ALTERATION OF ETHANOL-INDUCED CHANGES IN LOCOMOTOR BEHAVIOR BY DOPAMINE AGONISTS AND ANTAGONISTS IN SELECTIVELY BRED FAST AND SLOW LINES OF MICE

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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ABSTRACT

Catecholaminergic systems, particularly dopaminergic, have been suggested to be involved in mediating the locomotor activation produced by ethanol. The FAST and SLOW selectively bred lines of mice have been produced in two replicates for differential sensitivities to the stimulating effects of ethanol. Whereas FAST-1 and FAST-2 (sensitive) mice are stimulated by 2.0 g/kg ethanol, SLOW-1 and SLOW-2 (insensitive) mice are not stimulated, and are often depressed, by the same dose. It was hypothesized that if selective breeding of the FAST and SLOW lines produced differences in dopaminergic systems between the lines, manipulation of dopaminergic systems by dopamine receptor agonists and antagonists would alter the locomotor stimulation produced by ethanol in FAST mice, but would have little or no effect on the activity of ethanol-treated SLOW mice. Although the experimental design used in these experiments was, in retrospect, inadequate for detection of genetic differences between the lines, pilot data characterizing their responses to a wide range of dopaminergic agonists and antagonists suggest little difference in dopamine system functioning between FAST and SLOW mice. However, the results obtained with the FAST selected lines are consistent with the hypothesis that the stimulant effects of ethanol are mediated, at least in part, by dopaminergic systems.

Pretreatment with the dopamine antagonists haloperidol, SCH-23390, and raclopride, decreased ethanol-stimulated activity of FAST mice, while having no effects on locomotor activity on their own. However, this response to SCH-23390 was seen only in FAST-1 mice.

Coadministration of SCH-23390, a D1-specific antagonist, and raclopride, a D2 antagonist, produced greater decreases in ethanol-stimulated activity than either drug alone, suggesting that D1 and D2 receptors have additive or synergistic effects on the expression of this behavior. The activities of saline- and ethanol-treated SLOW mice were either unchanged, or exhibited parallel decreases in response to antagonist administration.

Pretreatment with the agonists, apomorphine (mixed), SKF-38393 (D1 agonist), and quinpirole (D2 agonist), produced changes in activity that were specific for each drug. Apomorphine generally had biphasic effects on the activities of saline- and ethanol-treated mice, producing slight decreases at a lower dose, and no change at a higher dose. However, saline-treated FAST-1 mice were not affected by apomorphine, while slight stimulation by ethanol was enhanced by apomorphine administration. SKF-38393 produced parallel increases in activity of saline-treated and ethanol-treated FAST mice, but blocked the depressant effects of ethanol in SLOW mice, and had no effect on the activity of saline-treated SLOW mice. Quinpirole, which had little effect on saline-treated animals in either line, also produced no significant changes in animals treated with ethanol. This result was replicated in the coadministration experiment, in which the activity of ethanol-treated FAST-1 mice was not changed by quinpirole, slightly increased by SKF-38393, and greatly increased by the combination of agonists. These data are in agreement with antagonist data, suggesting an additive or synergistic relationship of D1 and D2 receptors in mediating the stimulant effects of ethanol. The inability to detect enhancement of

ethanol-stimulated activity in FAST-2 mice may have been due to maximal stimulation by ethanol alone. In general, SLOW mice showed little change in response to agonist administration.

INTRODUCTION

Ethanol-Stimulated Activity

Although ethanol is classified as a sedative-hypnotic and has central depressant effects, it has also been shown to produce behavioral stimulant effects in animals and humans. The first demonstration of ethanol-induced stimulation was published in 1960 by Read, Cutting and Furst. "Gross activity" was measured on an activity table, an apparatus which consisted of a light plastic container supported by a spring lever. Movement of a mouse placed inside the container closed a microswitch, which activated an electrical counter. An ethanol volume of 5 ml/kg (ethanol concentration was not reported), injected subcutaneously into mice, produced marked stimulation with rapid onset, followed by the more typical depressant phase. Buckalew and Cartwright (1968) reported a dose-response curve for ethanol's effects on activity in albino rats. Low doses of ethanol (30% ethanol in saline) up to 1.2 g/kg were observed to produce increases in exploratory activity in a Skinner box, while doses of 1.8, 2.4, and 3.0 g/kg caused decreases in exploration. Data from gerbils were found to be consistent with data obtained from mice and rats. Low doses of ethanol (1.0 and 2.0 g/kg) produced increases in open-field activity (Järbe and Ohlin, 1977). Taken together, these studies are typical examples of the biphasic actions of ethanol as emphasized by Pohorecky (1977). Whereas high

doses of ethanol have sedative effects, the stimulant properties of ethanol are evident at low doses or at early time points after exposure to higher doses of ethanol.

Clinical studies have also produced evidence suggesting that moderate ethanol doses can elicit stimulant, along with euphoric effects, in human subjects. Ahlenius et al. (1973) asked subjects to rate themselves on the following behavioral and mood categories following oral ingestion of 200 ml of 43% (v/v) ethanol: "tired," "alert," "talkative," "elated," "happy," "subjective working capacity," "tense," and "restless." Talkativeness was considered to reflect the stimulant actions of ethanol while elation and happiness were taken as reflections of the euphoric effects of the drug. Subjects ingesting alcohol rated themselves to be more talkative, more elated, and happier than in a sober state. Non-intoxicated observers who were familiar with the subjects were also asked to give independent assessments of subjects' mood, and were in agreement with subjects' self-reports. These data are consistent with results reported by Ekman and colleagues (1963, 1964) who had originally developed this method of estimating general intoxication by subjective reports. In those studies, Ekman et al. also reported decreases in tension, tiredness, and restlessness at time points corresponding to the stimulant and euphoric effects. In a more recent study by Lukas and Mendelson (1988), subjects reported euphoric episodes beginning within 10 minutes after drinking a 0.695 g/kg ethanol dose (total of 350 ml solution containing grapefruit juice and 40% beverage ethanol), which continued for an additional 40 minutes. The increased incidence of subjective reports of euphoria (75% of

subjects who ingested alcohol compared to 0% in a placebo control group) closely paralleled the increase in plasma ethanol levels, EEG alpha activity, and plasma ACTH levels, suggesting to the authors that the "major physiological and behavioral concomitants of ethanol intoxication occur at relatively low blood ethanol levels (approximately 32 mg/dl) during the ascending phase of the blood ethanol curve."

Because of the similar patterns of stimulation seen between animals and humans, and because humans also report feelings of euphoria and well-being corresponding to the timecourse of stimulation, it has been suggested that ethanol-stimulated activity in animals can serve as a model of alcohol's euphoric effects in humans. Consistent with this suggestion, it has been postulated that the stimulant effects of ethanol are mediated by neural mechanisms that also mediate reward and reinforcement, which may be the basis of ethanol's abuse potential. This theory, termed the psychomotor stimulant theory of addiction (Wise and Bozarth, 1987), extends to all addictive drugs, postulating that " . . . the seemingly disparate phenomena of drug addiction, positive reinforcement, and psychomotor activation are homologous, resulting from activation of a common brain mechanism." An understanding of the mechanisms by which ethanol produces its stimulant effects may lead to a clearer understanding of the mechanisms underlying alcohol addiction.

The FAST and SLOW Lines of Mice

Selective Breeding

Selective breeding has been called "the most important application of quantitative genetics" (Falconer, 1989). Methods of selective

breeding have been employed for many years in the fields of agriculture, animal husbandry, and canine breeding to produce plants and animals with certain desirable traits. These methods have gained popularity in drug addiction research, and a number of sets of selected animal lines currently exist that differ with respect to a particular drug response. In general, a bidirectional selection procedure consists of measuring a phenotypic trait in individuals, and subsequently mating high scoring individuals with each other and low scoring individuals with each other. This process of testing offspring and mating extreme scorers is repeated with each generation so that, given some genetic control of the phenotype, the phenotypic means of the lines diverge. The basic effect of selective breeding is to change gene frequencies within a line so that nearly all genes relevant to the selected trait become homozygously fixed for one particular allele, while trait-irrelevant genes continue to segregate independently. In addition to their usefulness for identification of traits that may be genetically correlated with the selection phenotype, selected lines are excellent tools for identifying and studying possible physiological mechanisms underlying the trait under selection.

Selection of the FAST and SLOW Lines

Several years ago, a selective breeding program was initiated to develop lines of mice differing in sensitivity to the activating effects of ethanol (Crabbe et al., 1987). FAST (sensitive) mice are highly activated by acute administration of low doses of ethanol, as measured in automated open-field activity monitors. In contrast, SLOW

(insensitive) mice consistently show little or no activation by these same doses (Phillips et al., 1991). Using within-family selection, two genetically independent replicates of these lines (FAST-1, SLOW-1 and FAST-2, SLOW-2) were simultaneously derived from HS/Ibg mice, a genetically heterogeneous stock produced by crossbreeding 8 inbred mouse strains chosen for widely divergent genetic backgrounds. In addition, two control lines (CON-1 and CON-2) were randomly bred from this stock and maintained with the selected lines. Because these control lines were shared with another selective breeding project, they were tested for ethanol-stimulated activity every third generation. The FAST and SLOW lines are widely divergent in their responses to a low dose of ethanol, and are currently in the 28th generation of selection (see figure 1).

The selection protocol has been described in detail elsewhere (Crabbe et al., 1987). Briefly, each mouse is tested for activity on two consecutive days with a 24-hour intertest interval. All injections are given intraperitoneally (i.p.). In selected generations 0 - 5 (S_0 - S_5), mice were given saline injections on the first day, and after a 2-minute wait, were tested for 4 minutes in Lehigh Valley circular open-field activity monitors. These monitors are 61 cm in diameter and are transected by six pairs of radially oriented photocell beams and receptors. On day 2, mice were injected and tested exactly as they had been on day 1, except that they were given 1.5 g/kg of 20% v/v ethanol solution prepared in saline. Testing occurred under dim lighting conditions.

The selection protocol was altered beginning with generation S_6.

Figure 1. Response of FAST, SLOW, and CON replicate 1 (panel a), and replicate 2 (panel b) lines to 27 generations of selection for differential sensitivity to ethanol-induced stimulation. For most generations, mice were injected on day 1 with 2.0 g/kg EtOH and, after 2 minutes, placed in activity monitors for 4 minutes. On day 2, mice were tested in the same manner, except mice were injected with saline. ACT represents the difference between saline activity counts and ethanol activity counts collected 24 hours earlier. Each data point represents the entire population of first litter offspring of parents from the previous generation; SEM larger than symbol size are shown. (Phillips et al., unpublished).

