after more extreme treatments, with EtOH or convulsant drugs, are the scores above 2 seen: these do not require a spin to elicit the convulsion. It is possible that these different scores reflect different mechanisms rather than a single mechanism. It this were the case, the different patterns of genetic correlation of drug sensitivity variables with saline-, pyrazole- and EtOH withdrawal-stimulated HIC could reflect different mechanisms underlying the predominant type of HIC which is scored under these conditions. An explanation along these lines would have difficulty explaining the difference between saline- and pyrazole- stimulated patterns of correlations, but could explain the loss of significant correlation when the higher HIC scores characterizing EtOH withdrawal were assessed.

The results of Seale and coworkers, discussed in Section II.D.2., were not consistent with the present findings. Seale *et al.* (1987) demonstrated a significant difference in sensitivity to DMCM between CBA and SWR mice, while in the present experiments, CBA and SWR mice did not differ in response to DMCM. They found that these two strains were equally responsive to strychnine and picrotoxin, similar to the result found here. The discrepancy may reflect the

differences in method used to assess convulsant sensitivity (ip administration vs timed infusion), or in the different endpoints used. Seale *et al.* used tonic seizures and death as their endpoints for measuring sensitivity to all drugs, while in the present experiments, only myoclonus and face and forelimb clonus were recorded for DMCM.

Freund *et al.* (1987) studied the response of 3 inbred strains to bicuculline. This study was also discussed in section II.D.2. Bicuculline was administered by ip injection, and latency to seizure measured. The endpoints timed were clonus and THE. From the description given by these investigators, the clonus they measured was probably the equivalent of face and forelimb clonus measured in the present experiments. They found that for clonus, order of strain sensitivity (least to most) was C57 < DBA = C3H. In the present experiments, a similar result was seen: For face and forelimb clonus, ED for face and forelimb clonus after bicuculline for the strains was .712 ± .016(C57), .481 ± .015 (DBA) and .515 ± .030(C3H). For THE, Freund reported a different pattern, C57 = DBA < C3H. In the present experiments, ED for THE after bicuculline for these three strains was 1.29 ± .08(C57), 1.17 ± .07(DBA), and .90 ± .05(C3H). Thus, the results in the present studies appear to be consistent with some of the previous work in this area.

V. CONCLUSIONS

Studies of the effects of nine convulsant drugs on inbred mouse strains revealed two very interesting points. First, within a drug, different convulsant signs were controlled by different genes. An inbred strain that was very sensitive to a drug-induced myoclonus was not necessarily sensitive to tonic hindlimb extensor seizures elicited by that drug. Thus, independent mechanisms appear to regulate the different convulsant signs. On the other hand, some signs elicited by a drug were highly intercorrelated, and appeared to reflect the same mechanisms. These correlated signs tended to be temporally and sequentially proximate.

Second, the type of convulsant sign produced by a particular drug seemed to be the primary determinant of strain sensitivity. Thus, a strain that was sensitive (had a short latency to or low effective dose for sign) to one drug that induced face and forelimb clonus was sensitive to all drugs that induced face and forelimb clonus. Two categories of convulsant sign were identified, one characterized by face and forelimb clonus (Group 1), and one characterized by running/bouncing clonus and THE (Group 2). Group 1 convulsions may be mediated by forebrain mechanisms, while Group 2 may be based in the brainstem and reticular formation.

Severity of handling-induced convulsions in inbred mice treated with saline for three days correlated positively with sensitivity to Group 1 drugs. This suggests that HIC and face and forelimb clonus share a common mechanism of action.

Severity of HIC in inbred mice treated with pyrazole for three days correlated positively with sensitivity to some drugs in both groups. This suggests that the pyrazole-induced increase in sensitivity to HIC is mediated by mechanisms different from the those underlying either Group 1 or Group 2 variables.

Severity of HIC in inbred mice withdrawn after having been treated with ethanol for three days did not correlate with sensitivity to any convulsant drug. Thus, the genes responsible for differences in sensitivity to convulsant drugs and those responsible for differences in ethanol withdrawal do not appear to be the same.

The response of WSP and WSR selected lines to nine convulsant drugs suggested that sensitivity to three of the drugs (picrotoxin, 4-aminopyridine and CHEB) may be related to sensitivity to EtOH withdrawal. However, no evidence for a mechanism of action common to these three drugs was present, either with respect to Group 1/Group 2 distinction or in the convulsion-initiating mechanisms of each drug. If the drugs which appeared to be moderately related to EtOH

withdrawal severity are also considered (strychnine and kainic acid), it appears that sensitivity to Group 1 drugs is not genetically related to withdrawal.

WSP1 mice appeared to be generally more sensitive to convulsant drugs than WSR1 mice. This suggests that in selecting for EtOH withdrawal severity, we have also selected for general sensitivity to convulsions in this pair of lines. WSP2 and WSR2 mice show a drug specific pattern of sensitivity, and may be more useful for mechanism-oriented studies of sensitivity to convulsant drugs.

VI. REFERENCES

Adamson, J., and Budwiels, J.A. Sleep of dry alcoholics. *Archives of General Psychiatry* **28** (1973) 146-149.

Allan, A. and Harris, R.A. Involvement of neuronal chloride channels in ethanol intoxication, tolerance, and dependence. In: Galanter, M. (Ed.), *Recent Developments in Alcoholism,* Vol. 5. Plenum Press, 1987, 313-325.

Allan, A. and Harris, R.A. Anesthetic and convulsant barbiturates alter Gamma-aminobutyric acid-stimulated chloride flux across brain membranes. *Journal of Pharmacology and Experimental Therapeutics* **238** (1986) 763-768.

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

Andrews, P.R., Jones, G.P. and Lodge, D. Convulsant, anticonvulsant and anaesthetic barbiturates. 5-ethyl-5(3'-methyl-but-2'enyl)-barbituric acid and related compounds. *European Journal of Pharmacology* **55** (1979) 115-120.

