50 Sinclair, J. D., Ethanol-conditioned taste aversion to ethanol. Alcohol *1* (1984) 223–227.
51 Sinclair, J. D., The feasibility of effective psychopharmacological treatments for alcoholism. Br. J. Addict. *82* (1987) 1213–1223.
52 Sinclair, J. D., and Bender, D. O., Compensatory behaviors: Suggestion for a common basis from deficits in hamsters. Life Sci. *22* (1978) 1407–1412.
53 Sinclair, J.D., Hilakivi, L., and Sarviharju, M., Characteristics of the AA and ANA rat strains after revitalization. Acta pharmac. Toxic. *53* Suppl. II (1983) Abstr. No. 41.
54 Sinclair, J. D., and Tiihonen, K., Lack of alcohol-deprivation effect in AA rats. Alcohol *5* (1988) 85–87.
55 Sinclair, J. D., Viitamaa, T., and Hyytiä, P., Behavioral and color variations between rat lines developed for differential alcohol sensitivity. Alcohol Alcoholism, *Suppl. 1* (1987) 449–453.
56 Sinclair, J. D., and von Troil, S., Saccharin but not alcohol deprivation increases consumption in AA rats. Pharmac. Toxic. *63*, Suppl. II (1988) Abstr. No. 24.
57 Wise, R., and Bozarth, M. A., Actions of drugs of abuse on brain reward systems. Pharmac. Biochem. Behav. *13* (1984) 213–223.

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Selected mouse lines, alcohol and behavior

T. J. Phillips, D. J. Feller and J. C. Crabbe

VA Medical Center, Research Service (151W), 3710 S.W. U.S. Veterans Hospital Rd, Portland (Oregon 97201, USA), and Departments of Medical Psychology and Pharmacology, Oregon Health Sciences University, Portland (Oregon 97201, USA)

Summary. The technique of selective breeding has been employed to develop a number of mouse lines differing in genetic sensitivity to specific effects of ethanol. Genetic animal models for sensitivity to the hypnotic, thermoregulatory, excitatory, and dependence-producing effects of alcohol have been developed. These genetic animal models have been utilized in numerous studies to assess the bases for those genetic differences, and to determine the specific neurochemical and neurophysiological bases for ethanol's actions. Work with these lines has challenged some long-held beliefs about ethanol's mechanisms of action. For example, lines genetically sensitive to one effect of ethanol are not necessarily sensitive to others, which demonstrates that no single set of genes modulates all ethanol effects. LS mice, selected for sensitivity to ethanol anesthesia, are not similarly sensitive to all anesthetic drugs, which demonstrates that all such drugs cannot have a common mechanism of action. On the other hand, WSP mice, genetically susceptible to the development of severe ethanol withdrawal, show a similar predisposition to diazepam and phenobarbital withdrawal, which suggests that there may be a common set of genes underlying drug dependencies. Studies with these models have also revealed important new directions for future mechanism-oriented research. Several studies implicate brain gamma-aminobutyric acid and dopamine systems as potentially important mediators of susceptibility to alcohol intoxication. The stability of the genetic animal models across laboratories and generations will continue to increase their power as analytic tools.
Key words. Mouse lines; selective breeding; ethanol effects; pharmacogenetics; long-sleep mouse; short-sleep mouse.

General introduction

Selective breeding takes advantage of genetic variability and has been utilized for many years in the fields of agriculture and animal husbandry to produce plants and animals with desired characteristics. However, it is only within the last 25 years that this technique has been widely recognized as a particularly powerful one in the field of pharmacogenetics, principally for studying the drug, ethanol (EtOH). A number of rat and mouse lines are now available which are highly sensitive or insensitive to various effects of alcohol (EtOH). Most originated from genetically heterogeneous foundation populations whose individuals were screened for sensitivity to the relevant EtOH effects. Breeding pairs, chosen for extreme sensitivity or insensitivity, produced offspring who were themselves screened and selectively bred according to their relative sensitivities. This process continued for a number of generations until highly sensitive and insensitive lines were produced. The success of a selective breeding program attests to the presence of genetic factors influencing the response in question. The speed and pattern of divergence between the sensitive and insensitive lines provide some indication of the genetic complexity underlying the response.

This paper reviews work which has been performed using selected mouse lines. A large portion is devoted to Long-Sleep (LS) and Short-Sleep (SS) mice because they have been the most extensively investigated. To avoid redundancy in the literature, we provide a short summary of two recent reviews which included data from LS and SS mice, and more thoroughly address more recent work and research that has not been exhaustively reviewed elsewhere. Other research reviewed pertains to the select-

ed lines, WSP and WSR, COLD and HOT, FAST and SLOW, and SEW and MEW. These designations will be defined in later sections.

Long-Sleep (LS) and Short-Sleep (SS) mice

The derivation of the LS and SS mice has been described in detail and the reader is referred to McClearn and Kakihana[102]. An 8-way cross of inbred strains comprised the genetically heterogeneous (HS) foundation population. These strains were chosen from divergent genetic backgrounds to maximize genetic variability, an essential prerequisite for success in a selection program. Mass selection was used and the selection phenotype was duration of loss of the righting reflex (LORR). Animals with the longest durations were mated to each other to initiate the LS line and those with the shortest were mated to initiate the SS line.

The lines diverged rapidly, and virtually no overlap in LORR durations was present after 17 selected generations[102]. Even after many generations of relaxed selection, LS and SS mice have retained an approximately 8-fold difference in LORR duration after EtOH. In addition, LS mice regain the righting reflex at brain EtOH concentrations that are 1.5-fold lower than SS mice[132,133]. This measure directly indexes central nervous system (CNS) sensitivity to the drug, and we shall see in subsequent sections that the wide use of LORR duration, rather than brain EtOH concentrations, has occasionally led to interpretational difficulties which could have been avoided. These mice have been used primarily in investigations of mechanisms of EtOH's actions, frequently in the study of genetic correlation among EtOH-affected traits, and less frequently in studies of genetic architecture underlying EtOH-related phenotypes. Genetic differences between LS and SS mice have been studied by examining brain polypeptide variants using electrophoresis[73]. Finally, recent derivation of recombinant inbred strains from LS and SS mice will facilitate future studies of correlated traits and may help to identify single loci of importance[39].

Previous reviews of work using Long- and Short-Sleep mice

Two recent book chapters have reviewed a large proportion of the data which have been collected using LS and SS mice[33,40]. The reader is referred to these reviews for references not presented here, and for a discussion of issues studied before 1985. Crabbe and colleagues[33] characterized LS mice as generally EtOH-sensitive and SS mice as EtOH-insensitive. Consistent with this suggestion is the greater sensitivity of LS mice to LORR induced by EtOH and many other alcohols and CNS depressants, EtOH depression of body temperature, EtOH disruption of neuronal lipid membrane structure, EtOH elevation of plasma corticosterone levels, and EtOH depression of cerebellar Purkinje cell firing. Deitrich and Spuhler[40] discussed the importance of certain similarities between the two lines in excluding postulated mechanisms of EtOH action responsible for the LORR duration difference. For example, metabolism of EtOH is largely similar in the two lines and cannot account for the large LORR differences. In addition, the similar LORR responses of LS and SS mice to pentobarbital and halothane suggest that a common mechanism of action to induce LORR does not exist for EtOH and all other sedative-hypnotics. Recent developments in this area are reviewed in the next section.

Some instances of greater sensitivity displayed by SS mice, as compared to LS, were also noted in these reviews. SS mice exhibited greater EtOH preference drinking, more severe EtOH withdrawal-induced seizures and flurothyl-induced seizures, more severe morphine withdrawal, and greater locomotor activation at low EtOH doses. In addition, SS mice are more fertile, have higher reproductive fitness, and greater adrenal weight.

Generality of sensitivity to hypnotics

Many studies have been performed to characterize the generality of the difference in LORR sensitivity between LS and SS mice (table 1). Almost all of these studies have employed the duration of LORR to index sensitivity. The seminal study demonstrated that LS mice had longer LORR durations than SS mice when given methanol, butanol, or t-butanol, but not when given chloral hy-

Table 1. Loss of righting reflex sensitivity in LS and SS mice.

Agent	Relative sensitivity	References
Ethanol	LS > SS	47,54,88,131, many others
Methanol	LS > SS	54
n-Propanol	LS > SS	88
n-Butanol	LS > SS	54,88
t-Butanol	LS > SS	47,54
3-me-Butanol	LS > SS	88
Trichloroethanol	LS = SS	54
	LS > SS	96,128
Trifluroethanol	LS > SS	96
Tribromoethanol	LS > SS	96
Dichloroethanol	LS > SS	96
Acetaldehyde	LS > SS	47
Paraldehyde	LS = SS	54
	LS > SS	96,128
Urethane	LS > SS	96
Isoflurane	LS > SS	91,96
Enflurane	LS > SS	96
Halothane	LS = SS	10
Nitrous oxide	LS > SS	96
Gammabutyrolactone	LS > SS	48
Chlordiazepoxide	LS > SS	104
Flurazepam	LS > SS	95
Chloral hydrate	LS = SS	54
	LS > SS	96
Methyprylon	LS > SS	87
Barbital	LS > SS	76,96
Thiopental	LS > SS	104
Ethchlorvynol	SS > LS	87
Phenobarbital	LS > SS	96,104
Secobarbital	SS > LS	87
Pentobarbital	LS = SS	8,54
	LS > SS	8,76
	SS > LS	8,47,87,111,131

drate, paraldehyde, or trichloroethanol[54]. These animals were from the 14th selected generation. Later studies confirmed the differential sensitivity to alcohols[47], and extended this generalized response difference to include 3-methyl-butanol and propanol[88]. Sanders et al.[128] found that LS mice from the 19th to 21st selected generations had longer duration LORR than their SS counterparts when given trichloroethanol or paraldehyde. In the Erwin et al.[54] study, LS mice had non-significantly longer LORR responses than SS mice to chloral hydrate, paraldehyde, and trichloroethanol. This suggests that with increased divergence in response to 5 additional generations of selection for sensitivity to EtOH-induced LORR, the LS and SS mice had developed sensitivity to these other CNS depressants as well. Recent experiments have confirmed the greater sensitivity of LS mice to trichloroethanol, paraldehyde, and chloral hydrate[96] as well as t-butanol[47]. It has also been reported that LS mice are more sensitive than SS to the water-soluble depressant, methyprylon[87], several halogenated EtOH derivatives, urethane, and barbital[96], phenobarbital[96,104], acetaldehyde[47], chlordiazepoxide and thiopental[104], flurazepam[95], and the gaseous anesthetics, nitrous oxide, enflurane, and isoflurane[91].

LS mice are not, however, more sensitive than SS mice to all hypnotics and anesthetics. LS and SS mice do not differ in sensitivity to halothane[10]. Several investigators have reported that SS mice are impaired longer after pentobarbital than LS mice[47,87,111,131]. Erwin et al.[54] originally reported no difference between SS and LS mice in response to pentobarbital, and Alpern and McIntyre have reported LS to be more, less, or equally[7,8] affected by pentobarbital than SS mice, depending upon dose, sex and time of day. The only studies which have examined the most relevant variable, brain concentration of pentobarbital at time of awakening, have found the two lines to be approximately equally affected by equal brain concentrations of the drug[111,131]. The differences in duration of LORR apparently derive from the fact that LS mice have twice the body fat of SS mice and consequently eliminate the highly lipid soluble barbiturate from brain more rapidly[111]. That is, the LS and SS mice are equal in brain sensitivity to pentobarbital, but differ in metabolism of the drug.

In a series of elegant studies, Collins and his co-workers showed that relative sensitivity of LS and SS mice to the effects of sedative-hypnotics on the righting reflex varied as a function of the lipid solubility of the compound. Howerton et al.[88] showed that the difference in duration of LORR between LS and SS mice was greater for EtOH and propanol than for butanols, which have higher octanol/water partition coefficients. They next reported that the highly lipid-soluble compounds, ethchlorvynol, secobarbital or pentobarbital, produced longer impairment in SS than in LS mice[87]. A recent paper calculated the effective doses of several barbiturates, alcohols, and other depressants to produce an average sleep time of 60 min (ED_{60}) for each line[96]. When the ratios of the EDs in LS and SS for each compound were regressed on the log of their octanol/water partition coefficients, a significant correlation ($r = -0.68$) was revealed. This relationship offers clear evidence that the mechanism underlying the difference between LS and SS mice in LORR sensitivity to hypnotics involves the lipid solubility of the compounds. The regression line also suggests why results with pentobarbital have been more equivocal than those with other drugs. The ratio of EDs (LS/SS) predicted for EtOH or propanol is 1.6–1.9, a large difference. At the other end of the scale, the predicted ED ratios for pentobarbital, ethchlorvynol, and secobarbital are 0.85–0.95, only slightly less than 1.0; thus no difference between LS and SS mice would be expected[96].

The relative roles of metabolism and CNS sensitivity cannot be distinguished from the data available for most drugs, for the hypothesis of lipid solubility differences underlying the LS/SS difference in sensitivity is based entirely on analyses of duration of LORR. Differences in metabolism were found to account for the differences between LS and SS in LORR duration after pentobarbital, so they may underlie other differences as well. Although LS and SS mice differ slightly in EtOH metabolism, these differences cannot account for the large difference in EtOH sensitivity[40].

Neurochemical characterization of LS and SS mice

A number of neurochemical pathways have been investigated in attempts to account for the LORR duration difference between LS and SS mice. The present review will critically examine evidence, based on work in LS and SS mice and other selected lines, which implicates catecholamines and the GABA/benzodiazepine/picrotoxin receptor-chloride ionophore complex (GABA Complex) in at least partially mediating the effects of EtOH on LORR. In addition, more limited data addressing the involvement of adenosine, prostaglandins, neurotensin, and other neurotransmitters and neuromodulators in mediating these effects will be considered.

Catecholamines (CAs). Deitrich and Spuhler[40] reviewed some of the data which suggest that differences in either noradrenergic or dopaminergic pathways of LS and SS mice might partially account for their differential sensitivity to EtOH. Since that review was published, further data have been reported supporting this hypothesis; these data suggest a predominant role for dopamine (DA).

It was previously shown that the density of β-adrenergic receptors is lower in the cortex of LS, as compared to SS, mice. However, this result may have no functional significance since the β-adrenergic agonist, isoproterenol, stimulated cAMP accumulation to the same extent in both lines. There was no difference in either dopaminergic or muscarinic receptor density, but slightly greater cAMP accumulation was found in the corpus striatum of LS mice after DA stimulation[41].

EtOH could act either on the reuptake or release of CAs. The reuptake systems of the LS and SS mice respond similarly to EtOH [86, 135]. LS mice appear to be slightly more sensitive than SS mice to the effect of EtOH on K^+-stimulated norepinephrine (NE) release from cortical slices [84]. Since the magnitude of the effect is small, it is not clear what the significance is with respect to LORR durations. If either noradrenergic or dopaminergic pathways are involved in the differential response of LS and SS mice to EtOH, then it is possible that there may be a change in response to EtOH in the activity of enzymes required for synthesis of the CAs. The basal activity of brain tyrosine hydroxylase (TH), the rate limiting step in the synthesis of DA and NE, is the same in both lines when measured using an in vitro assay [9]. An increase in the activity of TH in the hypothalamus measured in vitro after an acute i.p. injection of EtOH correlates with the time at which the two lines regain their righting reflex, suggesting that CAs may play a role in recovery of the righting reflex.