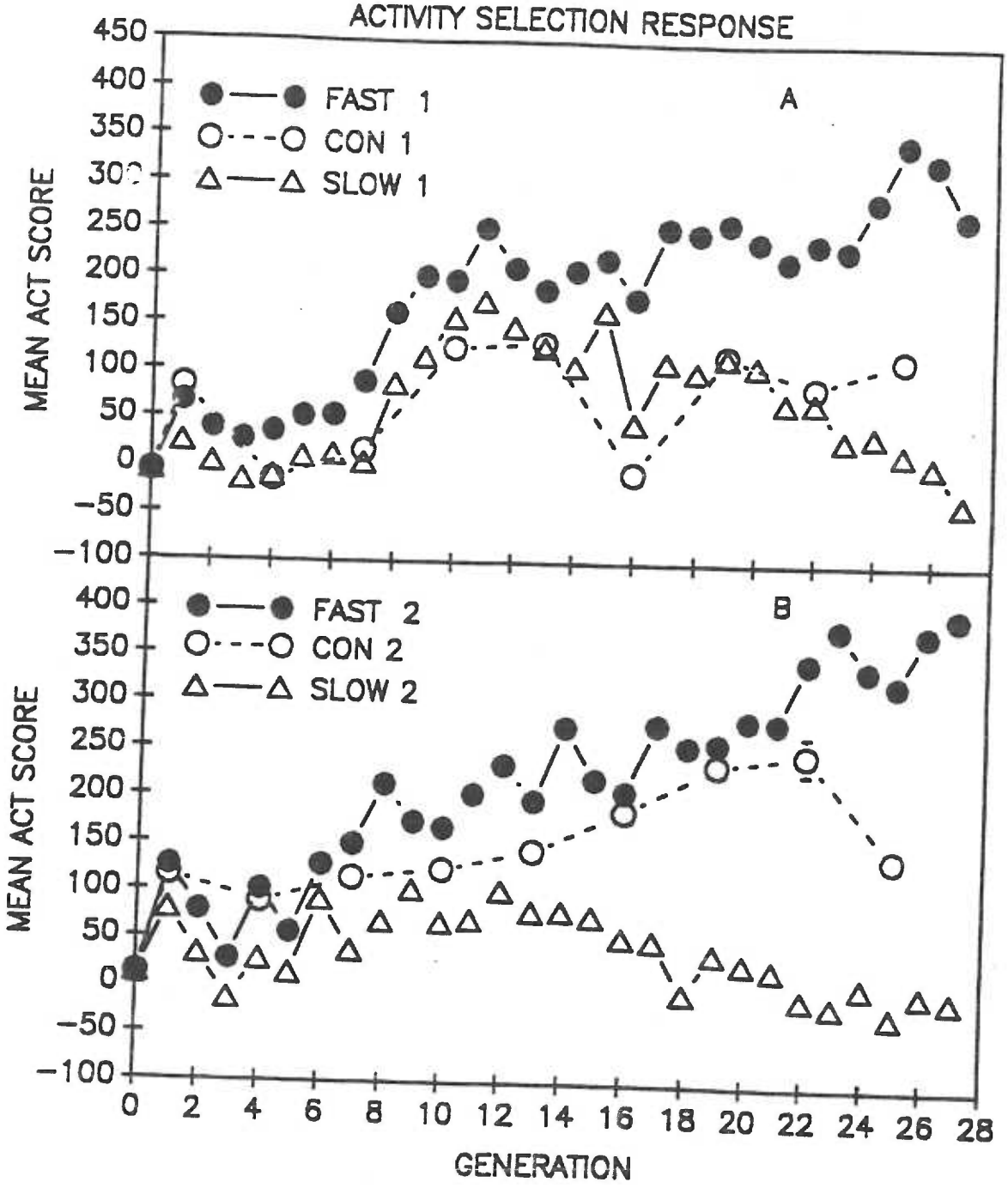
ACTIVITY SELECTION RESPONSE
FAST 1
CON 1
SLOW 1
A
MEAN ACT SCORE
450
400
350
300
250
200
150
100
50
0
−50
−100
FAST 2
CON 2
SLOW 2
B
MEAN ACT SCORE
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
GENERATION

Figure 1

The order of the days on which ethanol and saline were administered was reversed, the ethanol dose was increased from 1.5 g/kg to 2.0 g/kg (still 20% v/v), and light conditions during activity testing were changed from dim illumination to bright fluorescent light. These changes were motivated by data demonstrating that the latter conditions enhanced expression of ethanol-stimulated activity in Swiss, or FAST and SLOW mice (Crabbe et al., 1988). Throughout selection, the saline activity score has been subtracted from the ethanol activity score to produce an ACT score. The ACT score is the selection phenotype and generally reflects the effect of ethanol on locomotor activity. However, other factors may contribute to this score. These factors include the possible effects of previous exposure to handling and injection, previous exposure to the testing apparatus, differences in environmental conditions between day 1 and day 2 (e.g., temperature or noise levels), as well as the amount of stereotypy (e.g., grooming or sniffing) in which an animal engages.

A within-family breeding scheme has been used throughout selection. Within each replicated line, one male and one female from each of 9 families are chosen as parents and mated using a rotational breeding scheme, to produce the next generation. Individuals with the highest ACT scores are mated together to form the FAST lines, while individuals with the lowest (including negative) ACT scores are mated to produce the SLOW lines. Each of the lines is maintained as an independent breeding population, and the breeding scheme avoids brother-sister matings. Mice for the control lines are tested for activity responses to ethanol as described above, and individuals are randomly

bred (i.e., without regard to their ACT scores), avoiding brother-sister matings to produce nonselected lines.

Response to Selection

As seen in figure 1, a large response to selection occurred in the first generation. This large initial response, with little further divergence prior to protocol changes, suggests that the expression of ethanol-stimulated locomotor activity is primarily controlled by a single gene. The continued slow divergence between the lines after S_6, suggests that several genes with minor involvement in sensitivity to ethanol's locomotor effects were recruited after the change in the selection protocol, but have not yet become homozygously fixed in the lines.

The heritability of a trait reflects the proportion of phenotypic variance in that trait that can be presumed to be due to additive genetic variance. Heritability estimates can be derived in selective breeding programs from the calculated response to selection (the change in population mean resulting from selection) and selection pressure (the difference between the mean of the parents selected to produce offspring and the mean of the population from which parents were drawn). Phillips et al. (1991) reported heritability estimates (h^2) for 17 generations of FAST and SLOW lines. For the FAST-1 and FAST-2 lines, heritabilities were calculated to be $h^2 = 0.14$ and 0.17, respectively; for SLOW-1 and SLOW-2 lines, $h^2 = -0.12$ (effectively zero) and 0.05, respectively. These estimates of heritability are comparable to those obtained for other highly successful selection studies (McClearn and Kakihana, 1981;

Phillips et al., 1990).

Interpretation of Results with Selected Lines

When a selective breeding program has successfully produced lines which differ with respect to a specific phenotype, it then becomes of interest to identify correlated responses to selection. Because selection produces lines homozygous at gene loci relevant to the selected trait, other traits with common genetic influence can be identified. For example, if FAST and SLOW mice, which are being selected for differential sensitivity to the locomotor stimulant effects of ethanol, were found to differ in locomotor responses to dopaminergic drugs, differential sensitivity to dopaminergic drugs would constitute a correlated response to selection. The implication is that some or all of the genes involved in differential sensitivity to the stimulant effects of ethanol also influence dopamine system function.

Guidelines for interpretation of experiments using selectively bred lines have been suggested and discussed in detail (Crabbe et al., 1990). As pointed out by the authors, one issue that may cause problems in interpretation is that in selective breeding with a finite animal population, inbreeding inevitably occurs over successive generations of selection. The effect of inbreeding is to force all trait-relevant and irrelevant genes to the homozygous state. This leads to the possibility that due to chance fixation of alleles, lines will differ with respect to a trait that is not truly genetically correlated with the selection response. Development of replicate sets of selected lines from independent breeding populations can decrease the probability of finding

false genetic correlations due to inbreeding. It is unlikely that chance fixation of alleles for the same trait in the same direction would occur in both replicates; thus, differences found between the lines in both replicates are more likely to be genetically correlated with the selection phenotype.

The presence of strong, moderate, weak, or no evidence for genetic correlation may be concluded based on results from analysis of variance (ANOVA) grouped on line and replicate, if assumptions of ANOVA are met. In general, a significant difference between lines suggests moderate or strong evidence for a genetic correlation, depending upon the presence and the nature of a significant line by replicate interaction. When the line by replicate interaction is not significant, or when post-hoc analysis of a significant interaction between line and replicate reveals differences in both replicates, this may be regarded as strong evidence for a genetic correlation. A situation in which there is a main effect of line, and post-hoc analysis of a significant line by replicate interaction reveals differences in one replicate only, is considered to provide moderate evidence for a genetic correlation. Finally, the absence of line differences provides weak or no evidence for a genetic correlation, again depending upon the nature of the interaction between line and replicate. This manner of analysis of experiments using drugs with known mechanisms of action to characterize the locomotor responses of FAST and SLOW mice can aid in identification of correlated responses to selection, and can therefore lead to identification of substrates likely to be involved in determining sensitivity to ethanol-induced stimulation.

Neurochemical Basis of Ethanol-Stimulated Activity

Stimulation of Dopaminergic Activity by Ethanol

There is a considerable body of evidence that implicates catecholaminergic systems, particularly dopaminergic, in the mediation of ethanol-stimulated activity. Locomotor activity levels seem to be closely related to levels of dopamine present in the brain, and to dopaminergic transmission. Messiha et al. (1990) studied the interrelationships between spontaneous locomotor activity and whole brain biogenic amine concentrations by measuring dopamine, serotonin, and their metabolites in four strains of mice differing in levels of locomotor activity, and concluded that high levels of dopamine were correlated with high levels of spontaneous activity. Although acute ethanol treatment did not cause changes in dopamine levels measured in mouse brain homogenates (Alari et al., 1987), there is evidence to suggest that ethanol treatment produces changes in dopaminergic activity. For example, Carlsson and Lindqvist (1973) measured the effect of ethanol on hydroxylation of tyrosine and tryptophan (catecholamine precursors) in rats *in vivo*, and found increased synthesis. In addition, several studies have reported increases in dopamine metabolism/turnover, another index of dopaminergic activity, after acute administration of ethanol. Alari et al. (1987) found that ethanol (2.0 and 4.0 g/kg) caused a dose-dependent increase in the dopamine metabolites, 3,4-dihydroxyphenyl-acetic acid (DOPAC) and homovanillic acid (HVA), in mouse and rat brain homogenates of telencephalon plus diencephalon. Lucchi et al. (1983) observed increases in striatal DOPAC concentrations in rats previously given 3.0

g/kg ethanol. Engel et al. (1988) determined that 2.5 g/kg ethanol given to mice caused increases in DOPAC and HVA levels in homogenates of dopamine-rich limbic regions and striatum, and concomitant increases in locomotor activity.

Some *in vivo* evidence of dopamine system activation by ethanol has been provided by microdialysis experiments. Imperato and DiChiara (1986) measured the effect of ethanol administration on metabolite concentrations and dopamine release in freely moving rats by transcerebral dialysis. Ethanol doses which elicited behavioral stimulation in these animals (increases in incidence of rearing, grooming, and ambulation), also stimulated dopamine release and increased DOPAC and HVA concentrations in the nucleus accumbens. At higher doses (1.0 - 2.5 g/kg), dopamine, DOPAC, and HVA concentrations were increased in the caudate nucleus, as well as the nucleus accumbens. These doses produced sedation as well as activation, in accordance with the aforementioned biphasic time course. Interestingly, 5.0 g/kg, the highest dose of ethanol tested, produced a biphasic effect on dopamine release. Dopamine levels decreased during ethanol-induced hypnosis and increased during recovery to a sedated state. These results led the authors to suggest that the inhibitory effects of high doses of ethanol on dopamine release contribute to its sedative-hypnotic effects.

Effects of Interruption of Dopamine Function on Ethanol-Stimulated Activity

Various agents that block catecholamine synthesis or inhibit dopamine cell activity have been used to test the role of dopamine in

ethanol-induced locomotor stimulation. As an addition to their microdialysis experiments, Imperato and DiChiara (1986) administered γ-butyrolactone to rats given 0.5 g/kg ethanol. This inhibitor of dopamine cell firing and dopamine release completely abolished the behavioral activation seen in those animals; however, γ-butyrolactone also produced decreases in activity when administered by itself. When mice and rats pretreated with α-methyl-*p*-tyrosine (AMPT; an inhibitor of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis) were later assessed for locomotor activity after ethanol, the activation caused by ethanol was reduced to baseline levels, while AMPT alone had no significant effects on activity (Carlsson et al., 1972). In addition, this ethanol-stimulated activity could be partially restored in AMPT treated animals by administration of the dopamine precursor, L-dopa, in doses that by themselves did not influence activity (Engel et al., 1974). Pretreatment with AMPT was also shown to reduce ethanol-induced stimulation and euphoria in humans (Ahlenius et al., 1973). Pretreatment with nialamide, a compound that inhibits monoamine oxidase, also antagonized the excitatory effect of ethanol in mice tested in an open-field activity monitor (Ahlenius et al., 1974). This antagonism of stimulation by nialamide was postulated to be due to feedback inhibition of dopamine synthesis resulting from inhibition of monoamine oxidase. Data from the studies described above indicate that newly synthesized catecholamines may be involved in mediating ethanol-induced locomotor stimulation.