Andrews, P.R., Jones, G.P. and Poulton, D.B. Convulsant, anticonvulsant and anaesthetic barbiturates. *European Journal of Pharmacology* **79** (1982) 61-65.

Anderson, V.E., Hauser, W.A. and Rich, S.S. Genetic heterogeneity in the epilepsies. In: A.V. Delgado-Escueta, A.A. Ward, D.M. Woodbury and R.J. Porter (Eds) *Advances in Neurology* **44:** *Basic mechanisms of the epilepsies, Molecular and cellular approaches.* New York, Raven Press, 1986. pp 59-76

Ballenger, J.C. and Post, R.M. Kindling as a model for alcohol withdrawal syndromes. *British Journal of Psychiatry* **133** (1978) 1-14.

Baker, T. and Cannon, D.S. Potentiation of ethanol withdrawal by prior dependence. *Psychopharmacology* **60** (1979) 105-110.

Barbaccia, M.L., Bosio, A. and Lucchi, L. Neuronal mechanisms regulating ethanol effects on the dopaminergic system. *Life Sciences* **30** (1982) 2163-2170.

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

Begleiter, H., Porjesz, B., Hihari, B. and Kissin, B. Event related brain potentials in boys at risk for alcoholism. *Science* **225** (1984) 1493-1495.

Belknap, J. Genetic factors in the effects of alcohol: Neurosensitivity, functional tolerance, and physical dependence. In: H. Rigter and J.C. Crabbe, (Eds.) *Alcohol Tolerance and Dependence*, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, 157-180.

Belknap, J.K., Danielson, P.W., Lame, M. and Crabbe, J.C. Ethanol and barbiturate withdrawal convulsions are extensively codetermined in mice. *Alcohol* **5** (1988) 167-171.

Belknap, J.K., Laursen, S.E. and Crabbe, J.C. Mice bred to be either prone or resistant to ethanol withdrawal convulsions show closely parallel vulnerabilities with diazepam. *Life Sciences*, in press.

Belknap, J.K., Laursen, S.E. and Crabbe, J.C. Ethanol and nitrous oxide produce withdrawal-induced convulsions by similar mechanisms in mice. *Life Sciences*, 41 (1987) 2033-2040.

Ben-Ari, Y., Tremblay, D., Ritchie, G., Ghilini, G. and Naquet, R. Electrographic, clinical and pathological alterations following systemic administration of kainic acid, bicuculline or pentetrazole: Metabolic mapping using the deoxyglucose method with special reference to the pathology of epilepsy. *Neuroscience* **6**(7) (1981) 1361-1391.

Ben-Ari, Y. Limbic seizure and brain damage syndrome produced by kainic acid; mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* **14** (1985) 375-401.

Benardo, L.S. and Pedley, T.A. Basic mechanisms of epileptic seizures. *Cleveland Clinic Quarterly* **51** (1984) 195-203.

Berger, M.L., Lefauconnier, J.-M., Tremblay, E. and Ben-Ari, Y. Limbic seizures induced by systemically applied kainic acid: How much kainic acid reaches the brain? In R. Schwarcz and Y. Ben-Ari, (Eds) *Advances in Experimental Biology and Medicine* **203**: *Excitatory amino acids and epilepsy.* New York, Plenum Press, 1986, 199-209.

Bierley, R.A., Cannon, D.S., Wehl, C.K. and Dustman, R.E. Effects of alcohol on visually evoked responses in rats during addiction and withdrawal. *Pharmacology, Biochemistry and Behavior* **12** (1979) 909-915.

Blaustein, M.P. and Ector, A.C. Barbiturate inhibition of calcium uptake by depolarized nerve terminals in vitro. *Molecular Pharmacology* **11** (1975) 369-378.

Blizard, D.A. and Bailey, D.W. Genetic correlations between open-field activity and defecation: Analysis with the CXB recombinant-inbred strains. *Behavior Genetics* 9(5) (1979) 349-357.

Boisse, N. and Okamoto, M. Ethanol as a sedative-hypnotic; comparison with barbiturates and non-barbiturate sedative-hypnotics. In: H. Rigter and J.C. Crabbe, (Eds.) *Alcohol Tolerance and Dependence*, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, pp 157-180.

Braestrup, C. Schmiechen, R., Neef, G., Nielson, M. and Petersen, E.N. Interaction of convulsive ligand with benzodiazepine receptors. *Science* **216** (1982) 1241-1243.

Branchey, M., Rauscher, G. and Kissin, B. Modifications in the response to alcohol following the establishment of physical dependence. *Psychopharmacology* **22** (1971) 314-322.

Brown, M.E., Anton, R.F., Malcom, R. and Ballenger, J.C. Alcohol detoxification and withdrawal seizures: Clinical support for a kindling hypothesis. *Biological Psychiatry* 23 (1988) 507-514.

Browning, R.A. Effect of lesions on seizures in experimental animals. In Fromm, H., Faingold, C.L., Browning, R.A. and Burnham, W.W. (Eds) *Epilepsy and the Reticular Formation: The role of the reticular core in convulsive seizures.* Alan R. Liss Co., New York, 1987, pp 137-162.

Browning, R.A. and Nelson, D.K. Variation in threshold and pattern of elicited seizures in rats dendent on site of stimulation. *Life Sciences* 37(23) (1985) 2205-2211.

Burnham, W.M. and Browning, R.A. The reticular core and generalized convulsions: A unified hypothesis. In Fromm, H., Faingold, C.L., Browning, R.A. and Burnham, W.W. (Eds) *Epilepsy and the Reticular Formation: The role of the reticular core in convulsive seizures.* Alan R. Liss Co., New York, 1987, pp 193-201.

Carey, G. Inference about genetic correlations. *Behavior Genetics* 18(3) (1988) 329-337.

Chance, M.R.A. The posture of a falling mouse. *British Journal of Animal Behavior* **1** (1953) 118-119.

Chweh, A.Y., Swinyard, E.A., Wolf, H.H. and Kupferberg, H.J. Effect of GABA agonists on the neurotoxicity and anticonvulsant activity of benzodiazepines. *Life Sciences* **36** (1985) 737-744.