Results obtained on in vivo rates of TH activity in the brains of LS and SS mice after acute EtOH injections [63] are not in agreement with data from in vitro measurements. In vivo baseline TH activity is the same in all brain regions of LS and SS mice with the exception of the cerebellum where it is higher in SS compared to LS mice. TH activity in the cerebellum is depressed longer by peripheral injections of EtOH in LS mice than in SS mice. EtOH had no effect on TH in the locus coeruleus, hypothalamus and frontal cortex of SS mice, but depressed activity in the LS mice [63]. However, it had been reported that EtOH increased TH rate in the hypothalamus of both lines when measured in an in vitro assay [9]. French and Weiner [63] speculate that the disparity in results might be due to an in vivo effect of EtOH either on the levels or uptake of tyrosine, or some other substrate required for TH function. Plasma levels of dopamine-β-hydroxylase (DBH), the enzyme which converts DA to NE, are lower in SS mice compared to LS mice [82]. The authors argue that this difference may be due to greater central noradrenergic activity if one assumes that plasma DBH is a relevant index of sympathetic outflow. They propose that the shorter LORR durations of SS mice are due to antagonism by a more active noradrenergic pathway. However, this hypothesis is not supported by their behavioral data in the HS mice, from which the LS and SS mice were derived. Horowitz et al. [82] showed that the inhibition of DBH by fusaric acid, which should decrease central noradrenergic activity, did not increase LORR durations induced by EtOH but, in fact, attenuated LORR. A later experiment using propranol, a β-adrenergic antagonist, corroborated these results [4]. Additionally, the adrenal cortices of LS and SS mice respond differentially to EtOH [61]. Basal TH activity, NE and epinephrine levels are greater in LS mice and acute EtOH injection transiently increases TH activity in SS, but not LS, mice. Finally, the ganglionic blocking agents, chlorisondamine and hexamethonium block the increase in TH activity and prolong LORR in SS mice.

Another strategy for assessing the potential role of CAs in altering the responses of LS and SS mice to EtOH is the use of drugs that deplete DA and NE. LS mice are more sensitive than SS mice to the effect of α-methyl-p-tyrosine (AMPT), an inhibitor of TH, to deplete brain CAs [60]. Furthermore, the time required for recovery to normal levels is longer in LS mice. These effects appear to be primarily due to greater accumulation, and slower clearance, of AMPT in the brains of LS mice. CA levels can be depleted by the i.c.v. administration of 6-hydroxydopamine (6-OHDA). Pretreatment of adult LS and SS mice with 6-OHDA caused an increase in LORR durations of SS, but not of LS, mice [53]. The magnitude of the effect was not large, possibly due to the limited depletion of the CAs. In contrast, the lesions decreased the hypothermic and hyperglycemic responses of LS, but not SS mice. French et al. [62] examined the role of catecholaminergic function in determining EtOH-induced LORR duration differences in LS and SS mice after three different drug treatments. Reserpine treatment decreased CA levels 25–50% in both lines. However, LORR durations of LS mice were decreased, while increases were observed for SS mice. Neonatal administration of 6-OHDA increased CAs in the locus coeruleus, decreased them in the cerebellum and caused no change in the hypothalamus. This produced only a 10% decrease in the mean LORR duration of LS mice, but a 200% increase in LORR duration in SS mice. Finally, AMPT reduced brain CAs by 30–50% in both lines, caused no change in LS LORR durations, but produced a mean increase of 45% in SS mice. The authors suggested that these results indicate a role for CA pathways in antagonizing EtOH's depressant effect in SS mice, and that these pathways are not recruited in LS mice to alter the depressant effects of EtOH.

The treatments just described altered the levels of both DA and NE; thus, it is not possible to determine if one or both pathways are involved in the differential response of LS and SS mice to EtOH. Spuhler et al. [138] selectively lesioned the noradrenergic pathways of LS and SS mice using DSP4. The greatest depletion was in the hippocampus (80–85%) and the smallest decrease was in the hypothalamus (30–34%). No changes in EtOH-induced LORR durations of either LS or SS mice were found as a result of DSP4 lesions. These results indicate that an alteration in noradrenergic pathways is probably not involved in the differential response of the two lines, suggesting that DA, but not NE, may differentially antagonize the LORR induced by EtOH in LS and SS mice. More studies with DA-specific agonists, antagonists, and lesioning agents are needed to support this suggestion. Furthermore, it has been suggested that CA function in LS mice resembles that induced by a state of hypothyroidism [143]. LS and SS mice have equivalent sensitivity to EtOH-induced LORR on postnatal day nine,

but differ thereafter. This development of differences in LORR sensitivity temporally correlates with the development of differences in free thyroxin index[42]. The CA data and the implications of hypothyroidism in LS mice could be quite wide-ranging[143].

The GABA Complex. Acute and chronic EtOH exposure have been demonstrated to change brain GABA concentrations, GABA release, and to affect the GABA Complex[77,89]. GABAergic pathways form a major inhibitory system in the brain. If this system is involved in the ataxic and sedative effects of EtOH, then one would predict that the selection of LS and SS mice might have altered some component of their GABAergic pathways. For example, the sensitive line (LS) might be hypothesized to have a larger releasable pool of GABA. The levels of GABA in different brain regions, however, are the same in LS and SS mice, and are equally elevated in both lines after an i.p. injection of EtOH[17]. Alternatively, EtOH could differentially alter synaptic levels of GABA by acting on either reuptake or releasing mechanisms. However, studies by Howerton et al.[86] found that the potency of EtOH in altering GABA reuptake could not account for the difference between LS and SS mice. On the other hand, Howerton and Collins[85] found that EtOH was more potent at inhibiting K^+-stimulated GABA release in cerebral cortex slices of LS mice relative to SS mice, but not in cerebellar tissue. This effect was observed at high concentrations of EtOH ($\geq$ 171 mM). Thus, studies involving only levels, uptake or release of GABA cannot explain the LORR sensitivity difference between LS and SS mice.

Another potential site for genetic regulation is the receptor ion channel complex. In addition to containing a binding site for GABA there are distinct binding sites for the benzodiazepines (BZs), picrotoxin, and barbiturates. Essentially, GABA binding causes opening of the channel pore permitting influx of Cl^-, which results in hyperpolarization of the neuron. Binding of BZs and barbiturates allosterically enhance this action of GABA, and there are allosteric interactions among all of the binding sites. EtOH itself may exert a direct effect on the GABA Complex[140], and potentiates GABA-stimulated Cl^- flux[77], but the direct effect is controversial[3].

Behavioral studies have shown that LS mice are more sensitive than SS mice to the disruptive effects of GABA-mimetics in a bar holding test[101]. In addition, GABA agonists and antagonists alter LORR durations of LS and SS mice with differing magnitudes, or even in opposite directions[50,118]. To produce such behavioral results, selection for differences in neural sensitivity to the depressant effects of EtOH may have resulted in changes in receptor or channel density, receptor affinity, or allosteric interactions between various components of the channel. The level of [^{3}H]flunitrazepam (a BZ agonist) binding was 2-fold greater in the midbrain, but not in other brain areas, of LS than of SS mice[99]. Although the density of [^{3}H]GABA binding sites was similar in the forebrain and cerebellum of LS and SS lines, the LS mice exhibited a 2–3-fold greater affinity for low affinity [^{3}H]GABA binding[100]. Allan and Harris[2] have shown that the binding of [^{3}H]muscimol, a GABA agonist which labels the high affinity GABA site, is the same in the cerebellum of LS and SS mice. On the other hand, GABA enhancement of [^{3}H]flunitrazepam binding to cortex and cerebellum is greater in the LS than in the SS line[98]. Two groups have shown that the rate of heat inactivation of BZ binding sites in cortical, but not cerebellar, membranes is greater in tissue from LS than from SS mice[97,105]. Also, increasing the incubation temperature from 0 to 37 °C decreased the potency of GABA to enhance [^{3}H]flunitrazepam binding to cortical membranes prepared from LS, but not SS mice[105], and ethyl β-carboline, an inverse agonist at the BZ site, was more potent at inhibiting the binding of [^{3}H]flunitrazepam in cortical tissue from the LS line[97].

There appear to be significant brain regional differences in the properties of the GABA Complex, since muscimol is more potent at both inhibiting [^{35}S]TBPS (a picrotoxin receptor ligand) binding and stimulating $^{36}Cl^-$ flux into microsacs prepared from the cerebellum of LS relative to SS mice, but not from the cortex. EtOH at relevant concentrations potentiated muscimol-stimulated $^{36}Cl^-$ uptake in LS, but not SS, mice[2]. However, when Harris and Allan[78] measured the density and affinity of flunitrazepam binding sites and the ability of flunitrazepam to potentiate GABA-activated chloride flux in membrane vesicles from LS and SS cortex, they reported no difference in receptor density or affinity. Yet, BZs induce a greater increase in muscimol-stimulated $^{36}Cl^-$ uptake in the LS line than in the SS line[77,78]. These results suggest that there is a difference in the coupling of the BZ site to the chloride channel and imply the presence of 'spare receptors' in the cortex of LS, but not SS, mice.

All of the GABA Complex studies discussed thus far have involved in vitro membrane preparations. Thus, the interpretation of these results may be complicated by variability in membrane preparation, temperature and buffer conditions. Miller et al.[107] examined the effect of EtOH or defeat stress on BZ receptors by measuring the specific regional uptake of [^{3}H]Ro15-1788 (a BZ receptor antagonist) into the brains of LS and SS mice. In contrast to the in vitro binding data, they reported more [^{3}H]Ro15-1788 binding in the cortex and hippocampus of untreated LS mice, compared to SS mice, and the administration of EtOH increased the binding of [^{3}H]Ro15-1788 more in the brains of SS mice. These results have been corroborated in a very recent in vitro study in which GABA enhancement of [^{3}H]flunitrazepam binding to cortical membranes from SS, but not LS, mice was increased by behavioral stressors or the in vivo administration of EtOH (Martin and Wehner, submitted).

SS mice have more severe alcohol withdrawal seizures than do LS mice[74], and are more sensitive to the convulsant bicuculline[119,142]. The density of [^{35}S]TBPS bind-

ing sites is two-fold greater in superior and inferior colliculi of SS than LS mice and, in addition, there is a negative genetic correlation between latency to bicuculline seizures and the B_{max} values for [^{35}S]TBPS in inferior colliculus in recombinant inbred strains from the SS and LS lines (Peris et al., submitted). However, LORR sensitivity was unrelated to TBPS density.

These results suggest that there are some differences in the properties of the GABA Complex between LS and SS mice, which may partially account for the differences existing between these selectively bred lines in their sensitivity to the ataxic and hypnotic effects of EtOH. Further indirect support for this hypothesis is provided by similar work using lines of mice selected for either sensitivity or resistance to the effect of diazepam on rotarod performance. Gallaher et al.[64] developed diazepam-sensitive (DS) and diazepam-resistant (DR) lines of mice based on duration of ataxia assessed with a rotating rod. Muscimol stimulated $^{36}Cl^-$ flux more in the DS line than the DR line[1]. The reponse to flunitrazepam was also greater in DS mice. More importantly, DS mice also exhibited sensitivity to EtOH enhancement of muscimol-stimulated chloride flux, while DR mice did not. These results are very similar to the LS/SS data and strongly suggest that the GABA Complex may mediate some of the ataxic and sedative effects of EtOH. Differences between the LS and SS mice in seizure susceptibility and EtOH withdrawal severity, mentioned in other sections, also may be partially mediated by differences in GABA Complex function.

Adenosine. The administration of adenosine and related analogs depresses both motor activity and body temperature, and increases the latency to pentylenetetrazole-induced convulsions in mice and rats[51]. These effects are analogous to those observed after the injection of EtOH. There is some evidence to suggest that the purinergic system might mediate some of the behavioral effects of EtOH. In support of this hypothesis, Proctor and Dunwiddie[123] have shown that the LS and SS mice are differentially sensitive to adenosine agonists and antagonists. LS mice exhibit a greater sedative and hypothermic response than SS mice to the adenosine agonist, L-phenylisopropyladenosine (PIA). Theophylline, an adenosine antagonist, is more potent at increasing a motor escape response in LS, compared to SS, mice. Both lines achieve similar brain levels of the drugs, which suggests that the effects are due to CNS sensitivity and not metabolic factors[122]. The greater sensitivity of LS mice to adenosine drugs could be explained by a higher receptor density or affinity for adenosine, for there are 29–33% more [^{3}H]PIA binding sites in the cortex and subcortical areas of LS than of SS mice. In addition, the affinity of [^{3}H]PIA is 31–36% higher in the cortex and cerebellum of LS mice[59].

It is not clear if there is a direct relationship between the actions of EtOH and adenosine. Not all of the effects of EtOH are observed after treatment with PIA, and adenosine antagonists do not block all EtOH effects. Furthermore, LS and SS mice made tolerant to EtOH do not show cross-tolerance to adenosine agonists[122]. The limited access of peripherally-administered adenosine analogs to brain tissue (as distinct from brain vasculature) suggest that some effects may be mediated by reflex CNS response to the peripheral vasodilatory effects of these compounds[121]. The available data, therefore, support a potential role for the adenosine system in at least indirectly mediating sensitivity to the sedative and hypothermic effects of EtOH in LS and SS mice and, perhaps, in mediating some of the intoxicating properties of EtOH.

Prostaglandins. Inhibitors of prostaglandin synthesis antagonize EtOH-induced LORR in both LS and SS mice[67], suggesting that prostaglandins may mediate sensitivity to EtOH. This possibility is supported by the finding that brain levels of both PGE and PGF are lower in SS, as compared to LS, mice[66]. Furthermore, the EtOH-induced elevation of prostaglandin concentrations, particularly PGF, is greater in LS than in SS mice, and consistently follows a time course roughly similar to the behavioral effects produced by EtOH[66,68].

Neurotensin (NT). The central administration of NT, a tridecapeptide found in mammalian brains, depresses a variety of physiological and behavioral measures, including body temperature, pain response, muscle tone and locomotor activity. NT increases the sensitivity of animals to the hypothermic and hypnotic effects of EtOH and, specifically, the sensitivity of SS, but not LS mice, to LORR produced by EtOH[57,109]. The enhancement of EtOH's effect by NT was additive with the coadministration of calcium[109], but only up to a certain dosage of NT, suggesting that EtOH may produce some of its effects in conjunction with the NT system, also involving alterations in intracellular calcium.

In an attempt to explain the greater response of SS mice to EtOH, Erwin and Jones (submitted) have found no difference in NT-like immunoreactivity between LS and SS mice in several brain areas, although SS mice were more sensitive to the analgesic and spontaneous locomotor activating effects of NT. More importantly, they (and Erwin and Korte[56]) have found that the density, but not the affinity, of [^{3}H]NT binding was greater in the frontal cortex (75%), cerebellum (34%) and striatum (29%) of SS mice, as compared to LS mice. The kinetics of binding, the inhibitory action of cations, and the binding of NT fragments and analogs to the receptor were identical for the LS and SS mice. The greater density of receptors in specific brain regions might account for the differential response of the two lines to NT, and suggests that at least some NTergic pathways may mediate some of EtOH's effects.

Other neurochemical systems. Another study has suggested that EtOH-induced LORR may be partially mediated by muscarinic cholinergic receptor processes[55]. These results, and the NT studies dicussed in the preceding section, may reflect differential mobilization of intracel-

lular calcium in the selected lines. This possibility is also supported by the finding that i.c.v. calcium enhances LORR sensitivity in SS more than LS mice, as indexed by blood EtOH concentration at LORR[108] or by inhibition of Purkinje cell firing[113]. Furthermore, cerebellar homogenates from SS mice had a two-fold greater affinity for IP_3 binding, suggesting a potentially greater release of intracellular calcium[110]. On the other hand, Daniell and Harris[37] tested synaptosomes isolated from LS and SS mice and found no differences in resting or KCl-stimulated concentrations of intracellular ionized calcium. EtOH increased resting, and decreased stimulated, calcium levels, but to an equal extent in both lines.

Earlier data indicating a greater sensitivity of membrane preparations from LS than from SS mice to the fluidizing properties of EtOH have been extended in recent work. Harris et al.[81] found that the fluidization differences were eliminated in the presence of high calcium concentrations. EtOH was found to influence the activity of only one of five enzyme activities (low K_i NaK-ATPase) in cortical membrane preparations to a greater extent in LS, than in SS, mice[19], reflecting a specificity of EtOH's effects to discrete domains of neural membranes[79].

The impact of chronic EtOH consumption by LS and SS mice on fine anatomical brain structure has received some recent attention. Thirty days of exposure to EtOH in a liquid diet resulted in a reduced number of basket cells in the dentate granule layer of the hippocampus in LS, but not SS, mice relative to controls[129]. After 90 days of EtOH exposure, electron microscopy revealed a significant effect of EtOH on dendritic spine density in the stratum oriens of the CA_1 hippocampal field of LS, but not SS, mice[130].