Dopamine Agonist and Antagonist Effects on Locomotor Stimulation

The development of highly receptor-specific drugs has been helpful in elucidating the role of dopamine receptors in ethanol-stimulated activity. Liljequist et al. (1981) found that when the dopamine receptor antagonists, pimozide or haloperidol, were administered with ethanol, ethanol-stimulated activity in NMRI mice was suppressed by doses that had no effect on locomotor activity when administered alone. Higher doses of the antagonists, which decreased activity levels on their own, caused even greater suppression of ethanol-stimulated activity. In order to investigate the contributions of the dopamine D1 and D2 receptor subtypes to ethanol-stimulated activity, Koechling et al. (1990) tested the effect of SCH-23390 (a D1 antagonist) or pimozide (a D2 antagonist) on ethanol-stimulated activity in mice. SCH-23390 reduced ethanol-stimulated activity only at doses which by themselves attenuated motor activity. However, pimozide attenuated ethanol-stimulated activity in doses which did not by themselves affect motor activity.

Interestingly, administration of low doses of the nonspecific dopamine agonist, apomorphine, has also been shown to attenuate ethanol-induced activation (Carlsson et al., 1974; Strömbom et al., 1977). Dudek et al. (1984) demonstrated this effect of apomorphine in the selectively bred Long Sleep and Short Sleep mice. As discussed in Carlsson et al., (1974), these low doses of apomorphine are thought to act like dopamine antagonists because of their action on presynaptic autoreceptors that serve as an inhibitory feedback mechanism, thus suppressing dopamine release.

In addition to their effects on ethanol-stimulated activity, dopamine antagonists have been demonstrated to block locomotor stimulation produced by other drugs. These drugs include amphetamine and methylphenidate (Mithani et al., 1986), cocaine (Delfs et al., 1990), nicotine (Corrigall and Coen, 1991), and diazepam (Söderpalm et al., 1991). Higher antagonist doses that did affect motor ability by themselves generally caused even greater suppression of drug-induced activation. On the other hand, the dopamine agonists, SKF-38393 and quinpirole, have been shown to enhance the stimulant effects of amphetamine in mice depleted of dopamine by reserpine pretreatment (Ross et al., 1989).

Relative Roles of Dopamine Receptor Subtypes in Expression of Locomotor Behavior

Experiments designed to assess the role of dopamine systems in mediating locomotor responses to drugs are complicated by evidence suggesting that dopamine receptor subtypes, most notably the D1 and D2 subtypes, may have distinct, but interdependent, roles in mediating the expression of motor behavior. Reductions in motor activity, and in sniffing, rearing, and grooming behaviors, have been reported in mice after administration of the D2 agonists, quinpirole (0.01 - 0.4 mg/kg), and pergolide (0.025 - 0.04 mg/kg), and by the putatively D2 autoreceptor-selective agonists (-)-3-PPP and B-HT 920 (Jackson et al., 1989). Interestingly, the effects of these drugs were significantly, although not completely, reversed by each of the D1 agonists, CY-208243 and SKF-38393. The authors suggested that the depressant effects of D2 agonists were due to occupation of autoreceptors, which are generally

thought to be of the D2 receptor subtype (Kebabian and Calne, 1979; Mereu et al., 1983; Napier et al., 1986). The subsequent reduction in dopamine availability at postsynaptic D1 receptors allowed stimulation of D1 receptors by specific agonists to restore motor ability (also discussed in Jackson et al., 1988). Alternatively, D1 and D2 receptors may have distinct roles due to their different cellular effects. D1 and D2 receptors are part of a large family of receptors that are coupled to G-proteins which initiate a cascade of cellular responses (see reviews by Civelli et al., 1991, and Sibley and Monsma, 1991). D1 receptors are coupled to a G-protein (G_s) which stimulates adenylate cyclase activity to increase cAMP concentrations, while D2 receptors are coupled to G-proteins (G_i) that inhibit production of cAMP by inhibiting the activity of adenylate cyclase (see also Seeman and Niznik, 1988, and Stoof and Kebabian, 1981). In turn, cyclic AMP levels affect a number of cell functions by directly interacting with ion channels, or by stimulating protein kinase A to activate various enzymes by phosphorylation.

Despite the seemingly opposite responses that appear to be mediated by D1 and D2 receptors, it also appears that stimulation of both receptors is necessary for full expression of locomotor behavior (Braun and Chase, 1986; Walters et al., 1987). While SKF-38393 (D1), quinpirole (D2), or pergolide (D2) administration alone had no effect on activity levels of mice pretreated with reserpine (depletes catecholamine stores) and AMPT (inhibits synthesis of catecholamines), coadministration of quinpirole or pergolide with SKF-38393 resulted in significant activity increases in these animals. These data, along with several other studies (e.g. Jackson and Hashizume, 1986; Pichler and

Pifl, 1989; Rubenstein et al., 1988; Starr and Starr, 1987) support the notion that D1 and D2 receptors are interdependent, and that neither completely controls expression of locomotor behavior. In addition, it has been suggested that D1 receptors play a permissive role in D2 receptor-mediated behaviors. For example, D1 receptor stimulation by SKF-38393 enhanced stereotypy produced by the D2 agonist quinpirole in intact rats, and was necessary for expression of D2-mediated behaviors in animals depleted of endogenous dopamine (Longoni et al., 1987; White et al., 1988).

EXPERIMENTS: PHARMACOLOGICAL MANIPULATIONS

Rationale

The FAST and SLOW selected lines of mice differ markedly in their locomotor responses to low doses of ethanol. The success of the selection suggests that genetic factors are important in the expression of differential sensitivities to the locomotor effects of ethanol. Thus, the FAST and SLOW lines provide a good tool for determination of the biochemical and physiological mechanisms underlying this behavioral response. A substantial amount of evidence suggests that ethanol works centrally to produce its effects. As reviewed above, considerable evidence implicates the involvement of catecholamine systems, especially dopaminergic pathways, in ethanol-induced locomotor stimulation.

It was hypothesized that if selection differentially altered dopaminergic systems between the lines, FAST and SLOW mice would likely differ in their responses to manipulation of dopamine function. A

pharmacological approach was used, in which the effects of dopamine receptor specific agonists and antagonists on basal activity levels and on ethanol-induced activity changes were assessed. Based on literature reviewed above, it was hypothesized that if locomotor activation induced by ethanol in FAST mice is mediated by dopamine systems, pretreatment with dopamine antagonists would reduce or completely block ethanol-stimulated activity, even at antagonist doses that did not affect motor activity on their own. Given that SLOW mice do not show any appreciable stimulant response to ethanol, activity levels after ethanol treatment were not expected to change significantly in response to pretreatment with dopamine antagonists, particularly at doses chosen specifically to have little effect on basal activity levels.

As with the antagonists, it was predicted that the FAST and SLOW lines would differ from each other in their responses to agonist administration in the presence of ethanol. Specifically, ethanol-stimulated activity of FAST mice was expected to be sensitive to additional manipulation of the dopamine system, in the direction appropriate to the receptor subtype agonist being tested. Agonists that increase locomotor activity on their own at high doses were expected to enhance the stimulant response to ethanol, when administered in low doses that did not affect activity on their own. On the other hand, agonists that decrease locomotor activity on their own at high doses were expected to decrease or block ethanol-stimulated activity, at doses that did not affect activity on their own. Since there is little evidence for the involvement of dopamine systems in locomotor activity decreases produced by ethanol administration, it was hypothesized that

agonist administration would have no effect on the activity of ethanol-treated SLOW mice. Alternatively, it may be that direct stimulation or blockade of dopamine systems would increase or decrease the activity of SLOW mice, independent of the biological mechanism responsible for the depressant effects of ethanol. This result would provide some information regarding the sensitivity of dopaminergic systems in this line.

The studies described here explored the relative roles of the D1 and D2 receptor subtypes in the expression of ethanol-stimulated activity, using dopamine agonists and antagonists that are specific for either one or both subtypes. Selection of the FAST and SLOW mice for differential sensitivity to the locomotor stimulant effects of ethanol could have altered one or both of the dopamine receptor subtypes in these lines. Alternatively, it may be that selection did not alter dopaminergic systems, but altered other neurotransmitter systems involved in the pathways mediating sensitivity to the stimulant effects of ethanol. It has been demonstrated that ethanol interacts with GABAergic systems to produce some of its effects (e.g., Huidobro-Toro et al., 1987; Wood et al., 1989). In addition, it has been suggested that part of the pathway mediating stimulation of locomotor activity involves connections between γ-aminobutyric acid (GABA) containing neurons and dopaminergic neurons (Austin and Kalivas, 1991; Scheel-Krüger, 1978). Phillips et al. (1992) characterized the locomotor responses of FAST and SLOW mice to drugs of different pharmacological classes. The lines did not consistently differ in their responses to morphine, *d*-amphetamine, caffeine, or nicotine, but were found to differ in response to the

alcohols, barbiturates, and diazepam. The result that the FAST and SLOW lines differed in response to drugs that likely produce some of their effects via interactions with the GABA/benzodiazepine/chloride-ionophore receptor complex, but not to drugs with other mechanisms of action, led to the suggestion that selection of the FAST and SLOW lines had differentially altered some aspect of this receptor complex. The absence of a difference between the FAST and SLOW lines in locomotor response to several doses of amphetamine, a drug which has direct effects on dopamine systems, suggests that genetic selection of the FAST and SLOW lines has altered biological systems other than dopamine systems. However, this study does not represent exhaustive examination of dopaminergic systems in these mouse lines. The actions of amphetamine are specific to mechanisms of catecholamine release. The possibility that selection has differentially altered some other aspect of dopamine system functioning (e.g. dopamine receptors) between the lines warrants further examination.

Methods and Materials

Animals

Mice of both replicates of the FAST and SLOW selectively bred lines were used in all experiments, unless otherwise specified. As shown in Table 1, sex and selection generation varied depending upon availability. Ages of animals at testing ranged from 45 to 108 days. FAST and SLOW mice were bred in the Portland VA Animal Research Facility and kept on a 12 hour light/12 hour dark cycle with lights on at 6:00 a.m. Locomotor activity testing took place between 8:00 a.m. and 4:00

Table 1. Selection generation, sex, and age in days of FAST and SLOW mice for each drug tested. Drugs are listed in the order in which they are presented in the results section. Number of subjects are per line and replicate, except * are collapsed on replicate.

Table 1.

EXPERIMENT	DOPAMINE DRUG	GENERATION	SEX	AGE IN DAYS	NUMBER OF SUBJECTS
	Antagonists				
1	Haloperidol	S_{19-20}	Female	73-106	5-10
2	SCH-23390	S_{21-22}	Female	64-108	7-9
	FAST-2 only	S_{26}	Female	71-94	9-10
3	Sulpiride	S_{20}	Male	51-91	6-8
	FAST only	S_{21}	Female	56-81	6
	dose-response	S_{24}	Female	47-84	9-10
4	Raclopride	S_{26}	Female	53-97	8-10
	FAST-1 only	S_{26-27}	Female	54-56	8
5	SCH-23390 + Raclopride	S_{26-27}	Female	48-90	9-11
	Agonists				
6	Apomorphine	S_{24-25}	Male	50-87	9-10
7	SKF-38393	S_{19}	Female	86-130	10-14*
8	Quinpirole	S_{24}	Male	50-73	9-10
9	SKF-38393 + Quinpirole	S_{25}	Female	45-97	9-10

p.m. Animals were housed in clear polypropylene cages (28 x 18 x 13 cm) containing corn cob bedding that was changed twice weekly. Cages were placed in a filtered Thorens rack system or were covered with filter tops on open racks. Water and food were available *ad libitum*, except during activity testing. Mice were housed with littermates, dam, and sire until 21 ± 1 days of age, at which time they were weaned and housed 2-5 per cage with animals of the same sex, line, and replicate until testing. Ambient temperature in the colony room was controlled at 21 ± 2 °C.