Chopde, C.T., Brahmankar, D.M. and Shripad, V.N. Neurochemical aspects of ethanol dependence and withdrawal reactions in mice. *Journal of Pharmacology and Experimental Therapeutics* **200** (1977) 314-319.

Clemmesen, L. and Hemmingsen, R. Physical dependence on ethanol during multiple intoxication and withdrawal episodes in the rat: Evidence of a potentiation. *Acta Pharmacologia et toxicologie* **55** (1984) 345-350.

Cole-Harding, S., Wilson, J.R. and Schlesinger, K. Phenobarbital withdrawal in mice selectively bred for differences in ethanol withdrawal severity. Joint meeting of the American Medical Society on Alcoholism and Other Drug Dependencies and the Research Society on Alcoholism, San Francisco, Calif., USA, Apr. 18-22, 1986. *Alcoholism: Clinical and Experimental Research* **10(1)** (1986) 108.

Coleman, R.M., Pollak, C.P. and Weitzman, E.D. Periodic movements in sleep (nocturnal myoclonus): relation to sleep disorders. Annals of Neurology **8** (1980) 416-421.

Cooper, J.R., Bloom, F.E. and Roth, R.H. *The Biochemical Basis of Neuropharmacology* Oxford University Press, New York, 1986, 124.

Corley, R. and Allen, D. Multivariate diallel analysis of ethanol withdrawal symptoms in mice. *Alcoholism: Clinical and Experimental Research* **12**(1) (1988) 99-104.

Crabbe, J.C. Sensitivity to ethanol in inbred mice: Genotypic correlations among several behavioral responses. *Behavioral Neuroscience* **97**(2) (1983) 280-289.

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

Crabbe, J.C., Janowsky, J.S., Young, E.R. and Rigter, H. Handling-induced convulsions in twenty inbred strains of mice. Substance and Alcohol Actions/Misuse. 1 (1980) 149-153.

Crabbe, J.C. and Kosobud, A. Sensitivity and tolerance to ethanol in mice bred to be genetically prone or resistant to ethanol withdrawal seizures. *Journal of Pharmacology and Experimental Therapeutics* 239(2) (1986) 327-333.

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

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

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

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

Crabbe, J.C., Young, E.R. and Kosobud, A. Genetic correlations with ethanol withdrawal severity. *Pharmacology, Biochemistry and Behavior* **18** (1983c) 544-547.

Curtis, D.R., Duggan, A.W., Felix, D. and Johnston, G.A.R. GABA, bicuculline, and central inhibition. *Nature* **226** (1970) 1222-1224.

Dar, M.S. and Wooles, W.R. Striatal and hypothalamic neurotransmitter changes during ethanol withdrawal in mice. *Alcohol* 1 (1984) 453-458.

Dar, M.S. and Wooles, W.R. GABA mediation of the central effects of acute and chronic ethanol in mice. *Pharmacology, Biochemistry and Behavior* 22 (1985) 77-84.

DeFries, J.C. 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-NIAA Research Monograph No. 6). Washington D.C: U.S. Government Printing Office, 1981.

Deitrich, R.A. and Spuhler, K. Genetics of alcoholism and alcohol actions. In Smart, R.G., Cappell, H.C., Glaser, F.B., Isreal, Y., Kalant, H., Popham, R.E., Schmidt, W., and Sellars, E.M. New York, Plenum Press, 1984, 47-98.

Delgado-Escueta, A.V., Ward, A.A. Jr., Woodbury, D.M. and Porter, R.J. New wave of research in the epilepsies. In: A.V. Delgado-Escueta, A.A. Ward, D.M. Woodbury and R.J. Porter (Eds) *Advances in Neurology* **44:** *Basic mechanisms of the epilepsies, Molecular and cellular approaches.* New York, Raven Press, 1986, 3-56.

Dorow, R., Horowski, R., Paschelki, G., Amin, M. and Braestrup, C. Sever anxiety produced by FG 7142, a beta carboline ligand for benzodiazepine receptors. *Lancet* 2 (1983) 98-99.

Downes, H., Perry, R.S., Ostlund, R.E. and Karler, R. A study of the excitatory effects of barbiturates. *Journal of Pharmacology and Experimental Therapeutics* **175** (1970) 692-699.

Downes, H. and Williams, J.K. Effects of a convulsant barbiturate on the spinal monosynaptic pathway. *Journal of Pharmacology and Experimental Therapeutics* **168** (1969) 283-289.

Elmasian, R., Neville, H., Woods, D. Schukit, M. and Bloom, F. Event-related brain potentials are different in individuals at high and low risk for developing alcoholism. *Proceedings of the National Academy of Sciences (USA)* **79** (1982) 7900-7903.

Emmett-Ogglesby, M.W., Spencer, D.G. Jr., Lewis, M., Elmesallamy, F., Lal, H. Anxiogenic aspects of diazepam withdrawal can be detected in animals. European *Journal of Pharmacology* **92** (1983) 127-130.

Emmett-Ogglesby, M.W., Harris, C.M., Lane, J.D., Lal, H. Withdrawal from morphine generalizes to a pentylenetetrazol stimulus. *Neuropeptides* **5** (1984) 37-40.

Esplin, D.W. and Woodbury, D.M. Spinal reflexes and seizure patterns in the two-toed sloth. *Science* **133** (1961) 1426-1427.

Esplin, D.W. and Freston, J.W. Physiological and pharmacological analysis of spinal cord convulsions. *Journal of Pharmacology and Experimental Therapeutics* **130** (1960) 68-80.

Fahn, S. and Cote, L.J. Regional distribution of gamma-aminobutyric acid (GABA) in the brain of the Rhesus monkey. *Journal of Neurochemistry.* **15** (1968) 209-.

Falconer, D.S. *Introduction to Quantitative Genetics, 2nd Edition.* Longman, London and New York, 1983.