Finally, earlier studies of the effect of EtOH on the hypothalamic-pituitary-adrenal axis have been recently extended. Zgombick and his colleagues[145, 146] found that acute EtOH, but not halothane increased the adrenocortical and adrenomedullary response in LS mice, more than in SS mice. Administration of β-endorphin i.c.v. increased LORR sensitivity to EtOH in SS, but not LS mice[109]. Wand[141] treated LS and SS mice with EtOH for 4 or 7 days and examined several features of this axis, including proopiomelanocortin mRNA extracted from anterior pituitary, immunoprecipitated protein products, and serum corticosterone levels. Results suggested that EtOH acutely activated the axis in SS mice, and that activation persists after chronic treatment. LS mice, while acutely activated, showed reduced axis activity compared with controls after chronic EtOH treatment.

Summary of neurochemical results. Considering the diverse behavioral effects of EtOH in LS and SS mice, it would be unlikely that a single neuropharmacological pathway could be responsible for all of EtOH's actions. This is supported by the data reviewed above. For example, the differential sensitivity of LS and SS mice to adenosine drugs is on the same order of magnitude as that to EtOH, responses of the GABA Complex (and prostaglandin synthesis) appear to be systematically different in the two lines, and dopaminergic pathways may mediate the recovery from EtOH-induced LORR. Future work will need to address the relative importance of and interrelationship between the various pathways. The use of new techniques provided by molecular biology should provide additional strategies for understanding the biochemical changes produced by the selection paradigm.

Electrophysiological correlates

Data from different laboratories indicate that EtOH can produce either excitatory or inhibitory effects on neuronal firing, but the relationship of these results to the behavioral effects of EtOH is unclear. EtOH could have either a direct effect on the electrical properties of neurons or could be altering neuronal firing through synaptic mechanisms. Hunt[89] reviewed the results from a number of studies, and suggested that the evidence supporting a direct effect of EtOH on electrical conductance is inconclusive. As discussed in a previous section, the GABA Complex might be a good candidate for a synaptic site of action.

Sorenson et al.[137] demonstrated that pressure-ejected EtOH was 30-fold more potent at inhibiting spontaneous firing of cerebellar Purkinje fibers in LS mice, as compared to SS mice. The difference was seen during either spontaneous or evoked activity[136]. Mice from the HS population, which were used to generate the LS and SS lines and show an intermediate LORR duration, were also intermediate in their sensitivity to EtOH's inhibitory effect on Purkinje cell firing. Halothane inhibited Purkinje cell activity equally in the two lines, suggesting that the response to selection was not a nonspecific alteration in sensitivity to all anesthetics. The inhibitory effect of EtOH was specific to cerebellar Purkinje cells, and was not seen in hippocampal pyramidal cells[136]. The differential sensitivity of the LS and SS selected lines has been corroborated in HAS and LAS rat lines, selected to resemble LS and SS mice, respectively[116].

These in vivo data have been corroborated using an in vitro cerebellar slice preparation in which synaptic transmission was suppressed by elevating Mg^{2+} and decreasing Ca^{2+} concentrations[11]. Under these conditions, Purkinje cells from LS mice were approximately 5 times more sensitive than those from SS mice. This difference is smaller than that seen in in vivo studies, but strongly supports the original findings. The differential sensitivity of cerebellar Purkinje cells of LS and SS mice to EtOH is maintained when cerebellar tissue is transplanted into the anterior chamber of the eye of a mouse from the other selected line[115]. These results suggest that the sensitivity difference is intrinsic to cells of the cerebellum. Chronic treatment of LS and SS mice with EtOH leads to the development of behavioral tolerance to the LORR effect as assessed by blood EtOH concentrations[112]. Tolerance to the inhibitory effects of EtOH on cerebellar Purkinje

cell firing was also present both in vivo and in vitro. Interestingly, there was no apparent difference in the degree of tolerance between the lines [112]. Finally, HS, LS and SS mice were originally derived from the crossbreeding of 8 inbred mouse strains, and there was a strong genetic correlation ($r = 0.997$) between the mean sensitivity of the inbred strains to the effects of EtOH on LORR duration, and on the Purkinje cell firing rate [139]. This result was corroborated in a panel of inbred rat strains [90].

In summary, there is strong evidence which demonstrates a correlation between the behavioral depression produced by EtOH and the alteration in cerebellar Purkinje cell activity. The highly consistent results in selected lines from two species and panels of highly inbred strains of rats and mice make it virtually certain that a true, genetic correlation exists. However, these findings cannot completely account for the soporific effects of EtOH, since removal of the cerebellum in LS and SS mice does not completely abolish the differential sensitivity of the two lines [114].

EtOH effects on activity and coordination

Although there has been some disagreement among reports with regard to the relative baseline activities of LS and SS mice, results concerning EtOH effects on the locomotor activity of both lines have been largely consistent. In dose-response studies, LS mice exhibited little or no locomotor stimulation compared to vehicle-treated controls, while SS mice exhibited stimulation across a wide dose range. This is true of studies using photocell beam interruptions in an open field as the activity measure [45–47, 126], and in smaller, grid step-through chambers [18, 47, 49].

In studies requiring ambulatory coordination, LS mice were more uncoordinated under the influence of EtOH than were SS mice. LS mice committed more errors per activity count in a grid step-through test [18, 47, 49] and were much more inhibited by EtOH in an escape platform task [123]. One test of the discoordinating effects of EtOH, using a rotarod, failed to detect a difference between the lines [126]. However, another study requiring locomotion on a rod to escape cold water immersion did reveal greater EtOH impairment in LS mice [18].

Finally, the open-field locomotor activity of LS mice exposed in utero to EtOH was increased relative to sucrose controls [70]. However, the authors acknowledge the biasing effect of one litter with extreme scores. SS groups did not differ in activity. Average trials to criterion to learn a passive avoidance task was greater in prenatally exposed LS, but not SS, mice than in untreated, or sucrose-treated, controls [70]. These effects did not persist on a relearning test 24 h later.

Genetic correlation between EtOH's sedative and stimulating effects. The difference between LS and SS mice in EtOH-stimulated activity may be due to a pleiotropic action of genes affecting the selection phenotype. If so, this difference represents a genetically correlated response to selection. Alternatively, the EtOH-produced locomotor activity difference may be the result of inbreeding unrelated to selection. Results of several experiments designed to investigate the relationship between EtOH sedation and activation conflict. However, most of these experiments use phenotypic correlations to infer a genetic relationship. Due to the fact that phenotypic correlations derive from a combination of both environmental and genetic variation, such investigations are of questionable value in respect to tests of genetic relationships. For example, three such investigations, using genetically heterogeneous Swiss Webster mice [24], or the HS mice, progenitors of the LS and SS lines [124, 128], concluded that there was no relationship between sensitivity to EtOH's activating effects and the LORR selection phenotype. A fourth study used F_2 generation hybrids of the LS and SS lines and came to the opposite conclusion [44]. Finally, genetic correlations estimated from data with a limited number of inbred strains vary widely and have in some experiments approached, but never reached, significance [20, 24].

We believe that this issue has not yet been adequately investigated. As discussed in a previous section, blood or brain EtOH concentration at regaining the righting reflex is a more accurate measure of depressant sensitivity than is LORR duration, because it avoids the potential confounding factors of individual differences in EtOH metabolism, absorption or distribution. To date, unfortunately, this variable has not been used in correlational studies.

Neurotransmitters and activity. EtOH's effects on the activity and coordination of LS and SS mice have often been investigated by pharmacologically manipulating specific transmitter systems such as the GABAergic [101], purinergic [59, 123], and cholinergic [38] systems. However, most attention has been focused on dopaminergic systems [52].

Gamma-butyrolactone, which is thought to depress nigrostriatal DA neurons, was more effective in activating LS mice than SS mice [48]. At early time points, gamma-butyrolactone appeared to produce a depression in LS mice not seen in SS mice. However, large baseline activity differences between the lines at these time points make it difficult to interpret this result. Salsolinol, the condensation product of DA and acetaldehyde (an EtOH metabolite), activated SS mice at low doses and depressed LS mice at all doses; SS mice were also depressed at higher doses [18, 134]. Apomorphine, a DA receptor agonist, produced a similar dose-dependent depression in both mouse lines [46]. When coadministered with EtOH, apomorphine completely blocked the EtOH-induced depression of activity in LS mice and only partially blocked the EtOH-induced activation of SS mice [46]. Finally, haloperidol, a DA receptor antagonist, produced longer periods of catalepsy in SS than in LS mice [48].

Considered together with biochemical analyses of the brain DA systems of LS and SS mice (discussed elsewhere in this review), these data support some role for DA in mediating EtOH-induced activity differences between these mouse lines. However, the relationship seems to be a complex one which will require further consideration of pre- and post-synaptic receptor sites, receptor types and, certainly, brain localization. As there obviously are many neurochemical differences between LS and SS mice which are possibly related to EtOH's effects on activity, the absence of a specific EtOH receptor continues to complicate the process of unraveling this tangled web.

Specificity of EtOH's differential locomotor effects. The effects of drugs known to exert stimulatory effects at some doses, such as pentobarbital, paraldehyde, trichloroethanol, d-amphetamine, methanol, and t-butanol, as well as EtOH, have been examined on the locomotor activity of LS and SS mice [18, 45, 47–49, 126, 128]. SS mice were stimulated by all of these drugs. Depending on the study, LS mice also exhibited an increase in activity in the presence of each drug, with the exception of methanol. In all but two cases, however, SS mice were significantly more stimulated than were LS mice; trichloroethanol produced similar amounts of locomotor stimulation in both lines [128], and LS mice were more activated by d-amphetamine than were SS mice [48]. Therefore, differences between LS and SS mice in the locomotor stimulating effects of drugs do not appear to be limited to EtOH, or even to alcohols. Furthermore, the greater response of SS mice cannot be attributed to a general susceptibility to drug-induced stimulation, since d-amphetamine actually produced a greater stimulation in LS than in SS mice. In any case, these results suggest that there may be a common mechanism of action underlying the stimulatory effects of several of these agents. This would not be overly suprising, since most studies have been conducted with alcohols and barbiturates, which are known to have a high degree of pharmacological similarity and to show significant cross-tolerance.

Seizure susceptibility

Variability in the amount of locomotor stimulation induced by EtOH may reflect differences in CNS excitability. Another measure of CNS excitability which has been investigated in LS and SS mice is susceptibility to drug- or drug-withdrawal-induced seizures. Goldstein and Kakihana [74] demonstrated greater susceptibility of SS, as compared to LS, mice to EtOH-withdrawal seizures after EtOH dependence induction. More recently, due to growing support for a GABA Complex mediation of EtOH effects [94], and to the importance attributed to the GABA Complex in mediating seizure activity, seizure susceptibilities of LS and SS mice to agents with direct GABA Complex interactions have been a major research focus.

GABAergic agents. Pentylenetetrazole (PTZ) is thought to produce seizures via interactions with the picrotoxin site of the GABA Complex. LS and SS mice did not differ in ED_{50} values for PTZ-induced tonic hindlimb extensor seizures [74] or in ED_{50} values for clonic seizures [119]. However, LS mice have exhibited longer latencies to PTZ-induced myoclonus [119]. Using the latency to seizure measure, no line difference in sensitivity to PTZ-induced clonus [119], and greater sensitivity of LS mice (McIntyre and Alpern, submitted), have both been reported. Picrotoxin itself produced myoclonic and clonic seizures more quickly in SS than in LS mice [119]. SS mice were also more susceptible to seizures (myoclonus and clonus) induced by bicuculline (a GABA antagonist) than were LS mice [119, 142]. 3-Carbomethoxy-β-carboline (β-CCM), an inverse agonist at the BZ receptor, induced myoclonus more quickly in SS mice, but clonus more quckly in LS mice (McIntyre and Alpern, submitted). Finally, LS mice were more susceptible to convulsions induced by 3-mercaptopropionic acid (a competitive inhibitor of glutamic acid decarboxylase and a stimulator of GABA transaminase activity), than were SS mice [95, 99, 142]. These data are suggestive of a greater sensitivity for SS than for LS mice to GABAergic convulsants, but there are a number of inconsistencies. See table 2 for a summary of these results.

Other agents. Data are also available on seizure behavior produced by convulsants not known to interact directly with the GABA Complex. Latencies to flurothyl-induced

Table 2. Susceptibility to seizures and sensitivity to anticonvulsants in LS and SS mice.

Seizure-inducing treatment (seizure scored)	Susceptibility	Anticonvulsant and sensitivity	
Ethanol withdrawal after dependence			
(HIC)	SS > LS		
Pentylenetetrazole			
(tonic hindlimb extension)	SS = LS		
(myoclonus)	SS > LS	PH, Ro15-1788	SS = LS
(clonus)	LS > SS	PH, Ro15-1788	SS = LS
	LS = SS		
Picrotoxin			
(myoclonus)	SS > LS		
(clonus)	SS > LS		
Bicuculline			
(myoclonus)	SS > LS	EtOH	SS = LS
		PH, Ro15-1788	SS = LS
(clonus)	SS > LS	EtOH	SS = LS
		PH, Ro15-1788	SS = LS
B-CCM			
(myoclonus)	SS > LS		
(clonus)	LS > SS		
3-Mercaptopropionic acid			
(clonus)	LS > SS	FZ, DZ	SS > LS
Flurothyl			
(myoclonus)	SS > LS		
(clonus)	LS > SS		
	SS = LS	EtOH	SS = LS
Caffeine	SS > LS	PB, Ro15-1788	SS = LS
Nicotine	LS > SS	EtOH	LS > SS
Strychnine	SS > LS		
ECS			
(tonic hindlimb extension)	LS > SS	EtOH	SS = LS
		PB	LS > SS

PH, Phenobarbital; PB, Pentobarbital; DZ, Diazepam; FZ, Flurazepam; HIC, handling-induced convulsion; ECS, electroconvulsive shock. See text for applicable references.

myoclonus indicated greater sensitivity in SS than in LS mice, but the reverse was true for clonus [75, 76]. The results for flurothyl-induced myoclonus were corroborated in another study, but for clonus no line difference in sensitivity was found [127]. SS mice were more susceptible to seizures induced by caffeine and strychnine (McIntyre and Alpern, submitted), while LS mice were more susceptible to nicotine-induced convulsions [38]. Seizures have also been produced in LS and SS mice by transcorneally applied electroconvulsive shock (ECS), with LS mice being significantly more sensitive [21]. These results are also summarized in table 2. No general difference in seizure susceptibility between LS and SS lines appears strongly in these data; however, SS mice have been found consistently to be more sensitive to myoclonic seizures than LS mice.

Anticonvulsant sensitivity. The potencies of EtOH and other agents with anticonvulsant properties have been assessed in LS and SS mice against various convulsants (table 2). EtOH was clearly an effective anticonvulsant against ECS [21], nicotine (deFiebre and Collins, submitted), bicuculline [119] and flurothyl [127]. However, it's potency was equivalent for the two lines except against nicotine-induced seizures; in this case, the LS mice were much more sensitive. The BZs, flurazepam and diazepam, antagonized 3-mercaptopropionic acid seizures more effectively in SS than in LS mice [95, 142]. Pentobarbital antagonized ECS seizures [21] and flurothyl-induced convulsions [127] in these lines. No line difference in sensitivity was present for flurothyl seizure antagonism, but a greater sensitivity of LS mice to the anticonvulsant effect of pentobarbital against ECS was apparent. Phenobarbital antagonized PTZ-, caffeine-, and bicuculline-induced seizures equipotently in LS and SS mice. The BZ receptor antagonist, Ro15-1788, also antagonized seizures induced by these agents, but clearly with less potency than phenobarbital (McIntyre and Alpern, submitted). There was a strong trend toward greater effectiveness against bicuculline seizures in LS compared to SS mice, while the opposite was true for antagonism of PTZ seizures.