Measurement of Locomotor Activity

Omnitech Digiscan Animal Activity Monitors (Model CCDIGI) were used to assess activity. Each apparatus comprised a clear Plexiglas box (40 cm x 40 cm) in which mice were tested. A clear Plexiglas lid (44 cm^2) with 0.64 cm holes drilled 5 cm apart was placed on top of the box to prevent animals from escaping. This box was set inside a 40 x 40 cm square monitor, which has 8 photocell beams equally spaced along each of its 4 sides, approximately 2 cm above the floor of the box. There are an additional 8 photocell beams equally spaced along two parallel sides and placed 8 cm above the floor of the box for detection of vertical movements. The monitor was placed inside a black Plexiglas chamber which has a fluorescent light mounted high on the back wall, and a ventilation fan mounted on the rear right wall that also provides masking noise. The inside walls of the chamber are covered with foam material like that used in sound recording studios for outside sound attenuation. The following activity variables were automatically

recorded by computer during activity testing: horizontal activity counts, total distance traveled (cm), number of discrete horizontal movements, movement time, rest time, vertical activity (rearing), number of vertical/rearing movements, vertical time, stereotypy counts, number of stereotypic behaviors, stereotypy time, clockwise revolutions, anti-clockwise revolutions, margin distance, margin time, center distance, center time, and time spent in corners. Distance traveled was used in these studies as the measure of locomotor activity. Horizontal activity counts also measured locomotor activity; however, horizontal activity counts were accrued when lower photocell beams were interrupted, and an animal engaging in sniffing or grooming (stereotypic) behaviors can have high activity counts without engaging in forward locomotion. On the other hand, increases in distance traveled were recorded only when consecutive lower photocell beams were interrupted. Thus, distance traveled is thought to be a more accurate reflection of the amount of forward locomotion in which each animal engaged. All data were collected in 5 minute time blocks.

Experimental Protocol

The experimental protocol was identical for all experiments except coadministration studies and a sulpiride dose-response study. In the sulpiride dose-response study, animals were injected with vehicle, or one of four sulpiride doses, and tested in activity monitors on one day only. For all other studies in which a single dopamine drug was used, a design was employed for each drug in which each of three drug conditions (vehicle, dose 1 or dose 2) was paired with each of two ethanol

conditions (vehicle or 2.0 g/kg) and compared in each replicate of the FAST and SLOW lines (see Table 2 for experimental design). This design allowed assessment of drug effects on activity after saline injection (saline activity) as well as on activity after ethanol administration. As shown in Table 3, the coadministration studies involved the pairing of each of four drug conditions with each of two ethanol conditions, and comparison of treatment effects on both replicates of the FAST and SLOW lines. Four drug conditions were necessary in these experiments in order to evaluate the effect of vehicle alone, each drug alone, and the effect of the two drugs combined, on ethanol-stimulated activity and activity after saline administration. Specific drug doses, drug pretreatment times and duration of testing varied with the experiment and drug(s) used (see Table 4), but in all cases, mice were injected first with drug or vehicle, then injected with saline or ethanol and immediately placed in the activity monitors. Drug solutions were administered in a volume of 10 ml/kg; ethanol injection volumes were adjusted for body weight for delivery of 2.0 g/kg of 20% v/v ethanol in saline. All injections were given intraperitoneally. The test chamber was cleaned of fecal material and urine after each animal, wiped down with 10% isopropyl alcohol to remove scent cues, then dried thoroughly.

Results from previous literature suggest that an initial period of habituation was necessary to observe the effects of D1 agonists on motor behaviors (Molloy and Waddington, 1985; Starr, 1988; Starr and Starr, 1986). Whereas naive nonhabituated animals had higher levels of spontaneous sniffing, grooming, and locomotion, habituated animals displayed low levels of spontaneous behavior, thus making it possible to

Table 2. Experimental design for assessment of the effects of vehicle or two doses of a dopaminergic drug on saline- and ethanol-treated FAST and SLOW mice. The number of subjects (n) in each cell is representative, and does not reflect the actual number of animals in each experiment (see table 1).

Table 2.

	FAST MICE			SLOW MICE		
Replicate 1		Saline	Ethanol		Saline	Ethanol
	Vehicle	n = 10	n = 10	Vehicle	n = 10	n = 10
	Dose 1	n = 10	n = 10	Dose 1	n = 10	n = 10
	Dose 2	n = 10	n = 10	Dose 2	n = 10	n = 10
Replicate 2		Saline	Ethanol		Saline	Ethanol
	Vehicle	n = 10	n = 10	Vehicle	n = 10	n = 10
	Dose 1	n = 10	n = 10	Dose 1	n = 10	n = 10
	Dose 2	n = 10	n = 10	Dose 2	n = 10	n = 10

Table 3. Experimental design for assessment of the effects of D1 and D2 receptor drugs, alone or in combination, on saline- and ethanol-treated FAST and SLOW mice. The number of subjects (n) in each cell is representative, and does not reflect the actual number of animals in each experiment (see table 1).

Table 3.

	FAST MICE			SLOW MICE		
Replicate 1		Saline	Ethanol		Saline	Ethanol
	Vehicle	n = 10	n = 10	Vehicle	n = 10	n = 10
	Drug 1	n = 10	n = 10	Drug 1	n = 10	n = 10
	Drug 2	n = 10	n = 10	Drug 2	n = 10	n = 10
	Drug 1 + Drug 2	n = 10	n = 10	Drug 1 + Drug 2	n = 10	n = 10
Replicate 2		Saline	Ethanol		Saline	Ethanol
	Vehicle	n = 10	n = 10	Vehicle	n = 10	n = 10
	Drug 1	n = 10	n = 10	Drug 1	n = 10	n = 10
	Drug 2	n = 10	n = 10	Drug 2	n = 10	n = 10
	Drug 1 + Drug 2	n = 10	n = 10	Drug 1 + Drug 2	n = 10	n = 10

Table 4. Subtype specificity, doses used, and time between injections for all dopamine drugs tested. References for pretreatment times are also listed. Drugs are listed in the order in which they are presented in the results section. All doses are presented in mg/kg except apomorphine, which is presented in μmol/kg. Time between injections is presented in minutes. Duration of the activity test was 15 minutes for all drugs, except haloperidol and SKF-38393, which were tested for 30 minutes.

Table 4.

EXPERIMENT	DRUG	ACTION	DOSES (mg/kg)	PRETREATMENT TIME (min)	REFERENCES (pretreatment time)
	Antagonists				
1	Haloperidol	mixed or D2	0.08, 0.16	2	Okada et al., 1990
2	SCH-23390	D1	0.015, 0.03	30	Ross et al., 1989
	FAST-2 only		0.045	30	--
3	Sulpiride	D2	25, 50	2	--
	FAST only		50	60	Rubinstein et al., 1988
	dose-response		0, 12.5, 25, 50, 100	60	--
4	Raclopride	D2	0.25, 0.50	30	Köhler et al., 1985
	FAST-1 only		0.125	30	--
5	SCH-23390 + Raclopride	D1 + D2		30	--
	FAST-1, SLOW-1		0.015/0.1		
	FAST-2, SLOW-2		0.03/0.25		
	Agonists				
6	Apomorphine	mixed	2.0, 8.0 μmol/kg	3	Strömbom, 1976
7	SKF-38393	D1	10, 40	2	Ross et al., 1989
8	Quinpirole	D2	0.001, 0.005	3	Jackson et al., 1989b
9	SKF-38393 + Quinpirole	D1 + D2	10, 0.005	3	--

detect enhancement of activity, including locomotion, by the D1 agonist, SKF-38393. Based on these data, and because the selected phenotype of FAST and SLOW mice is based on a two-day protocol, experiments with agonists were conducted over the course of 2 days for each animal. In the interest of consistency, antagonists were similarly tested. The first day was an habituation day, on which all mice were tested after saline injections with the timecourse appropriate to the dopamine drug being tested. The second day involved both drug and ethanol injections.

Drug Selection

Drugs were chosen on the basis of their high affinity and specificity for dopamine receptors. In addition, they have been shown to affect locomotor behavior, and in some cases, have altered drug- or ethanol-stimulated activity.

Antagonists

Haloperidol is an antagonist with higher affinity for D2 than D1 dopamine receptors (Seeman and Niznik, 1988). However, there is evidence to suggest that only D2 receptors are functionally affected by haloperidol binding (Meshul et al., 1992). Ethanol-stimulated activity in NMRI mice was blocked by pretreatment with 0.04 and 0.08 mg/kg haloperidol, administered 30 minutes prior to ethanol (Liljequist et al., 1981). In addition, hyperactivity induced by coadministration of methamphetamine and chlordiazepoxide was decreased by 20-minute pretreatment with 0.1 mg/kg haloperidol (Okada et al., 1990). Ten-minute pretreatment with haloperidol (0.25 mg/kg) enhanced activity decreases produced by U50-488, a κ-opioid receptor agonist (Castellano

and Pavone, 1987).

SCH-23390 is the most potent D1-receptor-specific antagonist currently available, with an affinity for D1 receptors that is approximately 4500 times higher than for D2 receptors (Seeman and Niznik, 1988). Hoffman and Beninger (1985) demonstrated that it can decrease locomotor activity on its own. SCH-23390, administered 30 minutes prior to amphetamine, blocked amphetamine-induced locomotor stimulation (Ross et al., 1989), and blocked dopamine agonist-induced activity in mice when given 1 hour before testing (Jackson and Hashizume, 1987).

Sulpiride is a selective antagonist for D2 receptors with a K_D of 11 nM in canine striatum (Seeman and Niznik, 1988). Locomotor activation induced by pergolide in reserpinized and AMPT treated mice was blocked by 50 mg/kg sulpiride, when administered 60 minutes prior to testing (Rubinstein et al., 1988). In addition, sulpiride also blocked locomotor stimulation produced by administration of the D2 agonist, bromocriptine (Jackson and Hashizume, 1987).

Raclopride, is also a selective D2 antagonist, but is more soluble than sulpiride, and has a K_D of 1.2 nM (Köhler et al., 1985). Raclopride has been shown to block apomorphine-induced hyperactivity in rats (Ögren et al., 1986). In addition, low doses of raclopride injected directly into rat nucleus accumbens at 5 to 7 minutes prior to amphetamine, blocked amphetamine-induced stimulation, and decreased spontaneous locomotor activity when administered at higher doses (van den Boss et al., 1988). In mice, effects of raclopride have been demonstrated at 10 minutes (Hitzemann et al., 1991) to 1 hour after

injection (Eshel et al., 1990). However, it has been demonstrated that a majority (90%) of drug reaches rat brain by thirty minutes after intravenous injection (Köhler et al., 1985).

Agonists

Apomorphine is a mixed agonist with K_D values of 0.7 nM and 0.66 nM for D1 and D2 receptors, respectively (Seeman and Niznik, 1988). The effects of apomorphine on activity are biphasic, producing activity decreases at low doses, and increases to baseline at higher doses (see Dudek et al., 1984). This biphasic effect is thought to reflect the actions of apomorphine on pre- and post-synaptic receptors (DiChiara et al., 1978). Apomorphine is widely used in studies of drug effects on locomotor activity, and, as discussed previously, has been demonstrated to have effects on ethanol-stimulated activity when administered with ethanol (Carlsson et al., 1975), or 10 minutes before ethanol administration (Dudek et al., 1984). In addition, 0.025 to 0.4 mg/kg apomorphine given alone produced robust depressant effects on activity within 5 minutes of administration.

SKF-38393 is a selective D1 agonist with a K_D of 1.1 nM (Seeman and Niznik, 1988). It has been shown to affect locomotor activity in otherwise nondrug-treated animals. This agonist was shown to increase locomotor activity in mice pretreated with reserpine (Rubinstein et al., 1988), and reserpine plus AMPT (Ross et al., 1988). In addition, enhancement of amphetamine-stimulated behavior in mice depleted of endogenous dopamine was produced by simultaneous administration of SKF-38393 (Ross et al., 1989). In the studies described above, activity was

assessed immediately after injection.

Quinpirole (LY-171555), a D2-selective agonist with a K_D of 4.8 nM (Seeman and Niznik, 1988), was found to increase activity in mice whose catecholamine stores had been depleted by treatment with reserpine and AMPT (Ross et al., 1988). Simultaneous injection of quinpirole and amphetamine increased amphetamine-stimulated locomotor activity relative to amphetamine controls (Ross et al., 1989). However, Jackson et al. (1989) reported locomotor depression in mice as assessed by subjective scoring of immobility 30 minutes after quinpirole administration.

Drug Sources and Preparation

The racemic mixture of SKF-38393 N-allyl hydrochloride, (-)-quinpirole hydrochloride, and 2-hydroxypropyl-β-cyclodextrin were all purchased from Research Biochemicals, Inc. (Natick, MA). Apomorphine hydrochloride, sodium metabisulfite, and sulpiride were all purchased from Sigma Chemical Co., St. Louis, MO. Haloperidol was obtained from McNeil Pharmaceutical in the lactate salt form as a premixed solution of 5 mg haloperidol/ml vehicle. SCH-23390 and SCH-23390 maleate, both from Schering Corp., Bloomfield, N.J., were gifts from Drs. Aaron Janowsky and Charles Meshul, respectively. Raclopride was also a gift from Dr. Janowsky. Ethanol (200 proof) was purchased from Aaper Alcohol and Chemical Company, and from Pharmco Products, Inc.