File, S., Green, A.R., Nutt, D.J. and Vincent, N.D. On the convulsant action of Ro 5-4864 and the existence of a micromolar benzodiazepine binding site in rat brain. *Psychopharmacology* **82** (1984) 199-202.

Freed, W.J. and Michaelis, E.K. Glutamic acid and ethanol dependence. *Pharmacology, Biochemistry and Behavior* **8(5)** (1978) 509-514.

Friedman, H.J. Assessment of physical dependence on and withdrawal from ethanol in animals. In: H. Rigter and J.C. Crabbe, (Eds.) *Alcohol Tolerance and Dependence*, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, pp. 93-121.

Freund, R.K., Marley, R.J. and Wehner, J.M. Differential sensitivity to bicuculline in three inbred mouse strains. *Brain Research Bulletin* **18** (1986) 657-662.

Galindo, A. GABA-picrotoxin interaction in the mammalian central nervous system. *Brain Research* **14** (1969) 763-769.

Galindo, J. and Rudomin, P. Facilitation of synaptic activity in the frog spinal cord produced by 4-aminopyridine. *Neuroscience letters* **10** (1978) 299-304.

Goddard, G.V. Development of epileptic seizures through brain stimulation at low intensity. *Nature* **214** (1967) 1020-1021.

Goddard, G.V., Mcintyre, D and Leech, C. A permanent change in brain function resulting from daily electrical stimulation. *Experimental Neurology* **25** (1969) 295-330.

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

Goldstein, D.B. Convulsions elicited by handling: A sensitive method of measuring CNS excitation in mice treated with reserpine or convulsant drugs. *Psychopharmacologia* **32** (1973a) 27-32.

Goldstein, D.B. Inherited differences in intensity of alcohol withdrawal reactions in mice. *Nature* **245** (1973b) 154-156.

Goldstein, D.B. and Kakihana, R. Alcohol withdrawal reactions in mouse strains selectively bred for long or short sleep times. *Life Sciences* **17** (1975) 981-986.

Goldstein, D.B. and Kakihana, R. Alcohol withdrawal reactions and reserpine effects in inbred strains of mice. *Life Sciences* **15** (1974) 415-425.

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

Gonzales, R.A. and Leslie, S.W. [^{3}H]Adenosine uptake and release from synaptosomes. *Biochemical Pharmacology* 34(10) (1985) 1619-1625.

Greer, C.A., and Alpern, H.P. Mediation of myoclonic seizures by dopamine and clonic by acetylcholine and GABA. *Life Sciences* **21** (1977) 385-392.

Grieve, S.J., Griffiths, P.J. and Littleton, J.M. Genetic influences on the rate of development of ethanol tolerance and the ethanol physical withdrawal syndrome in mice. *Drug and Alcohol Dependence* **4** (1979) 77-86.

Grisar, R. Neuron-glia relationships in human and experimental epilepsy: A biochemical point of view. In: A.V. Delgado-Escueta, A.A. Ward, D.M. Woodbury and R.J. Porter (Eds) *Advances in Neurology* **44**: *Basic mechanisms of the epilepsies, Molecular and cellular approaches*. New York, Raven Press, 1986, 1045-1073.

Guerrero-Figueroa, R., Rye, M.M., Gallant, D.M. and Bishop, M.P. Electrographic and behavioral effects of diazepam during alcohol withdrawal stage in cats. *Neuropharmacology* **9** (1970) 143-150.

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

Harris, C.M., Emmett-Oglesby, M.W., Robinson, N.G. and Lal, H. Withdrawal from chronic nicotine substitutes partially for the interoceptive stimulus produced by pentylenetetrazol (PTZ). *Psychopharmacology* **90** (1986) 85-89.

Hegmann, J.P. and Possidente, B. Estimating genetic correlations from inbred strains. *Behavior Genetics* **11**(2) (1980) 103-114.

Hoffman, P.L. and Tabakoff, B. Ethanol's action on brain biochemistry. In: R.E. Tartar and D.H. Van Thiel (Eds), *Alcohol and the Brain; Chronic Effects*, New York, Plenum Medical Book Company, 1985, 19-68.

Holtman, J.R. and Richter, J.A. Increased release of [^{3}H]Acetylcholine *in vitro* from the mouse hippocampus by a convulsant barbiturate. *Neuropharmacology* **22** (1981) 1101-1108.

Hunt, W.A. The effect of ethanol on GABAergic transmission. *Neuroscience and Biobehavioral Reviews* **7** (1983) 87-95.

Hunt, W.A. and Majchrowicz, E. Alterations in the turnover of brain norepinephrine and dopamine in alcohol-dependent rats. *Journal of Neurochemistry* **194** (1974) 549-552.

Hunter, B.E., Boast, C.A., Walker, D.W., Zornetzer, S.F. and Riley, J.N. Ethanol dependence in the rat: Role of non-specific and limbic regions in the withdrawal reaction. *Electroencephalography and clinical Neurophysiology* **45** (1978) 483-495.

Hunter, B.E. and Walker, D.W. Ethanol dependence in the rat: Role of extrapyramidal motor systems in the withdrawal reaction. *Experimental Neurology* **62** (1978) 374-393.

Hupka, A.L., Williams, J.M. and Karler, R. Effects of convulsant barbiturates on vascular smooth muscle. *Journal of Pharmacy and Pharmacology* 21 (1969) 838-844.

Hutchins, J.R., Allen, D.L., Cole-Harding, S. and Wilson, J.R. Behavioral and physiological measures for studying ethanol dependence in mice. *Pharmacology, Biochemistry and Behavior* 15(1) (1981) 55-59.

Jaffe, J.H. and Ciraulo, D.A. Drugs used in the treatment of alcoholism. In: J.H. Mendelson and N.K. Mello (Eds.) *The Diagnosis and Treatment of Alcoholism, 2nd Edition*, New York, McGraw-Hill Book Co., 1985, pp 355-389.