Conclusions. Although the above data offer no clear indication of greater, general seizure susceptibility for either of the mouse lines, this is not surprising, since many agents with differing pharmacological mechanisms of action have been tested, and different seizure endpoints have been examined. A clear finding has been the greater susceptibility of SS mice to myoclonic seizures regardless of site of initiation. Greer and Alpern [6, 75] have suggested that myoclonus and clonus are controlled by neuropharmacologically distinct substrates. Specifically, they argue for a role for DA in mediating myoclonus, which would be consistent with a hypothesis of differences between LS and SS mice in DA systems, as previously discussed. A greater CNS excitability of SS mice, like that indicated by EtOH locomotor activation data, might apply to specific, as yet undetermined, modes of initiation.

EtOH self-administration and reinforcement

There is obvious interest in the potential relationship between genetically determined sensitivity to EtOH and the magnitude of EtOH's reinforcing effects. Several methods for assessing EtOH's reinforcing properties exist, but little genetic data have been available until recently. An early study reported sensitivity to EtOH as an unconditional stimulus in a test of conditioned taste aversion to saccharin solutions. LS and SS mice did not differ in sensitivity to the anhedonic effects of EtOH in this test [43]. In a test of withdrawal symptomatology in LS and SS mice forced to consume a liquid diet containing EtOH for 7 or 14 days, SS mice consumed more diet than did LS mice [69]. While this may have contributed to their generally more severe withdrawal symptoms, consistent with an earlier report [74], blood EtOH levels achieved in the lines did not differ systematically [69]. The most direct assessment to date of EtOH's reinforcing effects in LS and SS mice is an experiment in which LS and SS mice were initiated to bar press for access to EtOH. LS mice eventually bar-pressed consistently under an FR4 schedule to receive access to 8% EtOH solutions and attained average post-session blood EtOH concentrations of up to 300 mg/dl. SS mice responded sporadically, and achieved post-session EtOH levels of 60 mg/dl [65]. These very interesting results certainly warrant extension to delineate the relative importance of genetic and non-genetic variables controlling self-administration.

Withdrawal seizure-prone (WSP) and -resistant (WSR) mice

Development of the lines

We began ten years ago to develop an animal model of genetic susceptibility to develop severe and mild signs of withdrawal after chronic treatment with EtOH. Using within-family selective breeding, and starting with the HS mouse stock used to produce all of the selected lines discussed in this review, lines and replicates have been selected to be prone (WSP) and resistant (WSR) to the withdrawal seizures seen after chronic exposure to EtOH [32]. WSP and WSR lines now differ at least 10-fold in convulsion severity and in other EtOH withdrawal signs after identical chronic exposure to EtOH. To produce physical dependence, mice are exposed to EtOH vapor inhalation for 72 h. Daily injections of the alcohol dehydrogenase inhibitor, pyrazole, stabilize EtOH concentrations. After 72 h mice are removed from the vapor chambers and scored for withdrawal severity each hour for 15 h and at 24 h and 25 h using the handling induced convulsion (HIC) test. The details of these methods have been published [31]. One male and one female mouse from each of the 9 original families (litters) were chosen at random to constitute a control (Withdrawal Seizure Control: WSC) line. The WSC line thus serves as a control for the small but unavoidable degree of inbreeding. From the remaining mice, the highest-scoring male and

female from each family were chosen for the WSP line and the lowest-scoring male and female from each family were chosen for the WSR line. The experiment is replicated; thus, there are two WSP, two WSR, and two WSC lines, maintained and selected completely independently of one another.

Selection has progressed to generation S_{26} (G_{30}) and is continuing. Male and female WSP mice from each replicate were found to have significantly higher seizure scores than the respective WSR mice after 5 selected generations [30], and showed approximately 10-fold higher withdrawal scores than WSR mice after 11 selected generations [32]. Control lines showed intermediate responsiveness. In genetic terms, the total realized response to selection continued to increase during the first 11 generations as increasing cumulative selection pressure was applied. The estimated heritability, or percent of total variability in response that is genetically additive, is approximately 26%, which represents a substantial amount for a presumptively complex character. Inbreeding in the lines has been held to the relatively low level of approximately 1.5% per generation. A full report of the genetic parameters of the selection study has been published [32].

Periodically during the course of this project, we have directly compared WSP and WSR mice for severity of EtOH withdrawal signs after chronically exposing the animals to EtOH vapor for 72 h in the same inhalation chamber. No important further divergence between the WSP and WSR selected lines has occurred since the 11th selected generation. For the WSR mice, this is due to a floor effect, while in the WSP lines, there remains scalar room for higher scores, but they are simply not attained.

The second important result revealed by these studies derives from the fact that between generations G_{16} and G_{24} we relaxed selection for two generations (S_{22} G_{23} and S_{22} G_{24}). Animals were mated according to the scheme consistently used, but without regard to withdrawal score. A comparison of the selected lines in S_{22} G_{24} did not reveal any apparent decline in the difference between WSP and WSR lines, which suggests a fixation of almost all relevant alleles in the homozygous state.

Seizure susceptibility

We have been testing the possibility that the WSP and WSR lines might have been selected for general differences in CNS excitability, and that we were simply using EtOH withdrawal to reveal that difference. We predicted that if this were the case, WSP mice should have greater sensitivity to convulsant treatments than WSR mice. When tested in S_8–S_{10}, the ED_{50} to produce maximal tonic hindlimb extensor- (THE), PTZ-, picrotoxin-, bicuculline-, strychnine-, flurothyl- and ECS-induced seizures did not differ between WSP and WSR mice [106]. This is not the most sensitive method for testing the hypothesis, however, for more subtle differences in seizure susceptibility might be revealed by examining ED_{50} to produce threshold, rather than maximal, seizure signs. In recent generations, we have used two further methods to address this hypothesis.

First, we have administered convulsant agents by timed tail-vein infusion. This procedure allows an accurate determination of threshold dose for each animal and provides an estimate of the ED_{50} characterizing the line with a minimal number of animals. The choice of convulsants has been guided by the potential role of the GABA Complex as an important site in the WSP/WSR difference in EtOH withdrawal seizures. Thus, we have used picrotoxin receptor agonists, GABA receptor antagonists, BZ receptor inverse agonists, and drugs which act by mechanisms not known to be related to the GABA Complex. WSP mice were significantly more sensitive to picrotoxin seizures than WSR mice. Importantly, this difference was seen in both genetic replicates. Similar differences were seen in mg/kg dose infused, so the differences in latency are not likely attributable to differences in drug absorption or distribution. Parallel differences were seen in sensitivity to the drugs, CHEB (a convulsant barbiturate) and the potassium channel blocker, 4-aminopyridine. No apparent common mechanism of action can explain the similarity in response to these three drugs across selected lines.

In contrast, for several other drugs, a different pattern has been found. Strychnine is a glycine receptor antagonist. While WSP1 mice responded significantly more quickly than WSR1 mice, this difference was not present in the replicate WSP2 and WSR2 lines. This suggests that accidental fixation of genes in the WSP1/WSR1 mice has occurred, rather than indicating a true pleiotropic effect of some genes on EtOH withdrawal and strychnine sensitivity. A similar pattern of responses was found for kainic acid (an excitatory amino acid receptor agonist), DMCM (a BZ inverse agonist), and the functional GABA antagonists TBPS, PTZ, and bicuculline (manuscript in preparation). In all cases, WSP1 mice were more sensitive than WSR 1 mice, but the second replicate pair of selected lines did not differ.

These data suggest that there may be specific differences in seizure susceptibility that have been fixed in the WSP and WSR mice during the process of selection for EtOH withdrawal severity. They also suggest that the presumed mechanisms of action of the convulsant drugs tested may not be as clear as supposed. For example, picrotoxin, PTZ, and TBPS are all thought to act at the same binding site on the GABA Complex, but the pattern of genotypic differences among the four WSP/WSR mouse lines tested was not the same for these drugs. This finding was confirmed in studies with a panel of ten inbred strains (Kosobud and Crabbe, submitted).

We have also used the method of examining the proconvulsant effects of various agents by administering subconvulsant doses and measuring the severity of HICs in WSP and WSR mice. We administered picrotoxin i.p. in doses of 2–8 mg/kg to separate groups of WSP and WSR

mice. Before, and each 20 min after injection for 120 min, the mice were scored for HIC on a scale ranging from 0 to 4. Picrotoxin administered in subconvulsive doses exacerbated HIC in both lines of mice. WSP mice were more sensitive than WSR mice to this elevation in HIC[58]. Using this method, we have also tested proconvulsant effects of PTZ, bicuculline, strychnine, the BZ receptor inverse agonist, Ro15-4513, the glutamic acid decarboxylase inhibitor, 3-mercaptopropionic acid, and nicotine. WSP mice were more sensitive than WSR mice to all compounds, and all of these effects were equally displayed in mice of both replicates.

Differences in sensitivity to anticonvulsants

As discussed in the previous section, the WSP and WSR lines did not differ in sensitivity to maximal THE seizures elicited by ECS. However, EtOH pretreatment antagonized ECS-induced seizures to a much greater degree in WSR mice than in WSP mice[106]. This differential sensitivity in WSR mice generalized to several alcohols, to three barbiturates, and, interestingly, to three unrelated anticonvulsants[36]. We have subsequently determined that WSR mice are also more sensitive to the BZ, carbamazepine[27], and to ethosuximide. Thus, WSR mice are more sensitive to anticonvulsants of most major classes. The mechanisms underlying this line difference are unknown, and given the lack of clear understanding of the basis for ECS-induced seizures, we do not feel that pursuing anti-ECS effects is likely to be fruitful. Rather, we plan to examine EtOH's anticonvulsant effects against threshold seizures elicited by tail-vein or intracranial infusions of the agents as described in the previous section. Using similar logic, we hope to be able to isolate seizures induced by some agents which are more vulnerable to differential EtOH inhibition in the WSP and WSR lines.

Neurochemical and molecular studies on WSP and WSR mice

The GABA Complex. Given the interesting seizure susceptibility differences to GABA Complex compounds between WSP and WSR mice reported in the preceding section, we assayed binding characteristics of receptors in discrete brain areas using TBPS, a cage convulsant binding to the picrotoxin site of the GABA Complex. WSP and WSR mice were found to differ in neither K_D nor B_{max} for TBPS binding in frontal cortex, remainder of cortex, hippocampus, or cerebellum. Furthermore, there were no significant differences in BZ receptor affinity or density among the two WSP and two WSR mouse lines[58]. Since GABA enhances flunitrazepam binding through a coupling mechanism, we determined the sensitivity of whole-brain preparations from WSP and WSR mice to GABA-stimulated flunitrazepam binding, and found their sensitivities to be equivalent. Thus, initial attempts to identify the neurochemical substrates for differences in sensitivity to GABA Complex agents between WSP and WSR mice have been unsuccessful. A plausible explanation for this is that we are not using a fine enough level of anatomical resolution to detect the relevant differences. For example, J. K. Belknap has selectively bred mice for opiate sensitivity, and has found that the lines differ only slightly in μ-receptor binding characteristics when examined in brain-regional homogenate binding assays. However, μ-opioid receptor autoradiography has enabled him to detect a markedly enhanced concentration of μ-receptors in the dorsal raphe of the opiate sensitive line, as compared to the insensitive line (Belknap, Laursen, Sampson and Wilkie, submitted). For this reason, we intend to pursue the neurochemical differences between WSP and WSR mice with receptor autoradiography using labeled flunitrazepam, TBPS, and bicuculline.

We have also recently begun to study the neurochemistry of the GABA system at the chloride channel level, and found that non-EtOH treated WSP and WSR mice do not differ in GABA-induced chloride flux in mouse brain microsacs.

Hippocampal mossy fiber zinc content. Evidence has been accumulating which suggests a relationship between hippocampal mossy fiber zinc content and seizure activity. It has recently been shown that there is a high correlation between HIC scores after EtOH withdrawal and mean hippocampal mossy fiber zinc content (Feller and Savage, submitted). There is significantly less zinc in WSP than in WSC or WSR mossy fibers. It is not clear at this time if this difference in zinc content is directly involved in regulating the severity of EtOH withdrawal seizures. However, the high correlation observed, and the 70% reduction in WSP zinc content, indicate that differences in hippocampal mossy fiber function may be one important factor.

Protein variation in the WSP and WSR lines. Goldman and Crabbe[71] have investigated brain-specific protein genetic variants in the WSP, WSR and WSC lines from both replicates of the 11th selected generation. 2-D electrophoretic gels were prepared from whole brain homogenates for 8 mice per line, and 11 proteins were initially identified which displayed apparent genetic variability, where an acidic and a basic form of the protein had different electrophoretic mobilities in different inbred strains. Ten of the 11 proteins still showed apparent heterozygosity indicating that inbreeding was not complete in the selected lines. Two proteins showed clear evidence for allelic frequencies which correlated with genotype. For example, for one protein, the allelic frequencies for the basic allele were 0.13 and 0.21 in the prone lines, zero in both resistant lines, and 0.08 and 0.06 in the nonselected control lines. This suggests that this protein may play a role in determining genetic predisposition to develop EtOH dependence[71].

A similar gene frequency pattern was seen for another protein, which has been identified as LTW-4, a 28 kDa, pI 5.6 protein expressed in brain (where it accounts for 0.25% of total protein), liver, and kidney. Its function is

unknown, and it has been mapped to chromosome 1. Recent work has demonstrated an association between EtOH acceptance, a trait related to EtOH preference drinking, and the presence of the basic allele at this locus. Panels of inbred strains and recombinant inbred strains have both confirmed this association [72]. Further studies are in progress to explore these relationships in genetically segregating populations of mice.

Role of membrane fluidization. For many years, the dominant hypothesis of EtOH's mechanism of action was that its primary effect was to disorder neuronal membranes. This 'fluidization' was suggested to compromise normal function at critical receptor/ionophore complexes, leading to behavioral disruption. We tested the membrane hypothesis by feeding WSP and WSR mice a liquid diet containing EtOH to induce physical dependence. Synaptosomal membrane preparations from brains of dependent animals, or those which had been treated with a control diet, were assayed for intrinsic membrane order and for sensitivity to EtOH's effects on order in vitro using fluorescence polarization. Membranes from naive WSP and WSR mice had equivalent intrinsic rigidity. EtOH dependence increased membrane rigidity in both lines to an equivalent degree. Sensitivity of neuronal membranes to EtOH in vitro was also similar [80]. The lack of genetic differences in basal membrane fluidity and in sensitivity to EtOH or sodium valproate were verified using a different method, electron spin resonance [117]. These experiments do not disprove the membrane hypothesis, but they indicate that more sensitive measures may be necessary to reveal the subtle differences underlying the very large withdrawal severity differences.

Other withdrawal signs

There are many symptoms of alcohol withdrawal other than the seizures for which WSP and WSR mice were genetically selected. When mice from the WSP and WSR lines were rendered physically dependent in the same inhalation chambers, and compared systematically for a number of withdrawal signs after 22 selected generations, WSP mice of both replicates exhibited more severe Straub tail than WSR mice at several times during withdrawal. Similarly, WSP mice of both replicates had more pronounced hypothermia during EtOH withdrawal than WSR mice at 7 and 15 h after withdrawal of EtOH. Thus, some signs have co-segregated with HIC scores during the process of selective breeding, but other signs (e.g. activity reductions) do not differ between the lines [28]. It is interesting to note that the pattern of withdrawal signs now differing in WSP and WSR mice has changed over generations of continuing selection. After 11 generations of selection, WSP mice tended to exhibit greater levels of Straub tail then WSR mice, but the differences did not achieve statistical significance [93]. That the lines now differ significantly probably represents the gradual development of a correlated response to selection, implying the action of some of the same genes on both traits.

Role of EtOH metabolism and basal HIC scores

When we compared WSP and WSR lines in generation S_9, factors other than genetic predisposition to EtOH withdrawal severity could not account for the line difference. WSP and WSR mice from generations S_9 or S_{22} did not differ in distribution or elimination of an acute dose of EtOH [93]. Blood EtOH concentrations (BEC) during the 72-h period of exposure to EtOH vapor or during the early hours of withdrawal did not differ significantly in S_9 mice treated in the same inhalation chamber [93]. Therefore, the line differences in withdrawal severity were probably due to functional (CNS) rather than dispositional mechanisms such as line differences in rates of metabolism, absorption or distribution. However, in a comparison of S_{16} mice, we noted a tendency toward increased BECs in WSP mice during chronic exposure. More recently, we studied EtOH metabolism in mice of generation S_{22} during chronic exposure to EtOH vapor and during withdrawal. WSP mice of both replicates now accumulate significantly more EtOH during 72 h exposure than their respective WSR mice. This effect becomes more pronounced over days of exposure. However, there are still no differences in elimination of EtOH after animals are removed from the inhalation chambers.