SCH-23390, SCH-23390 maleate, and raclopride solutions were prepared by dissolving each drug in 1 ml of warm water, then diluting with 0.9% saline. Sulpiride was solubilized in 5% or 20% hydroxypropyl-

β-cyclodextrin in saline. Apomorphine was mixed with 0.1% sodium metabisulfite in saline to enhance drug stability. SKF-38393 and quinpirole were dissolved in saline alone. Haloperidol and ethanol were diluted with saline to appropriate concentrations. SKF-38393 and apomorphine solutions were kept on ice and protected from light to inhibit degradation. Haloperidol was also protected from light.

Statistical Analyses

Difference scores (delta distance in cm) were calculated for each animal by subtracting day 1 distance data from day 2 distance data (drug day - saline day). Negative scores resulted when distance traveled on day 2 was less than distance traveled on day 1, due to habituation to the testing environment or to depressant drug effects. For each experiment, difference scores were grouped on line, replicate, drug condition, and ethanol condition, and analyzed by ANOVA in order to detect differences in response between the lines. One of the main interests in these experiments was to determine whether the dopamine drug had any effect on ethanol-induced activity within each line, thus a separate ANOVA was performed for each of the lines, in some cases. In cases in which the patterns of response between the replicates did not differ significantly, data were collapsed on replicate, and analyzed grouped on drug condition and ethanol condition. When patterns of response differed between replicates, a separate ANOVA was performed for each replicate of each line, with data again grouped on drug condition and ethanol condition. A statistically significant interaction of drug by ethanol was further characterized by analysis of simple main effects,

followed by a Newman-Keuls test, when appropriate, to detect the sources of significance. Level of significance was set at $p < 0.05$ for all analyses.

Results

Results for all experiments are summarized in Table 5. A common data range could not be derived across experimental results that would best represent differences between groups, thus data are presented on different scales in the figures.

Experiment 1: Haloperidol

Data are presented as delta distance scores (see figure 2). Although published literature report pretreatment times of 10 minutes to 30 minutes, it was uncertain whether the responses of FAST and SLOW mice to haloperidol would be similar to those of nonselected mice. Thus, in this experiment, haloperidol was injected 2 minutes prior to saline or ethanol injection, and activity testing continued for 30 minutes. Cumulative data for the first 15 minutes are presented for comparison with the majority of experiments described here, in which test duration was 15 minutes. Analysis of variance grouped on line, replicate, haloperidol condition, and ethanol condition revealed that there was no significant effect of replicate, nor were there any interactions involving replicate, thus data are presented collapsed on this variable. A significant line by ethanol interaction ($F[1,165]=80.5$, $p < 0.001$) indicated that the FAST and SLOW mice behaved according to their respective selection responses; FAST mice were highly stimulated by

Table 5. Summary of results: the effects of dopaminergic drugs on saline- and ethanol-treated FAST and SLOW mice. Results are simplified for brevity, and are based on statistically significant outcomes (see text). Results marked with * were obtained with analyses collapsed on ethanol condition within that line, and consequently are presented only once in the column of results of saline-treated mice.

Table 5.

EXPERIMENT	DRUG	ACTION	EFFECT ON ACTIVITY (Saline)	EFFECT ON ACTIVITY (Ethanol)
	Antagonists			
1	Haloperidol	mixed or D2	FAST: No effect SLOW: No effect	FAST: Decrease SLOW: No effect
2	SCH-23390	D1	FAST-1: No effect SLOW-1: Slight decrease FAST-2: No effect SLOW-2: No effect	FAST-1: Decrease SLOW-1: Slight decrease FAST-2: No effect SLOW-2: No effect
3	Sulpiride	D2	FAST: No effect SLOW: No effect	FAST: No effect SLOW: No effect
4	Raclopride	D2	FAST-1: Slight decrease SLOW-1: Slight decrease FAST-2: No effect SLOW-2: No effect	FAST-1: Decrease SLOW-1: No effect FAST-2: Decrease SLOW-2: No effect
5	SCH-23390 + Raclopride	D1 + D2	FAST-1: D1: No effect* D2: No effect D1+D2: No effect SLOW-1: D1: No effect* D2: No effect D1+D2: No effect FAST-2: D1: No effect D2: No effect D1+D2: No effect SLOW-2: D1: No effect* D2: No effect D1+D2: No effect	FAST-1: ---- SLOW-1: ---- FAST-2: D1: Decrease D2: Decrease D1+D2: Large decrease SLOW-2: ----
	Agonists			
6	Apomorphine	mixed	FAST-1: No effect SLOW-1: Decrease FAST-2: Biphasic effects SLOW-2: Decrease	FAST-1: Increase SLOW-1: Decrease FAST-2: Biphasic effects SLOW-2: Decrease
7	SKF-38393	D1	FAST: Slight biphasic SLOW: No effect	FAST: Biphasic effects SLOW: Increase
8	Quinpirole	D2	FAST: No effect SLOW: No effect	FAST: No effect SLOW: No effect
9	SKF-38393 + Quinpirole	D1 + D2	FAST-1: D1: No effect D2: No effect D1+D2: No effect SLOW-1: D1: No effect* D2: Decrease D1+D2: No effect FAST-2: D1: Increase* D2: Decrease D1+D2: No effect SLOW-2: D1: Increase* D2: No effect D1+D2: Increase	FAST-1: D1: Increase D2: No effect D1+D2: Large increase SLOW-1: ---- FAST-2: ---- SLOW-2: ----

Figure 2. Effects of the dopamine antagonist, haloperidol, on saline-treated and ethanol-treated FAST (top panel) and SLOW (bottom panel) mice. On day 1, all animals received two injections of saline. On day 2, haloperidol (0.08 or 0.16 mg/kg) or saline was injected 2 minutes prior to saline or ethanol (2.0 g/kg, 20% v/v in saline) injection. Data are presented collapsed on replicate as the mean change in distance traveled (day 2 - day 1). Each data point represents 13-18 animals; SEM larger than symbol size are shown. ** $p < 0.01$ vs. ethanol-treated, zero-dose FAST mice.

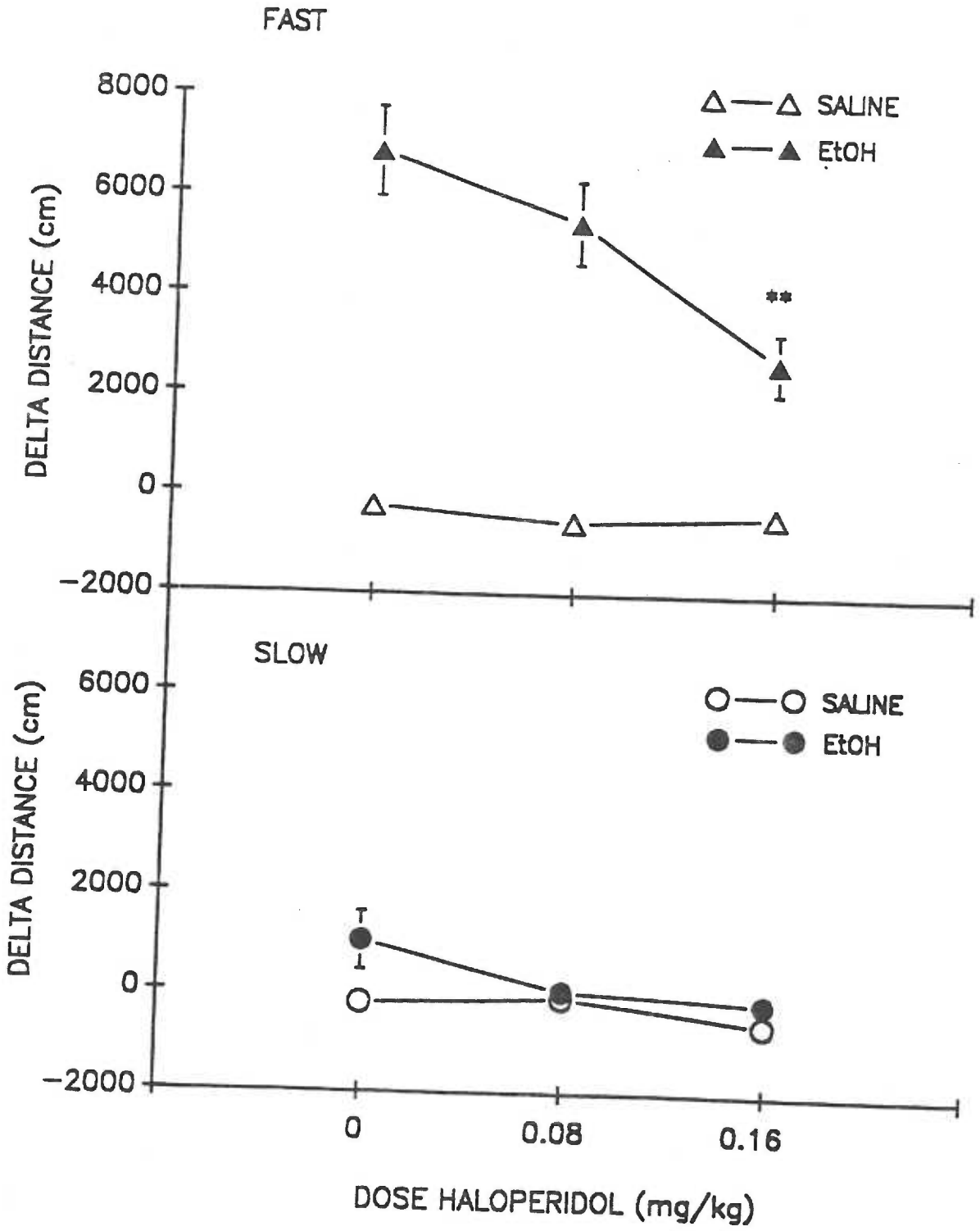
FAST
8000
6000
4000
2000
0
−2000
DELTA DISTANCE (cm)
SALINE
EtOH
**
SLOW
6000
4000
2000
0
−2000
DELTA DISTANCE (cm)
SALINE
EtOH
0
0.08
0.16
DOSE HALOPERIDOL (mg/kg)

Figure 2

ethanol, whereas SLOW mice were unaffected by the same dose of ethanol. Subsequent ANOVAs on FAST mice only (collapsed on replicate) revealed a significant interaction of haloperidol condition by ethanol condition ($F[2,90]=7.0$, $p < 0.001$). Simple main effects analysis revealed a significant effect of haloperidol administration on ethanol-stimulated activity of FAST mice ($F[2,90]=14.8$, $p < 0.001$), due to a significant reduction in the activity of mice given 0.16 mg/kg (Newman-Keuls test, $p < 0.01$). However, neither 0.08 mg/kg nor 0.16 mg/kg haloperidol affected the activity of saline-treated FAST mice ($F[2,90]=0.1$, NS). Similarly, there was no effect of haloperidol pretreatment on the activity of saline- or ethanol-treated SLOW mice.

These data indicate that pretreatment with haloperidol, a mixed dopamine antagonist with greater affinity for D2 than D1 receptors, can block ethanol-stimulated activity in FAST mice at doses that do not significantly affect locomotor activity. In addition, animals that are not stimulated by ethanol (SLOW mice), showed no response to dopamine receptor blockade by haloperidol.

Experiment 2: SCH-23390

SCH-23390, the D1 receptor antagonist, was administered in a dose of either 0.015 or 0.030 mg/kg 30 minutes prior to saline or ethanol treatment. Data are presented in figures 3a and 3b, as the change in distance traveled (day 2 - day 1). Informal examination of the data showed dissimilar response profiles between replicates of the FAST lines, in which ethanol-stimulated activity in replicate 1, but not replicate 2, mice was decreased by SCH-23390 administration. Analyses

Figure 3. Effects of the D1 antagonist, SCH-23390, on activity of saline-and ethanol-treated replicate 1 (a) and replicate 2 (b) FAST and SLOW mice. Data for FAST (upper panels) and SLOW (lower panels) mice are presented as the mean change in distance (delta distance) in cm. Mice were injected with saline or SCH-23390 (0.015 or 0.03 mg/kg), 30 minutes before saline or ethanol administration (2.0 g/kg) and a 15-minute activity test. Each data point represents 7-9 animals; SEM larger than symbol size are shown. * $p < 0.05$, ** $p < 0.01$ compared to ethanol-treated, zero-dose group.