Jensen, L.H., Petersen, E.N. and Braestrup, C. Audiogenic seizures in DBA/2 mice discriminate sensitively between low efficacy benzodiazepine receptor agonists and inverse agonists. *Life Sciences* 33 (1983) 393-399.

Kaij, L. Alcoholism in twins. *Studies on the Etiology and Sequels of Abuse of Alcohol*, Almvquist and Wiksell, Stockholm, 1960.

Katz, B. and Miledi, R. Spontaneous and evoked activity of motor nerve endings in calcium Ringer. *Journal of Physiology, London* 203 (1969) 686-706.

Kelley, J.T. and Reilly, E.L. EEG, alcohol and alcoholism. In Hughes, J.R. and Wilson, W.P., EEG and evoked potentials in psychiatry and behavioral neurology. Boston: Butterworths, 1983, 55-77.

Keppel, G. *Design and Analysis: A Researcher's Handbook.* Prentice Hall, Englewood Cliffs, 1982.

Kresch, M.J., Shaywitz, B.A., Shaywitz, S.E., Andersen, G.M., Lochman, J.L. and Cohen, D.J. Neurotransmitters in human epilepsy. In P.C. Jobe and H.E. Laird, *Neurotransmitters and Epilepsy*, Humana Press, Clifton N.J., 1987, 321-338.

Kosobud, A., Bodor, A.S. and Crabbe, J.C. Voluntary consumption of ethanol in WSP, WSC and WSR selectively bred mouse lines. *Pharmacology, Biochemistry and Behavior* **29** (1988) 601-607.

Kosobud, A. and Crabbe, J.C. Ethanol withdrawal in mice bred to be genetically prone or resistant to ethanol withdrawal seizures. *Journal of Pharmacology and Experimental Therapeutics* **238** (1986) 170-177.

Lal, H., Emmett-Oglesby, M.W., Spencer, D.G.Jr., Elmessallamy, F. Subjective signs of precipitated withdrawal from triazolam. *Federation Proceedings* **43** (1984) 931.

Lal, H., Harris, C.M., Emmett-Oglesby, M.W., Bhadra, S. and Benjamin, D. Ethanol withdrawal substitutes for an interoceptive discriminative stimulus IDS produced by the anxiogenic drug pentylenetetrazole. 71st Annual Meeting of the Federation of American Societies for Experimental Biology, Washington, D.C., USA, March 29-April 2, 1987. *Federation Proceedings* **46**(3) (1987) 712.

Leeb-Lundberg, F. and Olsen, R.W. Interactions of barbiturates of various pharmacological categories with benzodiazepine receptors. *Molecular Pharmacology* **21** (1982) 320-328.

Leimeignan, M. Analysis of the action of 4-aminopyridine on the cat lumbar spinal cord - I. Modification of the afferent volley, the monosynaptic discharge amplitude and the polysynaptic evoked responses. *Neuropharmacology* **11** (1972) 551-558.

Leimeignan, M. Analysis of the action of 4-aminopyridine on the cat lumbar spinal cord - II. Modification of certain spinal inhibitory phenomena, post-tetanic potentiation and dorsal root potential. *Neuropharmacology* **12** (1973) 641-651.

Leitch, G.L., Backes, D.J., Siegman, F.S. and Guthrie, G.D. Possible role of GABA in the development of tolerance to alcohol. *Experentia* **33** (1977) 496-497.

Liljequist, S. and Engel, J. The effect of chronic ethanol administration on central neurotransmitter mechanisms. *Medical Biology* **57** (1979) 299-210.

Lister, R.G. and Nutt, D.J. Is Ro 15-4513 a specific alcohol antagonist? *Trends in Neurosciences* **6** (1987)

Lloyd-Smith, D.C. and Gloor, P. Abnormal photic sensitivity in alcohol withdrawal. *Electroencephalography and Clinical Neurophysiology* **13** (1961) 496.

Loscher, W. 3-Mercaptopropionic acid: convulsant properties, effects on enzymes of the gamma-aminobutyrate system in mouse brain and antagonism by certain anti-convulsant drugs, aminooxyacetic acid and gabaculine. *Biochemical Pharmacology* **28** (1979) 1397-1407.

Lothman E.W and Collins, R.C. Kainic acid induced limbic seizures: metabolic, behavioral, electroencephalographic and neuropathological correlates. *Brain Research* **218** (1981) 299-318.

Luders, H., Lesser, R.P., Dinner, D.S. and Morris, H.H. Generalized epilepsies: a review. *Cleveland Clinic Quarterly* **51** (1984) 205-226.

Lundh, H. Effects of 4-aminopyridine on neuromuscular transmission. *Brain Research* **153** (1978) 307-318.

Lundh, H. Effects of 4-aminopyridine on statistical parameters of transmitter release at the neuromuscular junction. *Acta Pharmacologia et Toxicologia* **44** (1979) 343-346.

Lundh, H., Leander, S. and Thesleff, S. Antagonism of the paralysis produced by botulinum toxin in the rat. *Journal of Neurobiology* **32** (1977) 29-43.

Lundh, H. and Thesleff, S. The mode of action of 4-aminopyridine and quanidine on transmitter release from motor nerve terminals. *European Journal of Pharmacology* **42** (1977) 411-412.

Lyon, M.F. Hereditary absence of otoliths in the house mouse. *Journal of Physiology* **114** (1951) 410-418.

Marley, R.J. and Wehner, J.M. Correlation between the enhancement of flunitrazepam binding by GABA and seizure susceptibility in mice. *Life Sciences* **40** (1987) 2215-2224.

Marley, R.J., Gaffney, J.D. and Wehner, J.M. Genetic influences on GABA-related seizures. *Pharmacology, Biochemistry and Behavior* **24** (1987) 665-672.

Marsden, C.D., Hallett, M., Fahn, S. The nosology and pathophysiology of myoclonus. In: Marsden, C.D.and Fahn, F.S. (Eds) *Movement Disorders* London, Butterworth, 1982, 196-248.