The bases for the differences in the accumulation of EtOH during chronic exposure are not currently understood. They are not limited to the inhalation paradigm, and are likely not, therefore, due to differences in pyrazole sensitivity. J. K. Belknap has seen similar differences in WSP and WSR mice fed liquid diets containing EtOH (personal communication). The differences in EtOH accumulation are not large enough to account for the very great differences in EtOH withdrawal severity. As discussed in an earlier section, similar differences in EtOH pharmacokinetics arose in the later stages of the selection for EtOH's hypnotic properties in LS and SS mice. Since the WSP/WSR differences arose late in selection, after maximal separation of the WSP and WSR lines had been achieved, we do not feel that they are a critical genetic correlate of withdrawal severity; rather, they are a minor nuisance in attempts to use the WSP and WSR lines to address mechanistic questions.

In mice from S_9, we had reported small but consistent differences among the lines in either baseline HICs or in the very slight effect of pyrazole on HICs [93]. Specifically, WSP mice had higher basal scores and were slightly more affected by three days of pyrazole treatment (without EtOH) than were WSR mice. Examination of these differences in S_{22} revealed that they had become no larger. These differences are extremely small compared to the withdrawal seizure score differences seen between the two lines.

Neurosensitivity and tolerance to EtOH

Many investigators assume that the physiological substrates of alcohol sensitivity, tolerance, and dependence represent a continuum of increasing duration of drug

exposure. This reasoning suggests that study of the neurobiological changes underlying sensitivity to EtOH should elucidate the mechanisms of tolerance and dependence development, or vice versa. A series of experiments carried out with WSP and WSR mice suggest that this formulation is too simplistic. These studies revealed that genetic predisposition to develop EtOH withdrawal is not associated with genetic sensitivity to acute EtOH, or with EtOH tolerance development. WSP and WSR mice were equally stimulated by EtOH in an open field test[28]. They also have identical sensitivity to the acute hypothermic effect of EtOH[26]. Futhermore, the lines developed an equivalent magnitude of tolerance to the hypothermic effects of EtOH when EtOH was administered daily for three days. In addition, brain concentrations of EtOH at the time of LORR were virtually identical in the selected lines. Thus, intense selection for EtOH withdrawal severity differences has not been accompanied by the development of marked differences in sensitivity or tolerance to two other effects of EtOH[26]. This argues persuasively that the mechanisms underlying sensitivity, tolerance, and dependence are to a significant degree independent. Studies of genetic correlation among inbred strains support this interpretation[20, 25, 35].

Genetic cross-susceptibility to other drugs

It was of interest to determine whether genetic sensitivity to withdrawal from EtOH conferred similar sensitivity to other drugs known to be cross-dependent with EtOH. We rendered WSP and WSR mice physically dependent on phenobarbital by feeding them a drug admixed chow. During the ensuing withdrawal period, WSP mice achieved significantly greater withdrawal scores than WSR mice on several variables including handling seizures, even though they had achieved similar brain barbiturate concentrations[13]. We also examined cross-susceptibility to diazepam. WSP and WSR mice from the 5th and 13th selected generations were fed diazepam diets for 7 days. When withdrawal was precipitated with the BZ antagonist, Ro15-1788, and HIC severity was scored, WSP mice had greater mean HIC scores than WSR mice during the 20-min test. Furthermore, the difference in the intensity of withdrawal between WSP and WSR mice was larger when the experiment was repeated in S_{13}. These results could not be attributed to differences in dose or metabolism of diazepam[15]. The increased divergence of sensitivity to diazepam is another clear example of the development of a correlated response to selection.

Similar experiments exposed WSP and WSR mice to the anesthetic gas, nitrous oxide (N_2O), for differing periods[14]. WSP mice showed more marked HIC than WSR mice after exposure to different concentrations of N_2O. Together, these results suggest that genes predisposing mice to develop physical dependence on EtOH also predispose to dependence on other CNS depressant drugs. Generality to all psychoactive drugs was not complete, however. Similar experiments with morphine diets revealed that WSR mice had more severe naloxone-precipitated morphine withdrawal than WSP mice after five selected generations. The morphine experiments should be repeated in mice of a current selected generation.

Preference for EtOH solutions

We have naturally been curious about the possibility that our WSP and WSR selected lines might differ in their propensity to self-administer EtOH orally. Accordingly, we tested mice from the 17th selected generation for three 8-day sessions. During these sessions, EtOH was offered versus tap water 24 h/day at concentrations of 2.2, 4.6 or 10.0% (v/v). WSR mice had a significantly higher preference for the 2.2% EtOH solutions than WSP mice; WSC mice tended to be intermediate. At higher concentrations, the preference of WSR2 mice remained elevated, while that of the other lines showed the expected decline in preference with increasing EtOH concentration. Results of these experiments suggest that some genes influencing EtOH withdrawal severity also influence voluntary EtOH drinking[92].

Summary

Alcohol dependence and withdrawal are clearly complex phenomena. The data reviewed in the preceding sections characterizing the WSP/WSR genetic animal model suggest that genes influencing EtOH withdrawal severity play a fairly specific role in modulating EtOH effects in mice. Although some evidence for single gene differences with measurable impact on the alcohol withdrawal syndrome has been revealed, the preponderance of data at this stage reveal a surprising lack of difference between WSP and WSR mice for most alcohol-related traits. This indicates a need to reconsider the relationships among sensitivity to, tolerance to, and physical dependence on EtOH. On the other hand, the fact that some traits differ markedly between WSP and WSR mice offers hope that those traits are importantly related to the underlying genetic susceptibility to dependence. The increasing interest of investigators in studying these and other selected lines of mice may lead to better articulation of the hypotheses regarding genetic predisposition to alcoholism.

Severe (SEW) and Mild (MEW) Ethanol Withdrawal mice

Another selective breeding program for severity of the EtOH withdrawal syndrome is in progress at the Institute for Behavioral Genetics in Boulder, Colorado. Development of this animal model of physical dependence on EtOH shares the ultimate goal of a greater understanding of alcoholism with the WSP/WSR breeding program. However, the methods used to obtain these two models have differed in many ways.

Treatment and testing

McClearn et al.[103] have published the detailed procedural methods used in the development of the replicated

SEW, MEW, and C (control) mouse lines. Animals are singly housed and physical dependence is produced over a period of 9 days by EtOH liquid diet administration in increasing concentrations up to a maximum of 35% EtOH-derived calories. Consumption is recorded, and animals are withdrawn from EtOH 6 h prior to behavioral testing. Each animal is scored on a battery of tests between the 6th and 7th hour after EtOH withdrawal. Tests include handling-induced convulsion severity, activity and anxiety variables in a hole-in-wall apparatus, body temperature, presence of defecation and urination, and quantification of vertical screen activity. The above scores and EtOH consumption are intercorrelated and entered into a principal component analysis. Selection indices are computed from the component score coefficients, providing quantification of severity of the EtOH withdrawal syndrome for each animal.

Selective breeding

The initial foundation population consisted of 30 litters. Ten litters were randomly chosen and a male and female was drawn from each litter and mated randomly to initiate Control line 1 (C-1). A female and male from each of these litters exhibiting the most severe EtOH withdrawal syndrome were chosen and mated inter se to initiate the SEW-1 line. Similarly, the MEW-1 line originated from the least severely affected animals of these litters. Replicate 2 lines (C-2, SEW-2, and MEW-2) were chosen identically from a separate set of 10 litters. In subsequent generations, within-family selective breeding has continued with each line constituting a closed breeding population[103].

SEW and MEW lines differed significantly after five selected generations[5]. After 10 generations of selective breeding, the SEW and MEW lines differed by about 1.2 standard deviation units. The response to selection has been largely unidirectional with realized heritabilities for the SEW and MEW lines of about 0.2 and 0, respectively[144]. Wilson and coworkers[144] have described a number of problems they have encountered during the course of this selection. Most critical have been the high death and infertility rates. In addition, one of the unselected control lines (C-1) consistently had milder EtOH withdrawal scores than either of the MEW lines, at least until generation 9. An association between severity of withdrawal response and EtOH consumption values may account for this difference. The C-1 line animals have the lowest consumption scores while those of the SEW lines consume the most. An increase in EtOH consumption by all lines with increasing generations of selection has also been noted and likely has an impact on fertility and death rates. By generation S_{10}, mice of all lines were consuming 35–50% more than animals of generation S_0. Wilson et al.[144] interpret this overall increase as an effect of natural selection for animals able to obtain adequate nutrition and survive to reproduce in the presence of alcohol toxicity. Sensitivity to EtOH's hypothermic and depressant effects was assessed in SEW and MEW mice of generation S_9[144]. A line difference in LORR duration and in BEC at waking was found, with SEW-1 mice exhibiting longer durations and lower BEC's than MEW-1 mice. However, this difference was not present between the replicate 2 lines. No apparent line differences in hypothermic response to EtOH were found.

Comparison of SEW/MEW and WSP/WSR selected lines

Recently, we directly compared mice from the two EtOH withdrawal selection programs (manuscript in preparation). Experimentally naive SEW, MEW, WSP, and WSR mice were made physically dependent using the 72-h EtOH inhalation technique described for the WSP/WSR selection. At various times on the day of EtOH withdrawal, tail-blood samples were taken for BEC determination, HIC and rectal temperatures were assessed, animals were observed for Straub tail and tremor, and climbing behavior and activity tests were performed.

Although all animals were housed in the same inhalation chamber and, therefore, should have been exposed to the same amount of EtOH, SEW and MEW mice attained much higher blood levels than did WSP and WSR mice. There were no differences in BEC between WSP/WSR lines or SEW/MEW lines.

WSP mice of both replicates exhibited larger HIC scores than WSR mice, as expected. However, a difference in HIC scores of SEW and MEW mice was only present for replicate 1 animals with SEW mice more affected. Perplexingly, this is the replicate set of lines that has diverged least during selection. When differences in HIC responses to pyrazole were accounted for, SEW and WSP mice (and MEW-2 mice) had similar HIC scores. HIC scores of WSR mice were much lower than those of all other lines.

Hypothermia was present in mice of all lines 3 h after EtOH withdrawal. WSP mice were more hypothermic than WSR, and SEW mice were more hypothermic than MEW. There were no apparent differences in thermal sensitivity between WSP/WSR and SEW/MEW mice. Little occurrence of Straub tail or tremor was observed in any of the animals, suggesting that they were not dependent enough to display these signs. Similarly, climbing behavior and locomotor activity were little affected during the course of withdrawal.

In the hole-in-wall apparatus, crossing between compartments was suppressed and latency to leave the dark was reduced during EtOH withdrawal, but headpoking behavior and the number of rearings were little altered. In addition, more time was spent in the dark compartments during withdrawal. There was no difference between WSP and WSR mice in the amount of crossing suppression. However, opposite to expectation, MEW-1 mice were more suppressed than SEW-1. This difference in magnitude of suppression was not seen in replicate 2 SEW and MEW mice. In general, mice of all lines exhibited similar amounts of suppression with MEW-1 being

the aberrant group. Latencies to leave the dark were decreased more in SEW than in MEW mice; again this difference was larger for replicate 1. This pattern of sensitivity was reversed for the WSP/WSR mice with the resistant mice exhibiting more greatly reduced latencies than the prone. There was no suggestion of a greater effect in lines of one selection compared to lines of the other. Lastly, there was a suggestion of a larger effect of withdrawal in SEW mice on time spent in the dark (a greater increase) compared to MEW, but this difference was not seen for WSP and WSR mice.

Conclusions

The SEW/MEW selection program has met with some success. The sensitive and insensitive lines have diverged in severity of the withdrawal syndrome and the latest evaluation indicates that the C-1 line may be approaching a more intermediate severity value [144]. However, the high death and fertility rates pose severe problems as do line differences in EtOH consumption during dependence induction. It might be argued that the difference between SEW and MEW mice in withdrawal severity is completely accounted for by differences in EtOH dose. We believe that administration of metered EtOH doses followed by withdrawal severity measurement is a critical experiment in the evaluation of the success of this program.

The comparative experiment with SEW/MEW and WSP/WSR mice is difficult to evaluate in reference to existing results with the SEW and MEW mice [144]. For one thing, mode of physical dependence induction was much different and the magnitude of dependence achieved may differ between methods. The most striking difference observed was the size of the line difference in HIC severity seen between lines within selection program. The difference was much larger between WSP and WSR mice and was not present in replicate 2 SEW and MEW mice. It would be of interest to use EtOH liquid diet to induce physical dependence concomitantly in SEW/MEW and WSP/WSR mice, with the testing methods used for the SEW/MEW selection.

The Boulder group recently reported that they are developing another model. Replicate High (HA_1, HA_2), Low (LA_1, LA_2), and Control (CA_1, CA_2) lines are being made physically dependent upon EtOH by a 12-day liquid diet regimen. Withdrawal severity is indexed by HIC during hours 0–8 of withdrawal. Response to selection after one generation suggests that this method may also yield significant genetic differences [16].

New mouse lines selected for sensitivity to EtOH

In addition to the withdrawal-seizure lines, we are responsible for two other selective breeding projects. We are developing a genetic animal model for sensitivity to a depressant effect of EtOH, acute hypothermia (HT). These lines are designated HOT (unresponsive) and COLD (responsive). The other set of lines is being selected for EtOH-stimulated locomotor activity in an open field (ACT), since this behavioral response may model the disinhibitory and/or euphoric effects of the drug. FAST mice are highly stimulated by EtOH, and SLOW mice show less activation, or even depressed activity, after EtOH.

Our methods for the development of these lines closely follow those for the WSP, WSR, and WSC lines. Two randomly-selected control lines (CON1 and CON2) are held in common for both selections. Each line comprises 9 families and we are currently in selected generations 14 and 15 for the activity and hypothermia lines, respectively.

HOT and COLD lines

Testing and selection procedure. HOT and COLD mice are tested for sensitivity to the acute hypothermic effect of EtOH [25]. After the baseline temperature is taken, each mouse is weighed and injected with EtOH (3 g/kg, 20% v/v in physiological saline, i.p.). Temperatures are taken again 30 and 60 min later, and a tail blood sample is taken for EtOH concentration assays at 60 min. The greater change from baseline is used to index sensitivity to EtOH in the COLD lines, and the smaller change is used in the HOT lines.

Selective breeding has increased the HT response to EtOH in the COLD lines but has not decreased it in the HOT lines. We test the CON lines each third generation. It is clear that there has been a large divergence in sensitivity to EtOH hypothermia in both sexes and both replicates of the experiment [29].

Estimation of genetic parameters. Heritability for the total realized response difference between HOT and COLD lines was 0.20 after 14 generations of selection, which implies that 20% of the total variance in EtOH-induced HT in mice is of additive genetic origin. This is due almost entirely to heritabilities of about 0.36 in the COLD lines. The inbreeding coefficient (F) is increasing at about the expected rate of 1.5% per generation. Thus, inbreeding at genetic loci irrelevant to the EtOH response probably plays a relatively minor role in determining differences between COLD and HOT lines.

Dose-effect relationships and EtOH metabolism. We have assessed the dose-dependence of the response to EtOH-induced HT in COLD and HOT mice repeatedly during the course of selection. When mice from both replicates of S_{11} were given 1–4 g/kg EtOH, COLD mice had greater HT responses than HOT mice. The average HT response of COLD mice was 3.6 °C compared to 1.8 °C for HOT mice after 3 g/kg EtOH. The magnitude of the difference seen at the lowest effective dose tested (2 g/kg) was less than at higher doses [23]. This may be because hierarchically-organized sets of thermoregulatory control mechanisms are challenged in dose-dependent fashion by EtOH. COLD and HOT mice might have devel-

oped differences only in the effector systems sensitive to higher doses of EtOH.