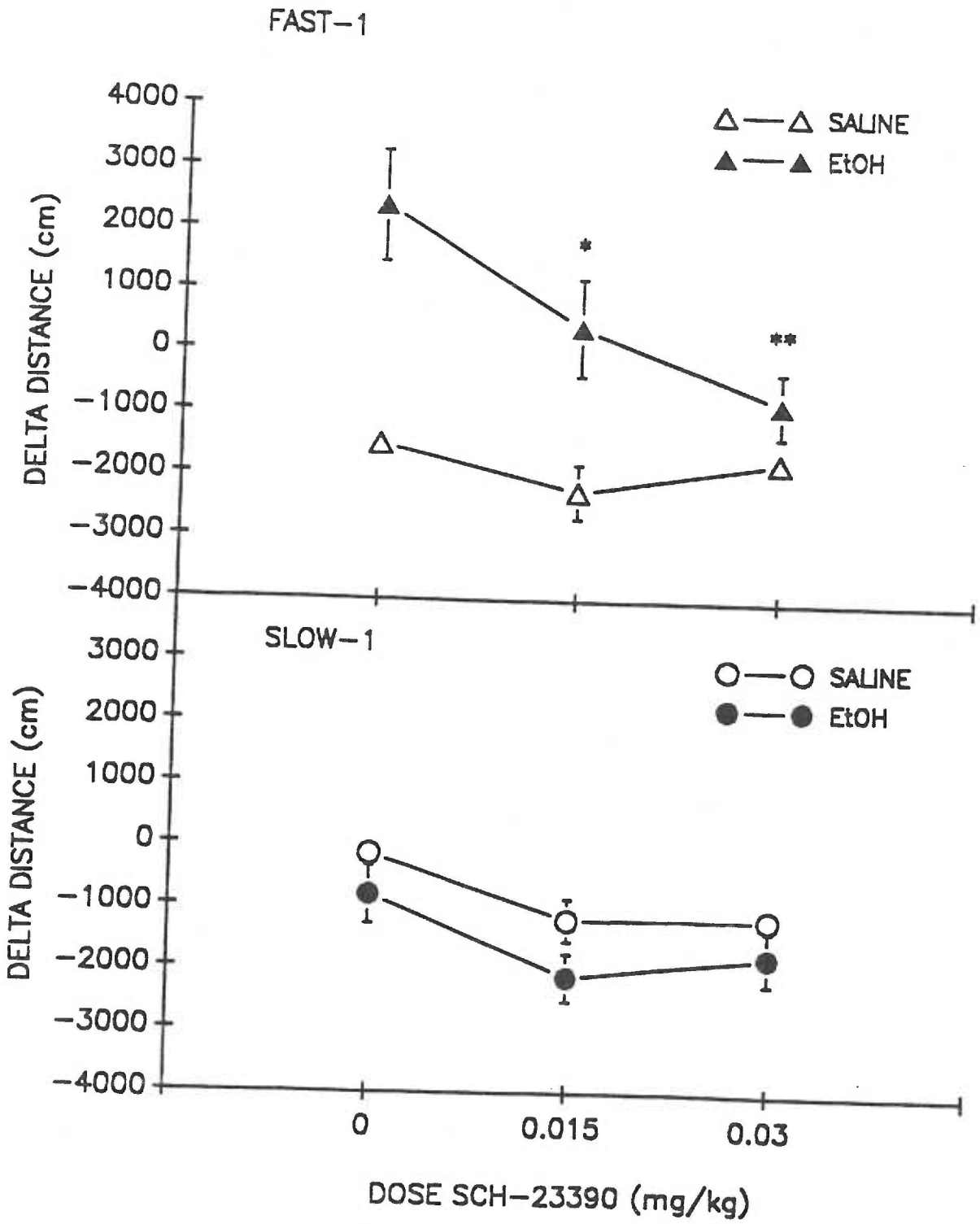
FAST–1
4000
3000
2000
1000
0
−1000
−2000
−3000
−4000
DELTA DISTANCE (cm)
SALINE
EtOH
*
**
SLOW–1
3000
2000
1000
0
−1000
−2000
−3000
−4000
DELTA DISTANCE (cm)
SALINE
EtOH
0
0.015
0.03
DOSE SCH–23390 (mg/kg)

Figure 3a

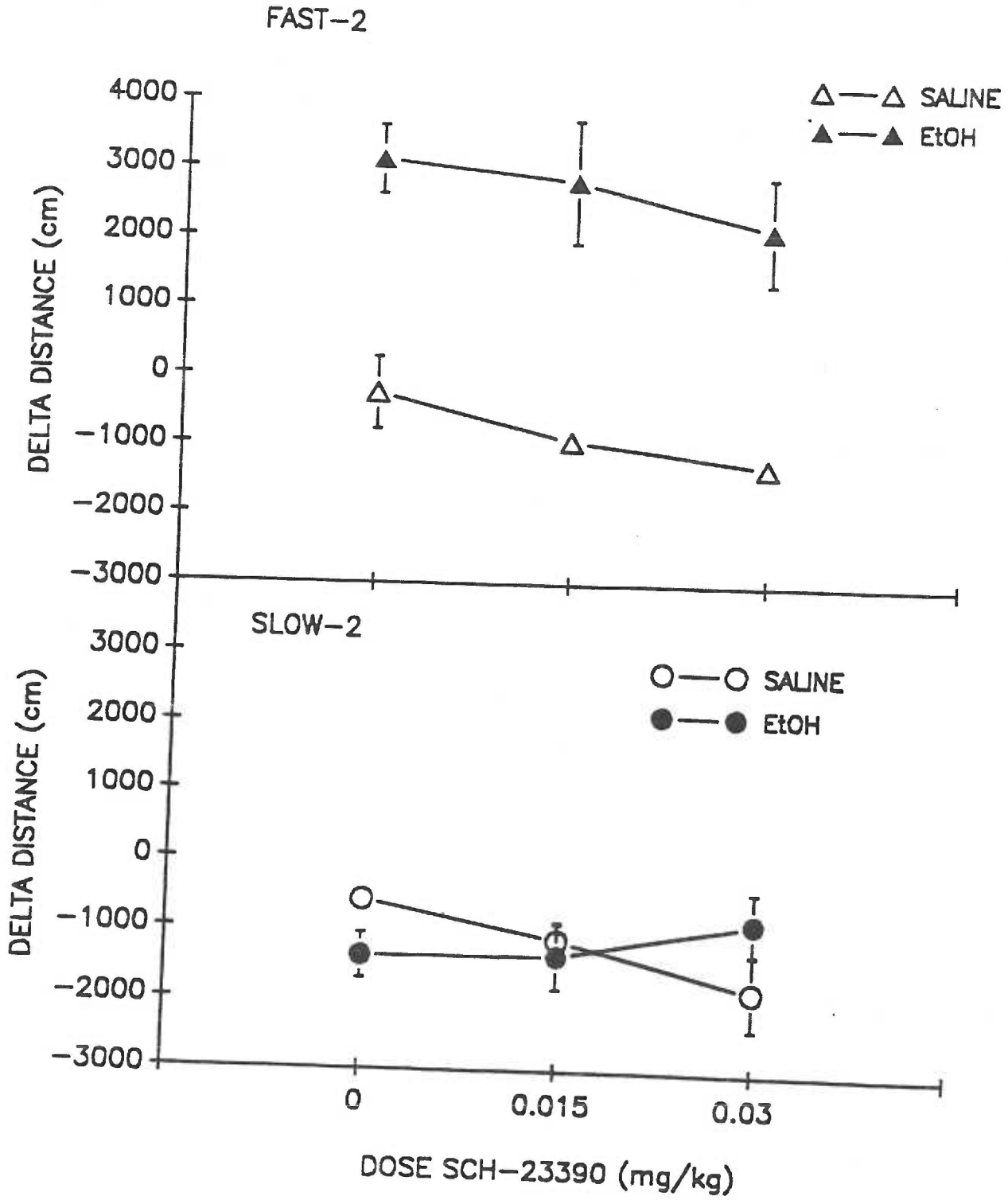
FAST–2
4000
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
SALINE
EtOH
SLOW–2
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
SALINE
EtOH
0
0.015
0.03
DOSE SCH–23390 (mg/kg)

Figure 3b

of variance on each line and replicate support these observations. As shown in figure 3a, SCH-23390 decreased the ethanol-stimulated activity of FAST-1 mice, without affecting saline activity. An ANOVA including data for only FAST-1 mice revealed significant effects of SCH-23390 ($F[2,39] = 5.1$, $p < 0.05$) and ethanol ($F[1,39] = 27.3$, $p < 0.001$), as well as a significant interaction between SCH-23390 and ethanol ($F[2,39] = 3.5$, $p < 0.05$). Subsequent analysis of the interaction revealed that administration of SCH-23390 significantly decreased ethanol-stimulated activity ($F[2,39] = 8.1$, $p < 0.01$), at both 0.015 mg/kg ($p < 0.05$) and 0.03 mg/kg ($p < 0.01$), whereas activity after saline injection was unaffected. In addition, there was no significant difference between saline-treated and ethanol-treated animals given 0.03 mg/kg, indicating that this dose of SCH-23390 completely blocked ethanol-stimulated activity. Although there appeared to be slight depressant effects of the antagonist on the activity of saline- and ethanol-treated FAST-2 mice (see figure 3b), this effect was not significant ($F[2,42] = 1.5$, $p = 0.24$).

The replicates of the SLOW lines also showed dissimilar responses to administration of SCH-23390. Both SCH-23390 administration and ethanol administration caused significant decreases in the activity of SLOW-1 mice ($F[2,41]=6.0$, $p < 0.01$; $F[1,41] = 6.3$, $p < 0.05$, respectively), however, the activity decreases of ethanol-treated animals were parallel to the activity decreases of saline-treated mice. Although the pattern of results seen in SLOW-2 mice (figure 3b) appeared to indicate slight depressant effects of SCH-23390 on the activity of saline-treated animals and a slight reversal of the depressant effect of

ethanol, analysis of data from SLOW-2 mice indicate that there were no significant effects of SCH-23390, and no significant interaction between the drug and ethanol.

Since FAST-2 mice were unaffected by doses of SCH-23390 that clearly affected FAST-1 mice, it was hypothesized that FAST-2 animals might be less sensitive to the effects of this antagonist. An experiment using FAST-2 female mice assessed this possibility. The maleate salt form of SCH-23390 was used in this experiment because SCH-23390 was not available at the time of this study. Initial pilot studies showed that 0.06 mg/kg of SCH-23390 maleate alone caused large decreases in locomotor activity; however, 0.03 and 0.045 mg/kg produced only marginal decreases. It is possible that the potency of the maleate form of SCH-23390 was different from that of SCH-23390, perhaps due to differing solubilities. However, the very slight depressant effects of 0.03 mg/kg SCH-23390 maleate observed in pilot studies appear to be consistent with those seen in the original study using SCH-23390. Since 0.03 mg/kg of SCH-23390 was previously ineffective in reducing ethanol-stimulated activity, the 0.045 mg/kg dose of SCH-23390 maleate was chosen. As in the original study, either saline or SCH-23390 was administered 30 minutes prior to a second injection of saline or ethanol, followed by a 15-minute activity test. Data are presented in figure 4. These animals were significantly stimulated by ethanol (main effect: $F[1,35]=98.6$, $p < 0.001$). There appeared to be a slight effect of SCH-23390 on ethanol-stimulated activity, however, the effect of drug ($F[1,35]=2.3$, $p=0.06$), and the interaction of drug and ethanol ($F[1,35]=0.8$, $p = 0.37$) were not significant.

Figure 4. Effects of SCH-23390 maleate (0.045 mg/kg) on activity of saline- and ethanol-treated FAST-2 mice. SCH-23390 was injected 30 minutes prior to ethanol (2.0 g/kg) or saline, followed by a 15-minute activity test. Each data point represents 9-10 animals; SEM larger than symbol size are shown.