Mason, C.R. and Cooper, R.M. A permanent change in convulsive threshold in normal and brain damaged rats with repeated small doses of pentylenetetrazol. *Epilepsia* **13** (1972) 663-674.

McClearn G.P. and Rodgers, D.A. Differences in alcohol preference among inbred strains of mice. *Quarterly Journal of Studies on Alcoholism* **20** (1959) 691-695.

McClearn, G.P., Wilson, J.R., Petersen, D.R. and Allen, D.L. Selective breeding in mice for severity of the ethanol withdrawal syndrome. *Substance and Alcohol Actions/Misuse* **3** (1982) 135-143.

McDonald, R.L. and McClean, M.J. Anticonvulsant drugs: Mechanisms of action. In: A.V. Delgado-Escueta, A.A. Ward, D.M. Woodbury and R.J. Porter (Eds) *Advances in Neurology* **44**: *Basic mechanisms of the epilepsies, Molecular and cellular approaches*. New York, Raven Press, 1986, pp 713-736.

McSwigan, J.D., Crabbe, J.C. and Young, E.R. Specific ethanol withdrawal seizures in genetically selected mice. *Life Sciences* **35** (1984) 2119-2126.

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

Meier-Ewert, K. and Broughton, R. Photomyoclonic response of epileptic and non-epileptic subjects during wakefulness, sleep and arousal. *Electroencephalography and clinical Neurophysiology* **23** (1967) 142-151.

Meves, H. and Pichon, Y. The effect of internal and external 4-aminopyridine on the potassium currents in intracellularly perfused squid giant axon. *Journal of Physiology London* **268** (1977) 511-532.

Michaelis, E.K., Michaelis, M.L. and Freed, W.J. Chronic ethanol intake and synaptosomal glutamate binding activity. In H. Begleiter (Ed.), *Biological Effects of Alcohol.* Advances in Experimental Medicine and Biology, Vol. 126. New York, Plenum Press, 1980.

Michaelis, E.K., Mulvaney, M.J. and Freed, W.J. Effects of acute and chronic ethanol intake on synaptosomal glutamate binding activity. Biochemical Pharmacology 27 (1978) 1685-1692.

Minium, E.W. *Statistical Reasoning in Psychology and Education.* New York, John Wiley and Sons, Inc., 1970.

Molgo, J., Leimeignan, M. and Lechat, P. Analysis of the action of 4-aminopyridine during repetitive stimulation at the neuromuscular junction. *European Journal of Pharmacology* **53** (1979) 307-311.

Molgo, J., Lundh, H. and Thesleff, S. Potency of 3,4-diaminopyridine and 4-aminopyridine on mammalian neuromuscular transmission and the effect of pH changes. *European Journal of Pharmacology* **61** (1980) 25-34.

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

Nadler, J.V. Minireview: Kainic acid as a tool for the study of temporal lobe epilepsy. *Life Sciences* **29** (1981) 2031-2042

Nordberg, A. and Wahlstrom, G. Tolerance, physical dependence and changes in muscarinic receptor binding sites after chronic ethanol treatment in the rat. *Life Sciences* 31 (1982) 277-287.

Nutt, D.J., Cowen, P.J. and Little, H.J. Unusual interactions of benzodiazepine antagonists. *Nature* **295** (1982) 436-438.

Obata, T. and Yamamura, H.I. The effect of benzodiazepines and Beta-carbolines on GABA-stimulated chloride influx by membrane vesicles from the rat cerebral cortex. *Biochemical and Biophysical Research Communications* **141** (1986) 1-6.

Olney, J.W., Rhee, V. and Ho, O.L. Kainic acid: A powerful neurotoxic analogue of glutamic acid. *Brain Research* **77** (1974) 507-512.

Olsen, R.W. GABA-benzodiazepine-barbiturate receptor interactions. *Journal of Neurochemistry* **37**(1) (1981) 1-13.

Olsen, R.W., Wamsley, J.K., Lee, R.J. and Lomax, P. Benzodiazepine/barbiturate/GABA receptor-chloride ionophore complex in a genetic model for generalized epilepsy. In: A.V. Delgado-Escueta, A.A. Ward, D.M. Woodbury and R.J. Porter (Eds) *Advances in Neurology* **44:** *Basic mechanisms of the epilepsies, Molecular and cellular approaches*. New York, Raven Press, 1986.

Pellmar, T.C. and Wilson, W.A. Synaptic mechanism of pentylenetetrazole: Selectivity for chloride conductance. *Science* **197** (1977) 912-914.

Penfield, W. and Erikson, T.C. Epilepsy and Cerebral Localization. Springfield, Ill. Charles C. Thomas Press, 1941.

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

Perry, T.L., Berry, K., Hansen, Diamons, S. and Mok, C. Regional distribution of amino acids in human brain obtained at autopsy. *Journal of Neurochemistry* **18** (1971) 513-

Petersen, E.N. DMCM: A potent convulsive benzodiazepine receptor ligand. *European Journal of Pharmacology* **94** (1983) 117-124.

Pincus, J.H. and Hsiao, K. Calcium uptake mechanisms affected by some convulsant and anticonvulsant drugs. *Brain Research* **217** (1981) 119-127.

Pinel, J.P.J. and Cheung, K.F. Controlled demonstration of metrazol kindling. *Pharmacology, Biochemistry and Behavior* **6** (1977) 599-600.

Pohorecky, L.A. Effects of ethanol on central and peripheral noradrenergic neurons. *Journal of Pharmacology and Experimental Therapeutics* **189** (1974) 380-391.

Poldrugo, F. and Snead, O.C.III. Electroencephalographic and behavioral correlates in rats during repeated ethanol withdrawal syndromes. *Psychopharmacology* **83** (1984) 140-146.

Prado de Carvalho, L., Grecksch, G., Chapouthier, G. and Rossier, J. Anxiogenic and non-anxiogenic benzodiazepine antagonists. *Nature* **301** (1983) 64-66.

Pranzatelli, M.R. and Snodgrass, S.R. The pharmacology of myoclonus. *Clinical Neuropharmacology* **8**(2) (1985) 99-130.