HOT and COLD mice do not differ significantly in BEC at 60 min after injection of a fixed dose of EtOH[23]. The rate of metabolism of a 3 g/kg dose of EtOH in mice from S_{10} differed only slightly between HOT and COLD mice. HOT mice had non-significantly lower brain EtOH concentrations than COLD mice only 3 and 4 h post-injection. Rates of metabolism in COLD and HOT mice were 0.86 and 1.01 mg/g brain/h. This small difference may be limited to higher doses of EtOH, and may be secondary to the line differences in body temperature at these doses[23].

Generalization to other alcohols and depressants. We thought it possible that selection for EtOH HT had altered sensitivity to the hypothermic effects of other depressants. Indeed, we have found that S_7 COLD mice were more sensitive than HOT mice to several straight-chain alcohols. They were also more sensitive to pentobarbital, phenobarbital, methyprylon (a water-soluble depressant), the highly lipid-soluble alcohol, ethchlorvynol, and diazepam. Thus, some genes controlling EtOH HT also control HT responses to other depressants, which in turn suggests commonality in their mechanism of action on the thermoregulatory system (Feller and Crabbe, submitted). However, generalization is not complete, for HOT and COLD mice have not differed in sensitivity to several other drugs affecting specific neurotransmitter systems.

As selection pressure was exerted each generation, the magnitude of the EtOH HT difference between COLD and HOT mice increased. The correlated sensitivity differences to other depressants were also augmented by additional selection (Feller and Crabbe, submitted). Such parallel development of a correlated response to selection in both pairs of selected lines offers very strong evidence for a true genetic correlation[83]. Another case where a genetically correlated response emerged in later generations of selection was that of the peripheral vasodilator, hydralazine. S_7 HOT and COLD mice did not differ in sensitivity to hydralazine HT. However, a significant difference was present in S_{11} mice. It appears that peripheral thermoregulatory mechanisms may now play a significant role in mediating the difference between HOT and COLD mice in sensitivity to EtOH (Feller and Crabbe, submitted).

Tolerance. We have tested the hypothesis that HOT and COLD mice might differ in their development of tolerance to EtOH HT[23]. When EtOH was given daily, the HT response of COLD mice was significantly attenuated by previous EtOH injections, while that of HOT mice remained approximately the same. COLD mice had significantly greater HT than HOT mice during the 4 days of EtOH exposure to induce tolerance, however, and HOT mice might have metabolized EtOH more rapidly than COLD mice. On the other hand, when different EtOH doses were given to equate HT response, COLD mice still developed tolerance, but no tolerance was seen in HOT mice. Thus, the failure of HOT mice to develop tolerance was not due to insufficient exposure to EtOH HT. In all of these experiments, BEC at the time of testing was assayed and found not to differ. We conclude that the differences between lines are in functional tolerance rather than due to metabolic factors[23]. Recent studies where animals are subjected to a cold ambient environment plus EtOH have found that HOT mice are capable of developing tolerance, but the magnitude of tolerance is still less than that of COLD mice similarly treated (unpublished).

Other correlated responses to selection. We have tested mice of both lines for severity of withdrawal, because earlier experiments with a number of inbred strains of mice had reported a negative genetic correlation between sensitivity to EtOH's initial hypothermic effects and the severity of EtOH withdrawal after cessation of forced EtOH inhalation[35]. HOT and COLD mice were exposed to identical regimens of forced EtOH exposure for 72 h and withdrawn, and their withdrawal seizures scored. There was more than a two-fold difference between HOT-1 and COLD-1 mice in handling-induced convulsion severity during withdrawal, but HOT-2 and COLD-2 mice did not differ[28]. The magnitude of difference in the first replicate set of HOT and COLD lines is suggestive of an important effect of a single locus. An earlier study with a panel of recombinant inbred strains had also hinted at an important single gene in this trait[31].

HOT and COLD mice have also been tested for locomotor activation after EtOH (2.0 g/kg, 20% v/v) and saline in an open field. While HOT-1 and COLD-1 mice did not differ in activity, HOT-2 mice showed 7–8-fold more activation than COLD-2 mice, and 2–3-fold more than either COLD-1 or HOT-1[28]. These results may also represent the influence of a single locus on EtOH ACT, a possibility raised by results of the selection study to be discussed next.

Finally, HOT and COLD mice were tested for development of a taste aversion conditioned by EtOH. Mice were allowed to drink sodium saccharin solutions and then injected with EtOH. On the following day, access to water only was offered, and no injections were given. This alternating pattern of conditioning days and water days led to the gradual reduction in saccharin intake. HOT mice of both replicates developed a more pronounced conditioned taste aversion than did COLD mice (Cunningham, Hallett and Nouth, unpublished observations).

Summary. Results with the HOT and COLD selected lines provide further evidence that sensitivity to the effect of EtOH on thermoregulation in mice is genetically mediated to an important degree. The sensitivity differences appear in both genetic replicates and have increased over generations of selection pressure; thus, the differences are genuine genetic effects as opposed to nonspecific effects of inbreeding. Results with HOT and COLD mice also confirm the genetic correlation between sensitivity

and tolerance to EtOH HT which was originally identified in studies with inbred strains of mice. Other behavioral responses to EtOH, such as withdrawal severity and locomotor activation, may share some genetic substrates in common with EtOH-induced HT, but such a common genetic determination does not appear to be substantial. The HOT and COLD selected lines should be a useful genetic animal model for future experiments designed to explore the mechanisms underlying hypothermic sensitivity and tolerance to EtOH.

FAST and SLOW mice

Testing procedure and selection response. As previously described[34], mice are tested in the colony room on two successive days at a 24 h intertest interval. At 2 min after injection, each mouse is placed in the middle of one of 2 Lehigh Valley open fields (61-cm diameter) and photocell beam interruptions are counted. On one test day, basal activity is assessed after administering the mouse a saline injection. On the other test day, each mouse is given an injection of EtOH and tested as described. Testing for generations S_0–S_5 was in the order saline-EtOH (1.5 g/kg) under dim ambient lighting conditions. Results of studies investigating ways to maximize the selection response led us to change to the testing order EtOH-saline, to an EtOH dose of 2.0 g/kg, and to bright light[22], beginning in S_6. The EtOH concentration was always 20% v/v, and the activity score (ACT) is always the difference between the saline and EtOH scores.

The resulting FAST and SLOW lines do not differ in response to saline. There was already divergence between each pair of FAST and SLOW lines in S_1, after only one generation of selection, which could indicate the significant role of a major locus. Immediately following the change in test parameters at S_6, there was again an increase in divergence between S_6 and S_7. Genetic selection has generated mouse lines that differ significantly in their response to EtOH in the open-field. We have found the FAST vs SLOW differences robust in other open-field apparatuses, using both repeated-testing (within-animal) and between-group designs.

Locomotor response to other drugs. We have examined the locomotor activating effects of diazepam, pentobarbital and two stimulants, caffeine and d-amphetamine, in FAST and SLOW mice. Overall, we have found that the clear difference between FAST and SLOW mice in EtOH-stimulated activity does not generalize to all other drugs with stimulant properties. Mice were from selected generations 11 or 12. Diazepam did not affect the activity of FAST and SLOW mice differently. However, because only very small activating-effects were seen at the doses used (2.5, 5, 10, and 20 mg/kg), we intend to extend our observations to other doses. Pentobarbital stimulated the activity of both mouse lines equally up to 20 mg/kg (i.p.) and reduced activity at 40 mg/kg. Caffeine (2.5–20 mg/kg) had similar small stimulant effects on the activity of FAST and SLOW mice. Finally, d-amphetamine had dose-dependent stimulant effects on mice of both lines, but FAST mice were more activated.

Collectively, these results do not support a hypothesis of greater general excitability of FAST mice. In addition, they suggest that the physiological mechanisms which mediate the activation produced by barbiturates and possibly BZs are not the same as those controlling EtOH-induced stimulation. Amphetamine interacts with DA systems which have been implicated as important in the activating effects of EtOH (as discussed in a previous section). Interestingly, d-amphetamine had the clearest differential effects on the activity of FAST and SLOW mice. This result is especially exciting because another stimulant-class drug that does not act via DA systems (caffeine) did not affect the lines differently. We are currently engaged in a similar study using apomorphine, a DA agonist. We are also looking at the effects of alcohols other than EtOH to assess a hypothesis of specificity of the FAST/SLOW difference to alcohols.

Dose-effect relationships and EtOH metabolism. Activity differences between lines (S_{11}) were tested for several EtOH doses and were most marked at lower doses. FAST mice were consistently more stimulated than SLOW mice, and the differences were largest within 10 min after injection, which corresponds to the time period examined for the selection criterion. In several experiments, brain or blood EtOH concentrations were determined after acute EtOH injections or chronic administration. No differences between FAST and SLOW mice of either replicate from S_6–S_7 were found. We conclude that selection for EtOH-induced ACT has not altered parameters of EtOH metabolism.

EtOH-induced ataxia. We examined ambulatory coordination after EtOH in several ways to compare the two lines. The grid test comprises a hardware cloth grid and measures stumbling errors per distance travelled as cases where the mouse's foot falls through the mesh. FAST mice made more errors in this apparatus than SLOW mice[120], but this difference may be apparent only after some EtOH doses. It is also greater in mice from the second replicate. FAST mice tested on a constant-speed rotarod after 2.5 g/kg EtOH were more ataxic than SLOW mice. However, when mice were tested on a static rod for latency to lose balance after 2.0 g/kg EtOH, there were no differences. These mice also did not differ in sensitivity on a larger rod that was accelerated at a constant rate. In summary, there may be a genetic correlation between sensitivity to EtOH-induced ACT and EtOH-induced ataxia, but the results for several tasks were not entirely consistent. This may be because the tasks assess different neural substrates; alternatively, the apparent genetic correlation may be weak or spurious.

Other correlated responses to selection. Sensitivity to the hypnotic effects of EtOH was studied in FAST and SLOW mice injected with 4 g/kg EtOH. S_7–S_8 FAST mice of replicate 2 were more sensitive than SLOW mice to EtOH-induced LORR for they had significantly lower

BEC values at regaining the righting reflex. The lines did not differ either in latency to LORR or in duration of LORR[120], but these measures include variables related to absorption, distribution, and elimination of EtOH as well as neurosensitivity. A more recent test of S_{13} animals revealed no line difference in sedative sensitivity as indexed by BEC at regaining righting. The sensitivities of FAST and SLOW mice to the sedative effects of pentobarbital were also similar (Phillips, unpublished). Lastly, FAST and SLOW mice did not differ in magnitude of EtOH-induced HT[120]. Although genes underlying EtOH activation may also influence sensitivity to EtOH sedation, the current evidence does not strongly support this suggestion.

In a study of EtOH-preference drinking like that described for WSP and WSR mice, utilizing increasing EtOH concentrations in water, small time-dependent differences were found between FAST and SLOW mice in the amount of EtOH consumed with FAST consuming more.

Summary

Selection for sensitivity and resistance to EtOH-induced HT has produced robust responses to selection in both replicate sets of lines. While it has been possible to select COLD mice for increased HT, the HOT lines have not developed much smaller HT responses than control mice over generations. A number of correlated responses to selection have been documented in the HOT and COLD lines. The HT difference generalizes to several other alcohols and depressants, suggesting that these compounds produce HT by the same mechanisms as EtOH. Generalization is not complete, however: the HOT and COLD lines do not differ in sensitivity to a number of agents producing HT through presumably specific effects on neurotransmitter receptors. HOT mice develop a less marked tolerance to EtOH HT than do COLD mice.

The response to selection has also been asymmetrical in FAST and SLOW mice. FAST lines have shown increasing activation over generations of selection, while SLOW lines have shown no decrease in response. One SLOW line is, in fact, increasing in responsiveness, despite selection against this character. Consistent with the lesser degree of divergence in the selected lines, the existence of correlated responses to selection is less clearly revealed in FAST and SLOW lines. Some evidence supports the development of a positive correlation between activation and ataxia. FAST mice may be more sensitive than SLOW mice to amphetamine- and diazepam-induced activation. There is no consistent difference between the lines in sensitivity to EtOH-induced hypothermia, LORR, or in sensitivity to pentobarbital-induced LORR. The general results so far in both selections are encouraging, and we expect that future experiments will use these lines to address questions regarding the relevant mechanisms of action of EtOH.

General conclusions

The studies reviewed above represent the cumulation of almost 15 years of work with lines of mice selected for genetic susceptibility to different effects of ethanol. They exemplify several powerful features of the selective breeding method as used to study drug responses. Moreover, they suggest several specific phenomena which may have practical relevance for our understanding of EtOH's actions in the nervous system. Finally, they illuminate several lacunae where further experiments could provide very useful information, some of which have already been mentioned.

Advantages of selectively bred lines

Use in building model systems. This strength derives from the power of selected lines to detect correlated responses to selection. One clear example from the reviewed literature is the enormous difference in cerebellar Purkinje cell sensitivity to EtOH inhibition between LS and SS mice. The specificity of this difference, its magnitude, and its robust nature across preparations, suggest that the neurobiological mechanism underlying sensitivity to EtOH hypnosis must involve this cell system. Another striking correlated response is the fact that WSP mice are more susceptible than WSR mice to withdrawal from BZs and barbiturates as well as EtOH, which suggests that there may be a common genetic substrate for susceptibility to dependence on at least these addictive drugs.

Cumulative nature of research. Studies with mature selected lines, which have reached their selection limit, may be compared across laboratories and generations for many years. Once again, our developing understanding of the mechanisms underlying sensitivity to EtOH's hypnotic effects is largely thanks to the longevity of the LS/SS lines, and their increasing use.

Use as a tool for hypothesis generation. The ease with which one can initially probe selected lines for potential correlated responses makes them an ideal tool for genetic hypothesis generation[12]. Putative genetic correlations can then be tested by more laborious methods (testing other selected lines, estimating correlations from multigenerational populations or panels of inbred strains). A clear example is the verification of the correlation between Purkinje cell sensitivity and LORR duration after EtOH. LS/SS mice were found to differ originally, and subsequent research with HAS/LAS rat lines and panels of inbred strains of both rats and mice corroborated the difference.

Specific results of potential importance

The importance of EtOH metabolism. In all selections to date, substantial differences in CNS sensitivity to EtOH have been produced by selection before any differences in EtOH elimination have emerged. The fact that with continued selection such differences in metabolism do eventually appear does not affect the principal finding that pharmacodynamic and pharmacokinetic aspects of

EtOH responses are under differential genetic control. It suggests that future selection experiments should closely monitor metabolism during the course of selection, and consider eliminating metabolic differences as they emerge.

Tolerance and dependence are genetically distinct. Early work in a single strain of mice suggested that tolerance to, and dependence upon, EtOH could be pharmacologically dissociated. 6-OHDA lesions blocked tolerance development while not affecting withdrawal[125]. That the WSP and WSR mice do not differ markedly in tolerance to two effects of EtOH also supports this distinction. Clearly, the conceptualization of tolerance and dependence as early and late stages of a single physiological continuum should be abandoned.

Mechanisms of drug effects are not uniform across drugs with the same presumptive mechanisms of action. Cross-tolerance among sedative-hypnotic agents does not necessarily predict common mechanisms of action. For example, cross-tolerance has been noted between EtOH and barbiturates, yet LS and SS mice do not differ in CNS sensitivity to the sedative effects of pentobarbital. Reports that LS mice are not uniformly more sensitive to all sedative-hypnotic drugs than are SS mice virtually proves that these drugs have distinct mechanisms of action. Similar results showed that COLD mice were more sensitive to hypothermic effects of some, but not all drugs than HOT mice, and that FAST mice were more activated than SLOW mice by some, but not all drugs. Even more strikingly, WSP and WSR mice were not similarly sensitive to seizures produced by 3 drugs presumably acting at the same receptor site on the GABA Complex. The response specificity of the selected lines is thus a formidable analytic tool with sufficient sensitivity to detect subtle neurochemical differences.