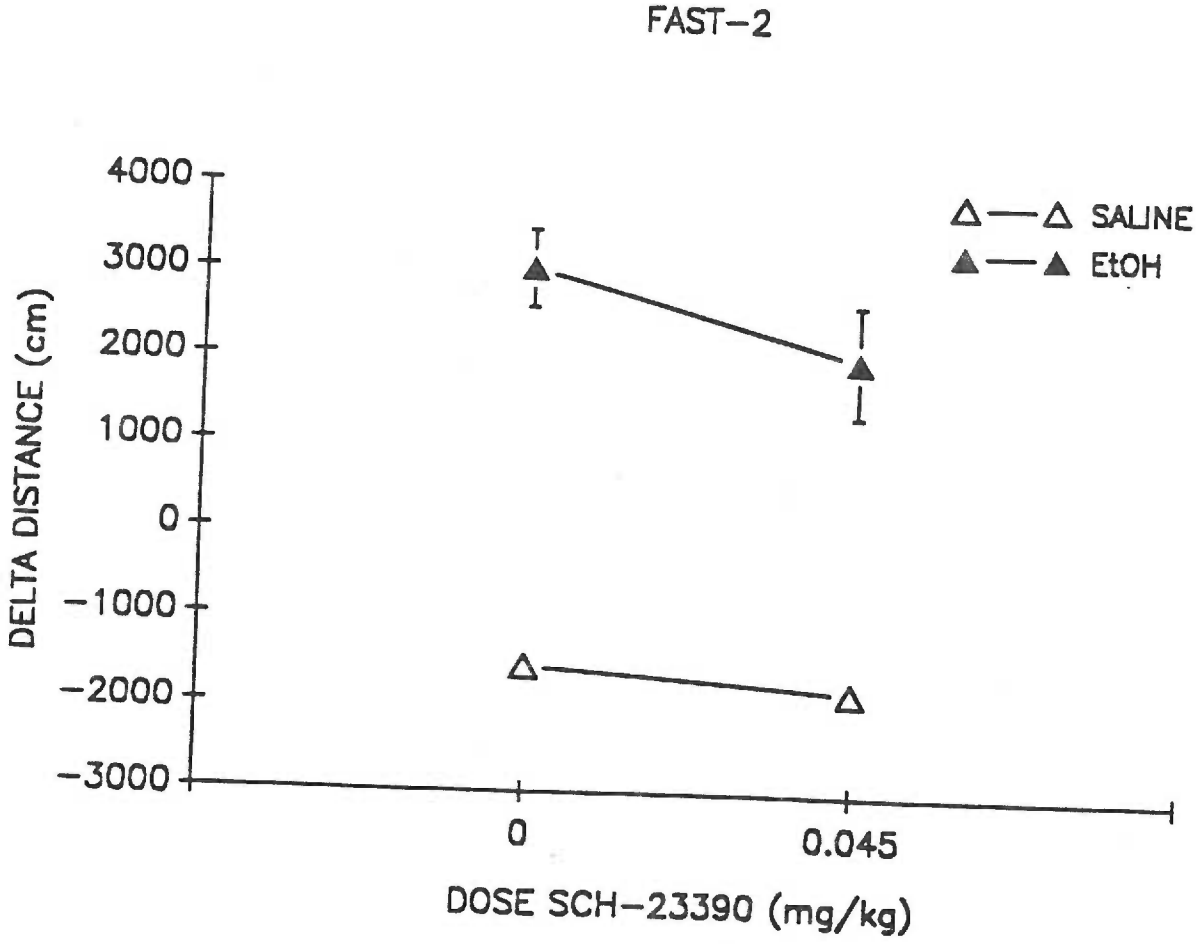
FAST–2
4000
3000
2000
1000
0
–1000
–2000
–3000
DELTA DISTANCE (cm)
SALINE
EtOH
0
0.045
DOSE SCH–23390 (mg/kg)

Figure 4

In summary, SCH-23390 significantly decreased ethanol-stimulated activity in FAST-1, but not FAST-2 mice. The SLOW-1 mice also appeared to be more sensitive to this antagonist than SLOW-2 mice. These data suggest that D1 receptors may be unimportant or minimally important in mediating sensitivity to the stimulant effects of ethanol. This is consistent with the notion that D1 receptors play a permissive, but not a primary role, in the expression of locomotor activation; however, these data do not provide conclusive evidence.

Experiment 3: Sulpiride

The effect of sulpiride, a D2 antagonist, was assessed in this experiment (see figure 5). The drug was administered in doses of 25 or 50 mg/kg, two minutes prior to saline or ethanol. Data are presented collapsed on replicate as the change in distance traveled. Although there were significant effects of replicate ($F[1,160=9.5$, $p < 0.01$), as well as significant line by replicate and line by replicate by ethanol interactions ($F[1,160]=12.3$, $p < 0.001$; $F[1,160]=9.2$, $p < 0.01$, respectively). Ethanol decreased the activity of SLOW-1 mice more than the activity of SLOW-2 mice whereas FAST-2 mice were more stimulated by ethanol than FAST-1 mice. The replicates differed in magnitude of response, not in direction, and collapsing on replicate did not alter interpretation of the results. As expected, ethanol caused an increase in the locomotor activity of FAST mice, and a slight decrease in the activity of SLOW mice. These informal observations were supported by the results of an ANOVA. There were significant main effects of line ($F[1,160]=84.0$, $p < 0.001$), and ethanol ($F[1,160]=44.0$, $p < 0.001$), as

Figure 5. Effects of the D2 antagonist, sulpiride, on activity of saline- and ethanol-treated FAST and SLOW mice. Data are presented collapsed on replicate as change in distance (cm). Sulpiride (25 or 50 mg/kg) or vehicle (5% cyclodextrin in saline) was administered 2 minutes prior to saline or ethanol, followed by a 15-minute activity test. Each data point represents 14-17 animals; SEM larger than symbol size are shown.

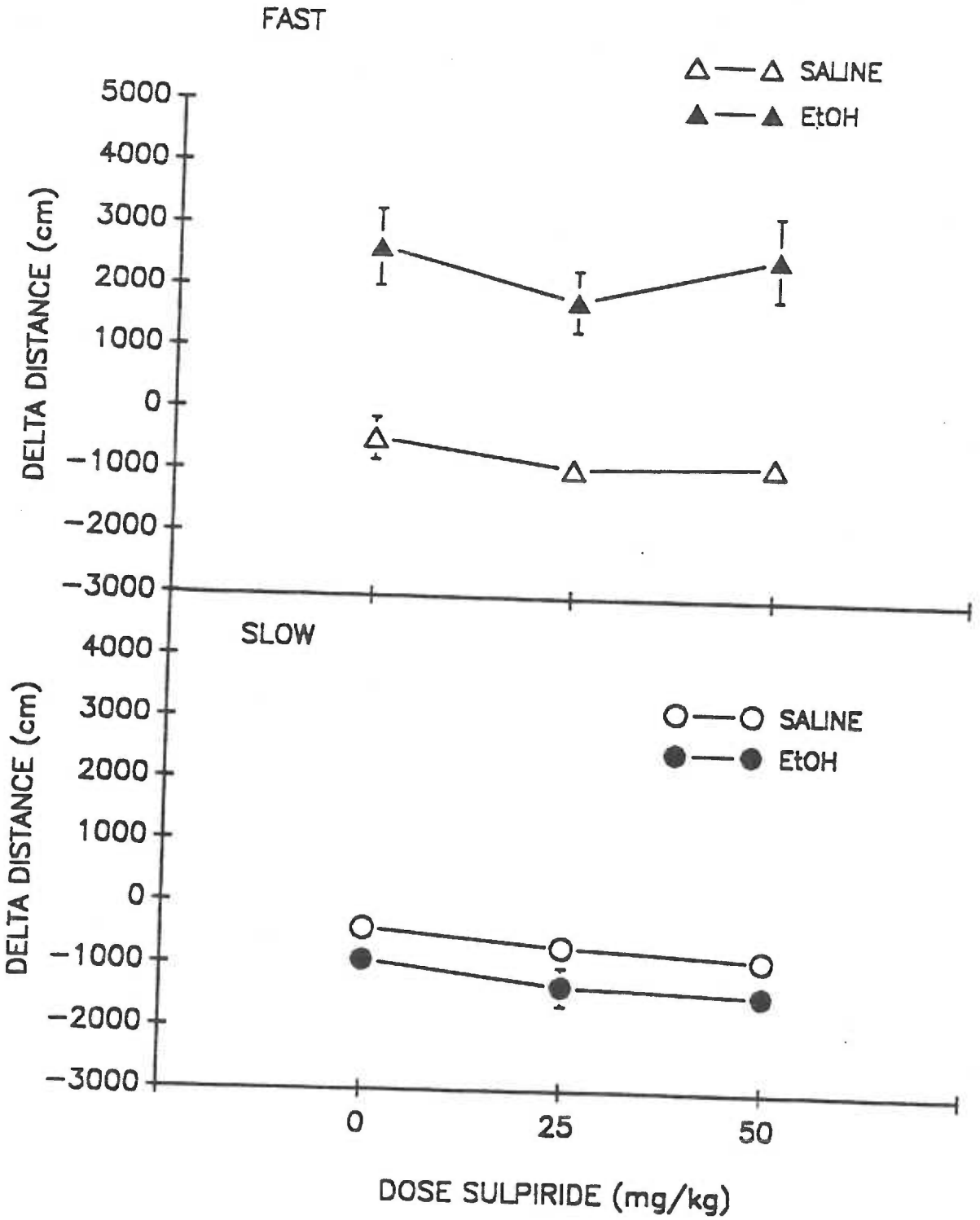
FAST
SALINE
EtOH
5000
4000
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
SLOW
SALINE
EtOH
4000
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
0
25
50
DOSE SULPIRIDE (mg/kg)

Figure 5

well as a significant line by ethanol interaction ($F[1,160]=91.2$, $p < 0.001$). However, there was no significant effect of sulpiride dose, nor were there any significant interactions involving sulpiride. Thus, this D2 antagonist had no effect on saline activity in either line, nor did it alter locomotor activity in response to ethanol in either line.

Despite its high specificity and potency *in vitro*, there is evidence, as discussed by Pinnock (1984) and Ögren et al. (1986), that the *in vivo* potency of sulpiride is poor, due to poor penetration of the blood-brain barrier. Thus, it is possible that the pretreatment time, or the doses used were not sufficient to detect an effect of this drug. Additional experiments involving manipulations of dose and pretreatment time were done in order to address the concern that the concentrations of sulpiride reaching the CNS were insufficient to effect any changes in the function of dopamine systems. The first of these studies involved manipulation of pretreatment time. The effect of 50 mg/kg sulpiride, injected 60 minutes prior to ethanol injection, on a 15-minute activity test in FAST-1 and FAST-2 mice was assessed. Animals were tested on one day only, thus data are presented as distance traveled, rather than as mean difference scores (see figure 6). In addition, because there were no significant effects of replicate, or any interactions involving replicate, data are presented collapsed on this factor. An ANOVA grouped on sulpiride and ethanol conditions revealed that FAST mice were significantly stimulated by ethanol ($F[1,44]=42.0$, $p < 0.001$), but were not affected by sulpiride treatment. Thus, 50 mg/kg sulpiride given 60 minutes prior to testing had no effect on saline-treated or ethanol-treated mice.

Figure 6. Effects of sulpiride (50 mg/kg) administered 60 minutes prior to saline or ethanol administration on activity of FAST mice. Sulpiride vehicle was 5% cyclodextrin in saline. Ethanol dose was 2.0 g/kg. Data are presented collapsed on replicate as distance traveled in cm (one day activity test). Each data point represents 12 animals (6 animals per replicate); SEM larger than symbol size are shown.