Rabin, R.A., Wolfe, B.B. and Dibner, Effects of ethanol administration and withdraws on neurotransmitter receptor systems in C57 mice. *Journal of Pharmacology and Experimental Therapeutics* **213** (1980) 491-496.

Racine, R.J. Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalography and clinical Neurophysiology* **32** (1972) 281-294.

Ramanjaneyulu R. and Ticku, M.K. Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine-GABA receptor-ionophore complex. European Journal of Pharmacology 98 (1984) 337-345.

Rawat, A.K. Brain levels and turnover rates of presumptive neurotransmitters as influenced by administration and withdrawal of ethanol in mice. *Journal of Neurochemistry* **22** (1974) 915-922.

Roberts, E. Failure of GABAergic inhibition: A key to local and global seizures. In: A.V. Delgado-Escueta, A.A. Ward, D.M. Woodbury and R.J. Porter (Eds) *Advances in Neurology* **44**: *Basic mechanisms of the epilepsies, Molecular and cellular approaches*. New York, Raven Press, 1986. pp 319-342.

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

Seale, T.W., Alba, K.A., Roderick, T.H., Rennert, O.M. and Carney, J.M. Different genes specify hyporesponsiveness to seizures induced by caffeine and the benzodiazepine inverse agonist, DMCM. *Pharmacology, Biochemistry and Behavior* **27** (1987b) 451-456.

Seale, T.W., Carney, J.M., Rennert, O.M., Flux, M. and Skolnick, P. Coincidence of seizure susceptibility to caffeine and to the benzodiazepine inverse agonist, DMCM, in SWR and CBA inbred mice. *Pharmacology, Biochemistry and Behavior* **26** (1987a) 381-387.

Seale, T.W., Johnson, P., Roderick, T.H., Carney, J.M. and Rennert, O.M. A single gene difference determines relative susceptibility to caffeine-induced lethality in SWR and CBA inbred mice. *Pharmacology, Biochemistry and Behavior* **23** (1985) 275-278.

Seale, T.W., Johnson, P., Carney, J.M. and Rennert, O.M. Interstrain variation in acute toxic response to caffeine among inbred mice. *Pharmacology, Biochemistry and Behavior* **20** (1984) 567-573.

Seyfried, T.N. Convulsive disorders. In *The Mouse in Biomedical Research* **IV** (1982) 97-124.

Skerritt, J.H. and MacDonald, R.L. Multiple actions of convulsant barbiturates on mouse neurons in cell culture. *Journal of Pharmacology and Experimental Therapeutics* **230** (1984) 82-88.

Snead, O.C., On the sacred disease: The neurochemistry of epilepsy. *The International Review of Neurobiology* **24** (1983) 93-180.

Squires, R.F., Saederup, E., Crawley, J.N., Skolnik, P. and Paul, S.M. Convulsant potencies of tetrazoles are highly correlated with actions on GABA/Benzodiazepine/Picrotoxin receptor complexes in brain. *Life Sciences* **35** (1984) 1439-1444.

Study, R.E. and Barker, J.L. Diazepam and (-)-pentobarbital: Fluctuation analysis reveals different mechanisms for potentiation of gamma-aminobutyric acid responses in cultured central neurons. Proceedings of the National Academy of Sciences (USA) 78(11) (1981) 7180-7184.

Sutton, I. and Simmonds, M.A. Effects of acute and chronic ethanol on the gamma-aminobutyric acid system in rat brain. *Biochemical Pharmacology* **22** (1973) 1685-1692.

Swinyard, E.A. and Toman, J.E.P. Effects of body temperature on of convulsive seizure in rats. *Journal of Pharmacology and Experimental Therapeutics* **154** (1948) 207-210.

Sytinsky, I.A., Guzikov, B.M., Gomanko, M.V. The gamma-aminobutyric acid (GABA) system in brain during acute and chronic ethanol intoxication. *Journal of Neurochemistry* **25** (1975) 43-48.

Szabo, G., Kovacs, G.L. and Telegdy, G. Increased sensitivity to picrotoxin as an index of physical dependence on alcohol in the mouse. *Drug and Alcohol Dependence 14 (1984) 187-195.*

Tabakoff B. and Hoffman, P.L. Alterations in receptors controlling dopamine synthesis after chronic ethanol ingestion. *Journal of Neurochemistry* **31** (1978) 1223-1229.

Tabakoff B. and Hoffman, P.L. Development of functional dependence on ethanol in dopaminergic systems. *Journal of Pharmacology and Experimental Therapeutics* **208** (1979) 216-222.

Tabakoff, B. and Hoffman, P.L. Neurochemical aspects of tolerance to and dependence on alcohol. In: B. Kissin, H. Begleiter (Eds), *The Biology of Alcoholism, Vol. 7*, New York, Plenum Press, 1983, 199-216.

Tabakoff B., Hoffman, P.L. and Moses, F. Neurochemical correlates of ethanol withdrawal: Alterations in serotonergic function. Journal of Pharmacy and Pharmacology 29 (1977) 471-476.

Tabakoff, B., Munoz-Marcus, M. and Fields, J.Z. Chronic ethanol feeding produces an increase in muscarinic cholinergic receptors in mouse brain. *Life Sciences* **25** (1979) 2173-2180.

Tenen, S.S. and Hirsch, J.D. Beta-carboline-3-carboxylic acid ethyl ester antagonizes diazepam activity. *Nature* **288** (1980) 609-610.

Thesleff, S. Aminopyridines and synaptic transmission. *Neuroscience* **5** (1980) 1413-1419.

Ticku, M.K. Interaction of depressant, convulsant, and anticonvulsant barbiturates with the [^{3}H]diazepam binding site of the benzodiazepine-GABA-receptor-ionophore complex. *Biochemical Pharmacology* **30** (1981) 1573-1579.

Ticku, M.K. The effects of acute and chronic ethanol administration and its withdrawal on gamma-aminobutyric acid receptor binding in rat brain. *British Journal of Pharmacology* **70** (1980) 403-410.