Genetic independence of determination of several responses to EtOH. We have reviewed data recording differences or similarities between lines of mice genetically selected for various effects of EtOH. One important conclusion which can be drawn from this information pertains to the genetic relatedness of different EtOH effects. Although LS and SS mice differ in sensitivity to almost every effect of EtOH for which they have been tested, this has not been the case for mouse lines selectively bred for sensitivity to EtOH's activating, hypothermic, or withdrawal effects. This suggests the absence of substantial genetic codetermination of sensitivity to these EtOH effects. It also suggests that some of the differences found between LS and SS mice are attributable to genetic loci other than those influencing sensitivity to EtOH's sedative effects. Inbreeding during the selection of the sleep lines could be a likely source of this result.

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1 Allan, A. M., Gallaher, E. J., Gionet, S. E., and Harris, R. A., Genetic selection for benzodiazepine ataxia produces functional changes in the gamma-aminobutyric acid receptor chloride channel complex. Brain Res. *452* (1988) 118–126.
2 Allan, A. M., and Harris, R. A., Gamma-amino butyric acid and alcohol actions: Neurochemical studies of long sleep and short sleep mice. Life Sci. *39* (1986) 2005–2015.
3 Allan, A. M., and Harris, R. A., Acute and chronic ethanol treatments alter GABA receptor-operated chloride channels. Pharmac. Biochem. Behav. *27* (1987) 665–670.
4 Allan, A. M., Horowitz, G. P., Isaacson, R. L., and Major, L. F., Adrenergic nervous system alteration and ethanol-induced narcosis in long-sleep and short-sleep mice. Behav. Neur. Biol. *42* (1984) 134–139.
5 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: clin. exp. Res. *7* (1983) 443–447.
6 Alpern, H. P., and Greer, C. A., A dopaminergic basis for the effects of amphetamine on a mouse 'preadolescent hyperkinetic' model. Life Sci. *21* (1977) 93–98.
7 Alpern, H. P., and McIntyre, T. D., Evidence that the selectively bred long- and short-sleep mouse lines display common narcotic reactions to many depressants. Psychopharmacology *85* (1985) 456–459.
8 Alpern, H. P., and McIntyre, T. D., Sedative-hypnotic anomalies related to dose of pentobarbital in long-sleep and short-sleep selectively-bred mice. Pharmac. Biochem. Behav. *25* (1986) 333–336.
9 Baizer, L., Masserano, J. M., and Weiner, N., Ethanol-induced changes in tyrosine hydroxylase activity in brains of mice selectively bred for differences in sensitivity to ethanol. Pharmac. Biochem. Behav. *15* (1981) 945–949.
10 Baker, R., Melchior, C., and Deitrich, R., The effect of halothane on mice selectively bred for differential sensitivity to alcohol. Pharmac. Biochem. Behav. *12* (1980) 691–695.
11 Basile, A., Hoffer, B., and Dunwiddie, T., Differential sensitivity of cerebellar Purkinje neurons to ethanol in selectively outbred lines of mice: maintenance in vitro independent of synaptic transmission. Brain Res. *264* (1983) 69–78.
12 Belknap, J. K., Genetic factors in the effects of alcohol: neurosensitivity, functional tolerance and physical dependence, in: Alcohol Tolerance and Dependence, pp. 157–180. Eds H. Rigter and J. C. Crabbe. Elsevier, Amsterdam 1980.
13 Belknap, J. K., Danielson, P. W., Lamé, M., and Crabbe, J. C., Ethanol and barbiturate withdrawal convulsions are extensively codetermined in mice. Alcohol *5* (1988) 167–171.
14 Belknap, J. K., Laursen, S. E., and Crabbe, J. C., Ethanol and nitrous oxide produce withdrawal-induced convulsions by similar mechanisms in mice. Life Sci. *41* (1987) 2033–2041.
15 Belknap, J. K., Crabbe, J. C., and Laursen, S. E., Ethanol and diazepam withdrawal convulsions are extensively codetermined in WSP and WSR mice. Life Sci. *44* (1989) 2075–2080.
16 Berta, J., and Wilson, J. R., Selection in mice for alcohol withdrawal seizures. Behav. Genet. (1989) in press.
17 Chan, A. W. K., Gamma aminobutyric acid in diffferent strains of mice. Effect of ethanol. Life Sci. *19* (1976) 597–604.
18 Church, A. C., Fuller, J. L., and Dudek, B. C., Behavioral effects of salsolinol and ethanol on mice selected for sensitivity to alcohol-induced sleep time. Drug Alcohol Depend. *2* (1977) 443–452.
19 Collins, A. C., Smolen, A., Wayman, A. L., and Marks, M. J., Ethanol and temperature effects on five membrane bound enzymes. Alcohol *1* (1984) 237–246.
20 Crabbe, J. C., Sensitivity to ethanol in inbred mice: genotypic correlations among several behavioral responses. Behav. Neurosci. *97* (1983) 280–289.
21 Crabbe, J. C., Belknap, J. K., and Young, E. R., Sensitivity to the anticonvulsant effects of ethanol and pentobarbital in mouse lines genetically selected for ethanol sensitivity. Alcoholism: clin. exp. Res. *13* (1989) 291–294.
22 Crabbe, J. C., Deutsch, C. M., Tam, B. R., and Young, E. R., Environmental variables differentially affect ethanol-stimulated activity in selectively bred mouse lines. Psychopharmacology *95* (1988) 103–108.
23 Crabbe, J. C., Feller, D. J., and Dorow, J. S., Sensitivity and tolerance to ethanol-induced hypothermia in genetically selected mice. J. Pharmac. exp. Ther. *249* (1989) 456–461.

24 Crabbe, J. C., Gray, D., Young, E., Janowsky, J., and Rigter, H., Initial sensitivity and tolerance to ethanol in mice: Correlations among open-field activity, hypothermia and loss of righting reflex. Behav. Neur. Biol. *33* (1981) 188–203.

25 Crabbe, J. C., Janowsky, J., Young, E., Kosobud, A., Stack, J., and Rigter, H., Tolerance to ethanol hypothermia in inbred mice: genotypic correlations with behavioral responses. Alcoholism: clin. exp. Res. *6* (1982) 446–458.

26 Crabbe, J. C., and Kosobud, A., Sensitivity and tolerance to ethanol in mice bred to be genetically prone (WSP) or resistant (WSR) to ethanol withdrawal seizures. J. Pharmac. exp. Ther. *239* (1986) 327–333.

27 Crabbe, J. C., and Kosobud, A., Alcohol withdrawal seizures: genetic animal models, in: Alcohol and Seizures. Eds R. J. Porter, R. H. Mattson and J. A. Cramer. (1989) in press.

28 Crabbe, J. C., Kosobud, A., Feller, D. J., and Phillips, T. J., Use of selectively bred mouse lines to study genetically correlated traits related to alcohol, in: Biomedical and Social Aspects of Alcohol and Alcoholism, pp. 427–430. Eds K. Kuriyama, A. Takada and H. Ishii. Excerpta Medica, Amsterdam 1988.

29 Crabbe, J. C., Kosobud, A., Tam, B. R., Young, E. R., and Deutsch, C. M., Genetic selection of mouse lines sensitive (COLD) and resistant (HOT) to acute ethanol hypothermia. Alcohol Drug Res. *7* (1987) 163–174.

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

31 Crabbe, J. C., Kosobud, A., Young, E., and Janowsky, J., Polygenic and single-gene determination of response to ethanol in BXD/Ty recombinant inbred mouse strains. Neurobehav. Toxic. Terat. *5* (1983) 181–187.

32 Crabbe, J. C., Kosobud, A., Young, E. R., Tam, B. R., and McSwigan, J. D., Bidirectional selection for susceptibility to ethanol withdrawal seizures in *Mus musculus*. Behav. Genet. *15* (1985) 521–536.

33 Crabbe, J. C., McSwigan, J. D., and Belknap, J. K., The role of genetics in substance abuse, in: Individual Differences in Substance Abuse, pp. 13–64. Eds M. Galizio and S. A. Maisto. Plenum Publ. Corp., New York 1985.

34 Crabbe, J. C., Young, E. R., Deutsch, C. M., Tam, B. R., and Kosobud, A., Mice genetically selected for differences in open-field activity after ethanol. Pharmac. Biochem. Behav. *27* (1987) 577–581.

35 Crabbe, J. C., Young, E. R., and Kosobud, A., Genetic correlations with ethanol withdrawal severity. Pharmac. Biochem. Behav. *18* (Supp. 1) (1983) 541–547.

36 Crabbe, J. C., Young, E. R., Tam, B. R., Kosobud, A., Belknap, J. K., and Laursen, S. E., Genetic differences in anticonvulsant sensitivity in mouse lines selectively bred for ethanol withdrawal severity. J. Pharmac. exp. Ther. *239* (1986) 154–159.

37 Daniell, L. C., and Harris, R. A., Effect of chronic ethanol treatment and selective breeding for hypnotic sensitivity to ethanol on intracellular ionized calcium concentrations in synaptosomes. Alcoholism: clin. exp. Res. *12* (1988) 179–183.

38 de Fiebre, C. M., Medhurst, L. J., and Collins, A. C., Nicotine response and nicotinic receptors in long-sleep and short-sleep mice. Alcohol *4* (1987) 493–501.

39 DeFries, J. C., Wilson, J. R., Erwin, V. G., and Petersen, D. R., LS × SS recombinant inbred strains of mice: Initial characterization. Alcoholism: clin. exp. Res. *13* (1989) 196–200.

40 Deitrich, R. A., and Spuhler, K., Genetics of alcoholism and alcohol actions, in: Research Advances in Alcohol and Drug Problems, vol. 8, pp. 47–98. Eds K. Smart, H. C. Cappell et al. Plenum Publ. Corp., New York 1984.

41 Dibner, M. D., Zahniser, N. R., Wolfe, B. B., Rabin, R. A., and Molinoff, P. B., Brain neurotransmitter receptor systems in mice genetically selected for differences in sensitivity to ethanol. Pharmac. Biochem. Behav. *12* (1980) 509–513.

42 Disbrow, J. K., Masserano, J. M., and Weiner, N., Thyroid status during postnatal maturation of the brain in mice genetically bred for differences in ethanol sensitivity. J. Pharmac. exp. Ther. *237* (1986) 874–880.

43 Dudek, B. C., Ethanol-induced conditioned taste aversions in mice that differ in neural sensitivity to ethanol. J. Stud. Alcohol *43* (1982) 129–136.

44 Dudek, B. C., and Abbott, M. E., A biometrical genetic analysis of ethanol response in selectively bred long-sleep and short-sleep mice. Behav. Genet. *14* (1984) 1–19.

45 Dudek, B. C., and Abbott, M. E., The relationship between ethanol-induced locomotor activation and narcosis in long-sleep and short-sleep mice. Alcoholism: clin. exp. Res. *8* (1984) 272–276.

46 Dudek, B. C., Abbott, M. E., Garg, A., and Phillips, T. J., Apomorphine effects on behavioral response to ethanol in mice selectively bred for differential sensitivity to ethanol. Pharmac. Biochem. Behav. *20* (1984) 91–94.

47 Dudek, B. C., Abbott, M. E., and Phillips, T. J., Stimulant and depressant properties of sedative-hypnotics in mice selectively bred for differential sensitivity to ethanol. Psychopharmacology *82* (1984) 46–51.

48 Dudek, B. C., and Fanelli, R. J., Effects of gamma-butyrolactone, amphetamine, and haloperidol in mice differing in sensitivity to alcohol. Psychopharmacology *68* (1980) 89–97.

49 Dudek, B. C., and Phillips, T. J., Locomotor stimulant and intoxicant properties of methanol, ethanol, tertiary butanol and pentobarbital in long-sleep and short-sleep mice. Subst. Alcohol Actions/Misuse *4* (1983) 31–36.

50 Dudek, B. C., and Phillips, T. J., Genotype-dependent effects of GABAergic agents on sedative properties of ethanol. Psychopharmacology (1989) in press.

51 Dunwiddie, T. V., and Worth, T., Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. J. Pharmac. exp. Ther. *220* (1982) 70–76.

52 Engel, J., and Liljequist, S., The involvement of different central neurotransmitters in mediating stimulatory and sedative effects of ethanol, in: Stress and Alcohol Use, pp. 153–169. Eds L. A. Pohorecky and J. Brick. Elsevier, Amsterdam 1983.

53 Erwin, V. G., and Cornell, K., Effects of 6-hydroxydopamine on brain catecholamines and on acute actions of ethanol in LS/Ibg and SS/Ibg mice. Alcoholism: clin. exp. Res. *10* (1986) 285–289.

54 Erwin, V. G., Heston, W. D. W., McClearn, G. E., and Deitrich, R. A., Effects of hypnotics on mice genetically selected for sensitivity to ethanol. Pharmac. Biochem. Behav. *4* (1976) 679–683.

55 Erwin, V. G., Korte, A., and Jones, B. C., Central muscarinic cholinergic influences on ethanol sensitivity in long-sleep and short-sleep mice. J. Pharmac. exp. Ther. *247* (1988) 857–862.

56 Erwin, V. G., and Korte, A., Brain neurotensin receptors in mice bred for differences in sensitivity to ethanol. Alcohol *5* (1988) 195–201.

57 Erwin, V. G., Korte, A., and Marty, M., Neurotensin selectively alters ethanol-induced anesthesia in LS/Ibg and SS/Ibg lines of mice. Brain Res. *400* (1987) 80–90.

58 Feller, D. J., Harris, R. A., and Crabbe, J. C., Differences in GABA activity between withdrawal seizure prone and resistant mice. Eur. J. Pharmac. *157* (1988) 147–154.

59 Fredholm, B. B., Zahniser, N. R., Weiner, G. R., Proctor, W. R., and Dunwiddie, T. V., Behavioural sensitivity to PIA in selectively bred mice is related to a number of A_1 adenosine receptors but not to cyclic AMP accumulation in brain slices. Eur. J. Pharmac. *111* (1985) 133–136.

60 French, T. A., Clay, K. L., Murphy, R. C., and Weiner, N., Alpha-methyl-para-tyrosine effects in mice selectively bred for differences in sensitivity to ethanol. Biochem. Pharmac. *34* (1985) 3811–3821.

61 French, T. A., Masserano, J. M., and Weiner, N., Ethanol-induced changes in tyrosine hydroxylase activity in adrenal glands of mice selectively bred for differences in sensitivity to ethanol. J. Pharmac. exp. Ther. *232* (1985) 315–321.

62 French, T. A., Masserano, J. M., and Weiner, N., Further studies on the neurochemical mechanisms mediating differences in ethanol sensitivity in LS and SS mice. Alcoholism: clin. exp. Res. *12* (1988) 215–223.

63 French, T. A., and Weiner, N., Effect of ethanol on tyrosine hydroxylation in brain regions of long and short sleep mice. Alcohol *1* (1984) 247–252.

64 Gallaher, E. J., Hollister, L. E., Gionet, S. E., and Crabbe, J. C., Mouse lines selected for genetic differences in diazepam sensitivity. Psychopharmacology *93* (1987) 25–30.

65 George, F. R., Genetic tools in the study of drug self-administration. Alcoholism: clin. exp. Res. *12* (1988) 586–590.

66 George, F. R., and Collins, A. C., Ethanol's behavioral effects may be partly due to increases in brain prostaglandin production. Alcoholism: clin. exp. Res. *9* (1985) 143–146.

67 George, F. R., Howerton, T. C., Elmer, G. I., and Collins, A. C., Antagonism of alcohol hypnosis by blockade of prostaglandin synthesis and activity: Genotype and time course effects. Pharmac. Biochem. Behav. *19* (1983) 131–136.

68 George, F. R., Ritz, M. C., Elmer, G. I., and Collins, A. C., Time course of ethanol's effects on brain prostaglandins in LS and SS mice. Life Sci. *39* (1986) 1069–1075.

69 Gilliam, D. M., and Collins, A. C., Quantification of physiological and behavioral measures of alcohol withdrawal in long-sleep and short-sleep mice. Alcoholism: clin. exp. Res. *10* (1986) 672–678.

70 Gilliam, D. M., Stilman, A., Dudek, B. C., and Riley, E. P., Fetal alcohol effects in long- and short-sleep mice: Activity, passive avoidance and in utero ethanol levels. Neurotoxic. Terat. *9* (1987) 349–357.

71 Goldman, D., and Crabbe, J. C., Use of chromosomally mapped and identified mouse brain proteins for behavioral genetic analysis of alcoholism. Prog. Neuropsychopharmac. Biol. Psychiat. *10* (1986) 117–190.

72 Goldman, D., Lister, R. G., and Crabbe, J. C., Mapping of a putative genetic locus determining ethanol intake in the mouse. Brain Res. *420* (1987) 220–226.

73 Goldman, D., Nelson, R., Deitrich, R. A., Baker, R. C., Spuhler, K., Markley, H., Ebert, M., and Merril, C. R., Genetic brain polypeptide variants in inbred mice and in mouse strains with high and low sensitivity to alcohol. Brain Res. *341* (1985) 130–138.

74 Goldstein, D. B., and Kakihana, R., Alcohol withdrawal reactions in mouse strains selectively bred for long or short sleep times. Life Sci. *17* (1975) 981–985.

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

76 Greer, C. A., and Alpern, H. P., Paradoxical effects of d-amphetamine upon seizures susceptibility in 2 selectively bred lines of mice. Devl Psychobiol. *13* (1980) 7–15.

77 Harris, R. A., and Allan, A. M., Neurochemistry of brain chloride channels: Genetic variation in modulation by GABA agonists, alcohol and benzodiazepines, in: Chloride Channels and Their Modulation by Neurotransmitters and Drugs, pp. 189–198. Eds G. Biggio and E. Costa. Raven Press, New York 1988.

78 Harris, R. A., and Allan, A. M., Genetic differences in coupling of benzodiazepine receptors to chloride channels. Brain Res. *490* (1989) 26–32.

79 Harris, R. A., Burnett, R., McQuilkin, S., McClard, A., and Simon, F. R., Effects of ethanol on membrane order: Fluorescence studies, in: Alcohol and the Cell, pp. 125–135. Annals N.Y. Acad. Sci., vol. 492. New York 1987.

80 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 Sci. *35* (1984) 2601–2608.

81 Harris, R. A., Zaccaro, L. M., McQuilkin, S., and McClard, A., Effects of ethanol and calcium on lipid order of membranes from mice selected for genetic differences in ethanol intoxication. Alcohol *5* (1988) 251–257.

82 Horowitz, G. P., Dendel, P. S., Allan, A. M., and Major, L. F., Dopamine-β-hydroxylase activity and ethanol-induced sleep time in selectively bred and heterogeneous stock mice. Behav. Genet. *12* (1982) 549–561.

83 Horowitz, G. P., and Dudek, B. C., Behavioral pharmacogenetics, in: Behavior Genetics: Principles and Applications, pp. 117–154. Eds J. L. Fuller and E. C. Simmel. Lawrence Erlbaum Assoc., Hillsdale, New Jersey 1983.

84 Howerton, T. C., and Collins, A. C., Ethanol-induced inhibition of norepinephrine release from brain slices obtained from LS and SS mice. Alcohol *1* (1984) 47–53.

85 Howerton, T. C., and Collins, A. C., Ethanol-induced inhibition of GABA release from LS and SS mouse brain slices. Alcohol *1* (1984) 471–477.

86 Howerton, T. C., Marks, M. J., and Collins, A. C., Norepinephrine, gamma-aminobutyric acid, and choline reuptake kinetics and the effects of ethanol in long-sleep and short-sleep mice. Subst. Alcohol Actions/Misuse *3* (1982) 89–99.

87 Howerton, T. C., O'Connor, M. F., and Collins, A. C., Lipid solubility is correlated with hypnotic and hypothermic responses of long-sleep (LS) and short-sleep (SS) mice to various depressant drugs. J. Pharmac. exp. Ther. *227* (1983) 389–393.

88 Howerton, T. C., O'Connor, M. F., and Collins, A. C., Differential effects of long-chain alcohols in long- and short-sleep mice. Psychopharmacology *79* (1983) 313–317.

89 Hunt, W. A., Alcohol and Biological Membranes. Guilford Press, New York 1985.

90 Johnson, S. W., Hoffer, B. J., Baker, R., and Freedman, R., Correlation of Purkinje neuron depression and hypnotic effects of ethanol in inbred strains of rats. Alcoholism: clin. exp. Res. *9* (1985) 56–58.

91 Koblin, D. D., and Deady, J. E., Anaesthetic requirement in mice selectively bred for differences in ethanol sensitivity. Br. J. Anaesth. *53* (1981) 5–10.

92 Kosobud, A., Bodor, A. S., and Crabbe, J. C., Voluntary consumption of ethanol in WSP, WSC and WSR selectively bred mouse lines. Pharmac. Biochem. Behav. *29* (1988) 601–607.

93 Kosobud, A., and Crabbe, J. C., Ethanol withdrawal in mice bred to be genetically prone (WSP) or resistant (WSR) to ethanol withdrawal seizures. J. Pharmac. exp. Ther. *238* (1986) 170–177.

94 Kulonen, E., Ethanol and GABA. Med. Biol. *61* (1983) 147–167.

95 Marley, R. J., Freund, R. K., and Wehner, J. M., Differential response to flurazepam in long-sleep and short-sleep mice. Pharmac. Biochem. Behav. *31* (1988) 453–458.

96 Marley, R. J., Miner, L. L., Wehner, J. M., and Collins, A. C., Differential effects of central nervous system depressants in long-sleep and short-sleep mice. J. Pharmac. exp. Ther. *238* (1986) 1028–1033.

97 Marley, R. J., Stinchcomb, A., and Wehner, J. M., Further characterization of benzodiazepine receptor differences in long-sleep and short-sleep mice. Life Sci. *43* (1988) 1223–1231.

98 Marley, R. J., and Wehner, J. M., GABA enhancement of flunitrazepam binding in mice selectively bred for differential sensitivity to ethanol. Alcohol Drug Res. *7* (1986) 25–32.

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

100 Marley, R. J., and Wehner, J. M., Gamma-aminobutyric acid receptor binding in long-sleep and short-sleep mice. Life Sci. (1989) in press.

101 Martz, A., Deitrich, R. A., and Harris, R. A., Behavioral evidence for the involvement of gamma-aminobutyric acid in the actions of ethanol. Eur. J. Pharmac. *89* (1983) 53–62.

102 McClearn, G. E., and Kakihana, R., Selective breeding for ethanol sensitivity: Short-sleep and long-sleep mice, in: Development of Animal Models as Pharmacogenetic Tools, pp. 147–159. Eds G. E. McClearn, R. A. Deitrich and V. G. Erwin. U.S. Dept. of Health and Human Services Research Monograph No. 6, Publication No. (ADM) 81-1133, Washington 1981.

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

104 McIntyre, T. D., and Alpern, H. P., Thiopental, phenobarbital, and chlordiazepoxide induce the same differences in narcotic reaction as ethanol in long-sleep and short-sleep selectively-bred mice. Pharmac. Biochem. Behav. *24* (1986) 895–898.

105 McIntyre, T. D., Trullas, R., and Skolnick, P., Differences in the biophysical properties of the benzodiazepine/gamma-aminobutyric acid receptor chloride channel complex in long-sleep and short-sleep mouse lines. J. Neurochem. *51* (1988) 642–647.

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

107 Miller, L. G., Greenblatt, D. J., Barnhill, J. G., and Shader, R. I., Differential modulation of benzodiazepine receptor binding by ethanol in LS and SS mice. Pharmac. Biochem. Behav. *29* (1988) 471–477.

108 Morrow, E. L., and Erwin, V. G., Calcium influence on neuronal sensitivity to ethanol in selectively bred mouse lines. Pharmac. Biochem. Behav. *24* (1986) 949–954.

109 Morrow, E. L., and Erwin, V. G., Calcium influence on neurotensin and β-endorphin enhancement of ethanol sensitivity in selectively bred mouse lines. Alcohol Drug Res. *7* (1987) 225–232.

110 Morrow, E. L., Erwin, V. G., and Smith, T. L., (^{3}H)Inositol trisphosphate specific binding in cerebella of mouse lines selectively bred for differential ethanol sensitivity. Proc. West. Pharmac. Soc. *31* (1988) 269–271.

111 O'Connor, M. F., Howerton, T. C., and Collins, A. C., Effects of pentobarbital in mice selected for differential sensitivity to ethanol. Pharmac. Biochem. Behav. *17* (1982) 245–248.

112 Palmer, M. R., Basile, A. S., Proctor, W. R., Baker, R. C., and Dunwiddie, T. V., Ethanol tolerance of cerebellar Purkinje neurons from selectively outbred mouse lines: in vivo and in vitro electrophysiological investigations. Alcoholism: clin. exp. Res. *9* (1985) 291–296.

113 Palmer, M. R., Morrow, E. L., and Erwin, V. G., Calcium differentially alters behavioral and electrophysiological responses to ethanol

in selectively bred mouse lines. Alcoholism: clin. exp. Res. *11* (1987) 457–463.

114 Palmer, M. R., Olson, L., Dunwiddie, T. V., Hoffer, B. J., and Seiger, A., Neonatal cerebellectomy alters ethanol-induced sleep time of short sleep but not long sleep mice. Pharmac. Biochem. Behav. *20* (1984) 153–159.

115 Palmer, M. R., Sorensen, S. M., Freedman, R., Olson, L., Hoffer, B., and Seiger, A., Differential ethanol sensitivity of intraocular cerebellar grafts in long-sleep and short-sleep mice. J. Pharmac. exp. Ther. *222* (1982) 480–487.

116 Palmer, M. R., Wang, Y., Fossom, L. H., and Spuhler, K. P., Genetic correlation of ethanol-induced ataxia and cerebellar Purkinje neuron depression among inbred strains and selected lines of rats. Alcoholism: clin. exp. Res. *11* (1987) 494–501.

117 Perlman, B. J., and Goldstein, D. B., Genetic influences on the central nervous system depressant and membrane-disordering actions of ethanol and sodium valporate. Molec. Pharmac. *26* (1984) 547–552.

118 Phillips, T. J., and Dudek, B. C., Modification of ethanol effects by bicuculline: Genotype-dependent responses and inheritance. Psychopharmacology (1989) in press.

119 Phillips, T. J., Kim, D., and Dudek, B. C., Convulsant properties of GABA antagonists and anticonvulsant properties of ethanol in selectively bred long- and short-sleep mice. Psychopharmacology (1989) in press.

120 Phillips, T. J., Limm, M., and Crabbe, J. C., Correlated behavioral responses as potential predictors of sensitivity to ethanol's rewarding effects and addiction liability, in: Genetic Aspects of Alcoholism, pp. 197–206. Eds K. Kiianmaa, B. Tabakoff and T. Saito. The Finnish Foundation for Alcohol Studies, Helsinki 1989.

121 Phillis, J. W., Behavioral and other actions of adenosine in the central nervous system, in: Neurotransmitters and Cortical Function, pp. 403–414. Eds M. Avoli, T. A. Reader, R. W. Dykes and P. Gloor. Plenum Publ. Corp., New York 1988.

122 Proctor, W. R., Baker, R. C., and Dunwiddie, T. V., Differential CNS sensitivity to PIA and theophylline in long-sleep and short-sleep mice. Alcohol *2* (1985) 287–291.

123 Proctor, W. R., and Dunwiddie, T. V., Behavioral sensitivity to purinergic drugs parallels ethanol sensitivity in selectively bred mice. Science *224* (1984) 519–521.

124 Reed, T. E., Three heritable responses to alcohol in a heterogeneous randomly mated mouse strain. J. Stud. Alcohol *38* (1977) 618–632.

125 Ritzmann, R. F., and Tabakoff, B., Dissociation of alcohol tolerance and dependence. Nature *263* (1976) 418–420.

126 Sanders, B., Sensitivity to low doses of ethanol and pentobarbital in mice selected for sensitivity to hypnotic doses of ethanol. J. comp. Physiol. Psych. *90* (1976) 394–398.

127 Sanders, B., and Sharpless, S. K., Dissociation between the anticonvulsant action of alcohol and its depressant action in mice of different genotypes. Life Sci. *23* (1978) 2593–2600.

128 Sanders, B., Sharpless, S. K., Collins, A. C., McClearn, G. E., and Flanagan, C., Activating and anesthetic effects of general depressants. Psychopharmacology *56* (1978) 185–189.

129 Scheetz, A. J., Markham, J. A., and Fifková, E., Changes in the frequency of basket cells in the dentate fascia following chronic ethanol administration in mice. Brain Res. *403* (1987) 151–154.

130 Scheetz, A. J., Markham, J. A., and Fifková, E., The effect of chronic ethanol consumption on the fine structure of the CA_1 stratum oriens in short-sleep and long-sleep mice: short-term and long-term exposure. Brain Res. *409* (1987) 329–334.

131 Siemens, A. J., and Chan, A. W. K., Differential effects of pentobarbital and ethanol in mice. Life Sci. *19* (1976) 581–590.

132 Smolen, A., and Smolen, T. N., Demonstration of a threshold concentration for ethanol at the time of regaining the righting response in long-sleep and short-sleep mice. Alcohol Drug Res. *7* (1987) 279–283.

133 Smolen, A., Smolen, T. N., and van de Kamp, J. L., Sensitivity of inbred and selectively bred mice to ethanol. Alcohol *4* (1987) 57–62.

134 Smolen, T. N., and Collins, A. C., Behavioral effects of ethanol and salsolinol in mice selectively bred for acute sensitivity to ethanol. Pharmac. Biochem. Behav. *20* (1984) 281–287.

135 Smolen, T. N., Howerton, T. C., and Collins, A. C., Effects of ethanol and salsolinol on catecholamine function in LS and SS mice. Pharmac. Biochem. Behav. *20* (1984) 125–131.

136 Sorensen, S., Dunwiddie, T., McClearn, G., Freedman, R., and Hoffer, B., Ethanol-induced depressions in cerebellar and hippocampal neurons of mice selectively bred for differences in ethanol sensitivity: An electrophysiological study. Pharmac. Biochem. Behav. *14* (1981) 227–234.

137 Sorensen, S., Palmer, M., Dunwiddie, T., and Hoffer, B., Electrophysiological correlates of ethanol-induced sedation in differentially sensitive lines of mice. Science *210* (1980) 1143–1145.

138 Spuhler, K., Gerhardt, G., and Palmer, M. R., CNS monoamine levels and the effect of DSP4 on ethanol sensitivity in LS and SS mice. Alcohol *4* (1987) 419–424.

139 Spuhler, K., Hoffer, B., Weiner, N., and Palmer, M., Evidence for genetic correlation of hypnotic effects and cerebellar Purkinje neuron depression in response to ethanol in mice. Pharmac. Biochem. Behav. *17* (1982) 569–578.

140 Suzdak, P. D., Schwartz, R. D., Skolnick, P., and Paul, S. M., Ethanol stimulates gamma-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. Proc. natl Acad. Sci. *83* (1986) 4071–4075.

141 Wand, G. S., Ethanol differentially regulates proadrenocorticotropin/endorphin production and corticosterone secretion in LS and SS lines of mice. Endocrinology *124* (1989) 518–526.

142 Wehner, J. M., Martin, B. J., Marley, R. J., and Pounder, J. I., Behavioral studies of GABAergic responses in LS and SS mice: Are ethanol sensitivity and responses to GABAergic agents regulated by common mechanisms? in: Conference on Mechanisms of Initial Sensitivity to Ethanol. Ed. R. A. Deitrich. US Dept. of Health and Human Services. NIAAA Research Monograph (1989) in press.

143 Weiner, N., Disbrow, J. K., French, T. A., and Masserano, J. M., The influence of catecholamine systems and thyroid function on the actions of ethanol in long-sleep (LS) and short-sleep (SS) mice. Annals N.Y. Acad. Sci. *492* (1987) 375–383.

144 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. Behav. Genet. *14* (1984) 235–256.

145 Zgombick, J. M., and Erwin, V. G., Central mechanisms of ethanol-induced adrenocortical response in selectively bred lines of mice. Neuroendocrinology *46* (1987) 324–332.

146 Zgombick, J. M., Erwin, V. G., and Cornell, K., Ethanol-induced adrenomedullary catecholamine secretion in LS/Ibg and SS/Ibg mice. J. Pharmac. exp. Ther. *236* (1986) 634–640.

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