Ticku, M.K., Ban, M. and Olsen, R.W. Binding of [^{3}H]alpha-dihydropicrotoxinin, a gamma-aminobutyric acid synaptic antagonist, to rat brain membranes. *Molecular Pharmacology* **14** (1978) 391-402.

Ticku, M.K. and Burch, T. Alterations in gamma-aminobutyric acid receptor sensitivity following acute and chronic ethanol treatment. *Journal of Neurochemistry* 34 (1980) 417-423.

Ticku, M.K. and Olsen, R.W. Interaction of barbiturates with dihydropicrotoxinin binding sites related to the GABA receptor-ionophore system. *Life Sciences* **22** (1978) 1643-1652.

Ticku, M.K. and Maksay, G. Convulsant/depressant site of action at the allosteric benzodiazepine-GABA receptor-ionophore complex. *Life Sciences* **33** (1983) 2363-2375.

Unwin, J.W. and Taberner, P.V. Sex and strain differences in GABA receptor binding after chronic ethanol drinking in mice. *Neuropharmacology* **19** (1980) 1257-1259.

Velluz, L., Mathieu, J. and Jequier, R. Analeptiques respiratoires en serie barbiturique. Aspereu general. *Ann Pharm Fr* **9** (1951) 271-275.

Victor, M. and Adams, R.D. The effect of alcohol on the nervous system. In: H.H. Merritt and C.L. Hare (Eds) Metabolic and Toxic Disease of the Nervous System. *Research Publications - Association for Research in Nervous and Mental Disease* **32**, 526-573, 1953.

Volicer, L. GABA levels and receptor binding after acute and chronic ethanol administration. *Brain Research Bulletin* **5** (1980) 809-813.

Volicer, L. and Biagoni, T.M. Effect of ethanol administration and withdrawal on GABA receptor binding in rat cerebral cortex. *Substance and Alcohol Actions/Misuse 3 (1982) 31-39.*

Wagman, A.M. and Allen, R.P. Effects of alcohol ingestion and abstinence on slow wave sleep of alcoholics. In: M.M. Gross (Ed), *Alcohol Intoxication and Withdrawal: Experimental studies*, Vol. 2. Advances in experimental medicine and biology. New York, Plenum Press, 1975, 453-465.

Walker, D.W. and Zornetzer, S.F. Alcohol withdrawal in mice: Electroencephalographic and behavioral correlates. *Electroencephalography and clinical Neurophysiology* **36** (1974) 233-243.

Wallgren, H. Neurochemical aspects of tolerance to and dependence on ethanol. In: M.M. Gross (Ed), *Alcohol Intoxication and Withdrawal: Experimental studies*, Vol. 1. Advances in experimental medicine and biology. New York, Plenum Press, 1973, 15-31.

Werman, R., Davidoff, R.A and Aprison, M.H. Inhibitory action of glycine on spinal neurons in the cat. *Journal of Neurophysiology* **31** (1968) 81-95.

Wikler, A., Pescor, F.T., Fraser, H.F. and Isbell, H. Electroencephalographic changes associated with chronic alcohol intoxication and the alcohol abstinence syndrome. *American Journal of Psychiatry* **113** (1956) 106-114.

Willow, M., Bornstein, J.C. and Johnston, G.A.R. The effects of anaesthetic and convulsant barbiturates on the efflux of [^{3}H]-D-aspartate from brain minislices. *Neurosciences Letters* **18** (1980) 185-190.

Wilson, J.R. Erwin, V.G., DeFries, J.C., Petersen, D.R. and Cole-Harding, S. Ethanol dependence in mice: Direct and correlated responses to ten generations of selective breeding. *Behavior Genetics* 14(3) (1984) 235-255.

Winer, B.J. *Statistical Principles in Experimental Design.* New York, McGraw-Hill, 1971.

Wixon, H.N. and Hunt, W.A. Effect of acute and chronic ethanol treatment on gamma-aminobutyric acid levels and on aminooxyacetic acid-induced GABA accumulation. *Substance and Alcohol Actions/Misuse* 1 (1980) 481-491.

Wood, J.H., Hare, T.A., Glaeser, B.S., Ballenger, J.C. and Post, R.M. Low cerebral spinal fluid gamma aminobutyric acid content in seizure patients. *Neurology* **29** (1979) 1203-1208.

Wood, J.H., Hare, T.A., Glaeser, B.S., Brooks, B.R., Ballenger, J.C. and Post, R.M. Cerebrospinal fluid GABA variations with seizure type and cerebellar stimulation in man. *Brain Research Bulletin* **5**(2) (1980) 747-753.

Woodbury, D.W. Applications to drug evaulations. In: Purpura, D.P., Penry, J.K. Tower, D.B., Woodbury, D.M., and Walter, R.D., (Eds.) *Experimental Models of Epilepsy.* Raven Press, New York, 1972, 557-583.

Woodbury, D.W. Convulsant drugs: Mechanisms of action. In Glaser, G. H. Penry, J.K. and Woodbury, D.M., (Eds.) *Antiepileptic Drugs: Mechanisms of Action.* Raven Press, New York, 1980, 249-303.

Young, A.B. and Snyder, S.H. Strychnine binding associated with glycine receptors of the central nervous system. *Proceedings of the National Academy of Sciences USA* **70** (1973) 2832-2836.

Young, A.B. and Snyder, S.H. The glycine receptor: Evidence that strychnine binding is associated with the ionic conductance mechanism. *Proceedings of the National Academy of Sciences USA* **71** (1974a) 4002-4005.

Young, A.B. and Snyder, S.H. Strychnine binding in rat spinal cord membranes associated with the synaptic glycine receptor: Cooperativity of glycine interactions. *Molecular Pharmacology* **10** (1974b).

Zukin, S.R., Young, A.B. and Snyder, S.H. Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system. *Proceedings of the National Academy of Sciences USA* **71**(12) (1974) 4802-4807.