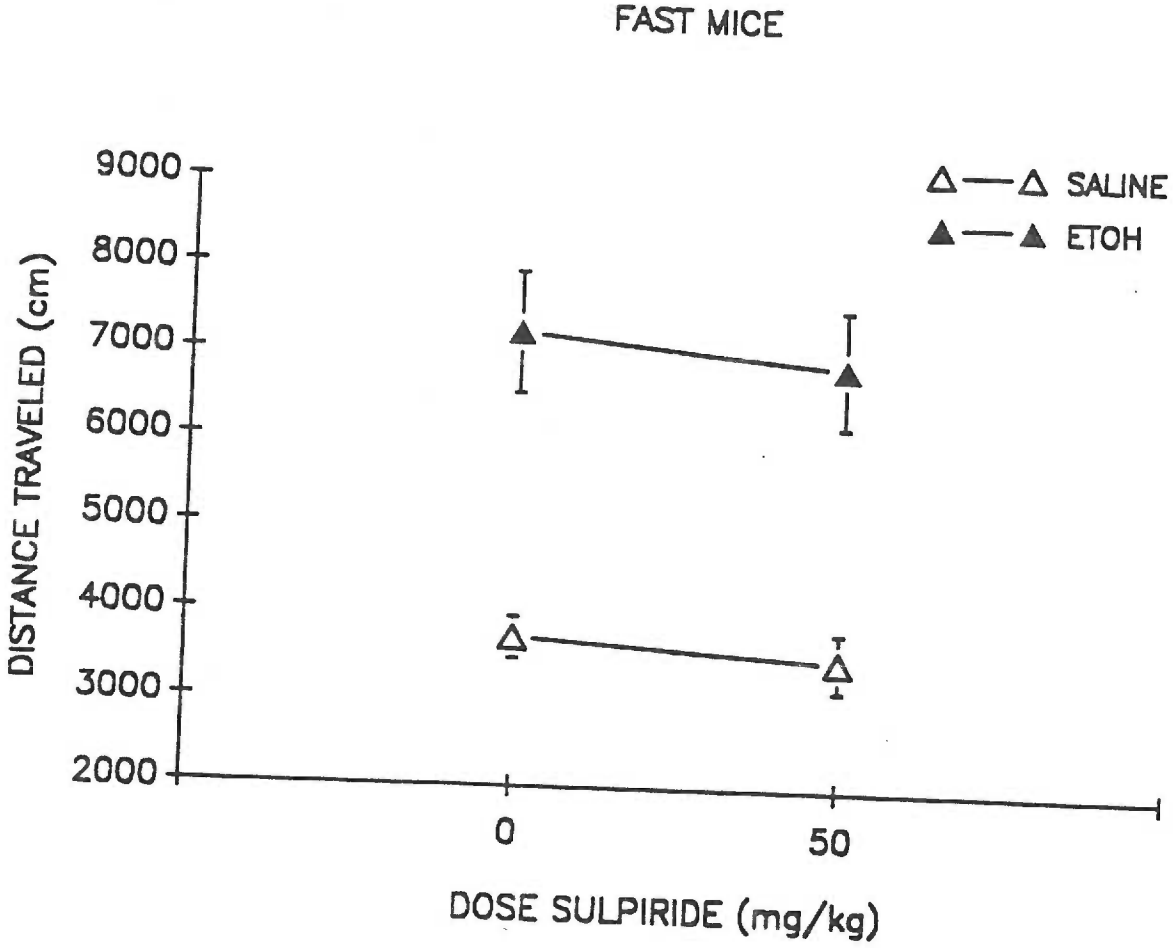
FAST MICE
SALINE
ETOH
9000
8000
7000
6000
5000
4000
3000
2000
DISTANCE TRAVELED (cm)
0
50
DOSE SULPIRIDE (mg/kg)

Figure 6

The original doses of sulpiride chosen for this study were based on published results (e.g. Rubinstein et al., 1988). However, to verify that the sulpiride doses used were not too low to cause changes in locomotor activity, a dose-response study was performed. Data for this study are presented as mean distance traveled in figure 7. FAST and SLOW female mice from both replicates were injected with vehicle (20% cyclodextrin in saline), 12.5, 25, 50, or 100 mg/kg sulpiride, 60 minutes prior to placement in an activity monitor. There were significant main effects of line, replicate, and dose ($F[1,162]=29.8$, $F[1,162]=49.2$, $F[4,162]=8.6$, respectively, $p < 0.001$ in all cases). There was also a significant line by replicate interaction ($F[1,162]=37.2$, $p < 0.001$), but interactions between line and dose, and line, replicate, and dose were not significant. FAST-1 mice had higher activity counts than SLOW-1 mice and both lines of replicate 2 mice at all doses, including saline. Although not significant, there was a trend towards a replicate by dose interaction ($F[4,162]=2.2$, $p = 0.07$). In general, it appeared that replicate 1 mice were more sensitive than replicate 2 mice to the effects of sulpiride.

In summary, these data seem to suggest that sulpiride can affect locomotor activity in FAST and SLOW mice. However, these slight effects appeared to be present only in replicate 1 animals. Although the replicates seem to differ in sensitivity to sulpiride, there did not appear to be differences between the lines within each replicate. Baseline differences like those seen between FAST-1 and SLOW-1 mice have been seen before in previous studies in both replicates (e.g., see Phillips et al., 1992), however, baseline differences between the lines

Figure 7. Effects of sulpiride (0, 12.5, 25, 50, or 100 mg/kg) on activity of FAST and SLOW mice. Replicate 1 data are presented as distance traveled (cm) in the upper panel; replicate 2 data are presented in the lower panel. Sulpiride or vehicle (20% cyclodextrin in saline) was injected 60 minutes prior to placement in an activity monitor. Activity data was recorded for 15 minutes. Each data point represents 9-10 animals; SEM larger than symbol size are shown.

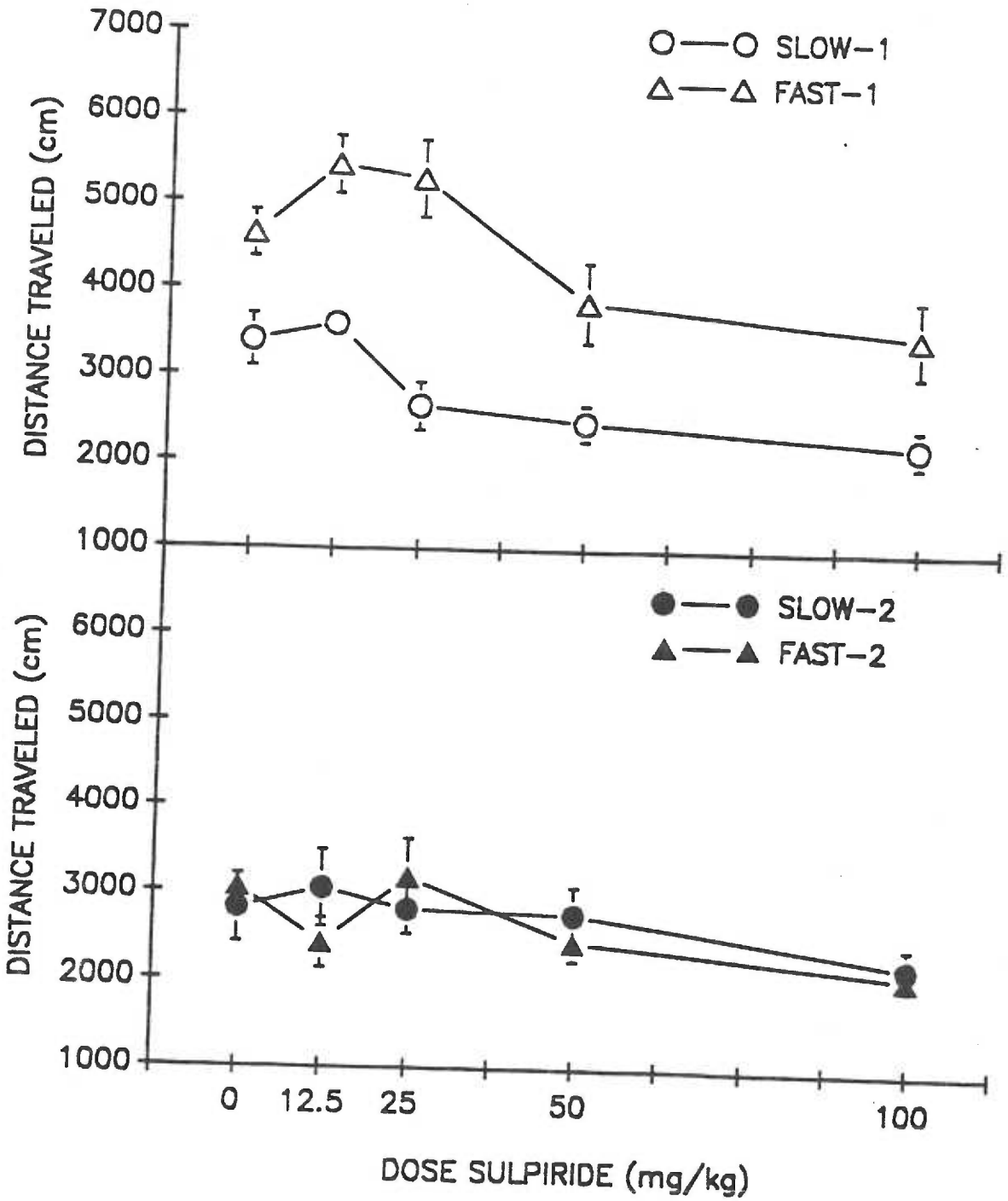
7000
6000
5000
4000
3000
2000
1000
DISTANCE TRAVELED (cm)
SLOW-1
FAST-1
SLOW-2
FAST-2
0
12.5
25
50
100
DOSE SULPIRIDE (mg/kg)

Figure 7

are not consistently demonstrated across experiments, nor do they occur in any particular direction. It is possible that the absence of a second injection in the dose-response study may have affected responses to sulpiride differently than in the studies with ethanol groups. However, data obtained from FAST and SLOW lines in other studies suggest that activity levels do not differ between mice given saline injections and mice that receive no injections (Phillips, T. J., personal communication). In addition, the main interest of the dose-response study was whether or not sulpiride had any effects on activity, not necessarily for comparison with previous data. It is possible that the slight effects of sulpiride seen in this dose-response study, but not in the other studies, were due to the vehicle having a higher concentration of cyclodextrin (20% vs 5%). This concentration of cyclodextrin was used in the dose-response study because it was observed that the 100 mg/kg dose of sulpiride was poorly solubilized in 5% cyclodextrin. The 20% cyclodextrin vehicle may have been better suited to keeping sulpiride in suspension at all dose concentrations, thus ensuring better and more consistent delivery of drug through the syringe. Because of the uncertainties associated with sulpiride's poor solubility and low bioavailability, it was considered preferable to continue these studies with raclopride, a more soluble and more potent D2 antagonist.

Experiment 4: Raclopride

In contrast to sulpiride, raclopride, a potent and specific D2 antagonist, has been shown to have high *in vivo* potency. Köhler at al (1985) demonstrated that, 30 minutes after intravenous injection in

rats, 90% of ^{3}H-raclopride had reached the brain in nonmetabolized form. In this experiment, raclopride was administered 30 minutes prior to ethanol injection, immediately followed by a 15-minute activity test. Analysis of variance grouped on line, replicate, raclopride condition and ethanol condition revealed a significant line by ethanol interaction ($F[1,200]=172.3$, $p < 0.01$), indicating the expected selection responses from the FAST and SLOW lines. In addition, there was a significant effect of replicate ($F[1,200]=13.7$, $p < 0.001$), which interacted with line ($F[1,200)=8.2$, $p < 0.001$), and with raclopride ($F[2,200]=3.8$, $p < 0.05$). Further examination of the results suggested that both FAST and SLOW mice of replicate 1 were more sensitive to the depressant effects of raclopride than were FAST and SLOW mice of replicate 2. Whereas saline activities of FAST-1 and SLOW-1 mice were reduced by both 0.25 and 0.5 mg/kg of the D2 antagonist, the activity of neither FAST-2 nor SLOW-2 animals was depressed by these same doses (see figures 8a and 8b). Separate ANOVAs on each line and replicate confirmed these observations. Ethanol significantly increased the activity of FAST-1 mice (main effect of ethanol, $F[1,53]=64.9$, $p < 0.001$). Although raclopride significantly decreased the activity of both vehicle and ethanol-treated mice (main effect of raclopride, $F[2,53]=22.3$, $p < 0.001$), there was a significant raclopride by ethanol interaction ($F[2,53]=3.9$, $p < 0.05$). There were parallel decreases in the activity of saline- and ethanol-treated animals produced by 0.25 mg/kg raclopride ($p < 0.05$, $p < 0.01$, respectively, compared to appropriate saline controls), however, 0.5 mg/kg produced additional decreases in ethanol-stimulated activity (0.25 vs. 0.5, $p < 0.01$), that were not seen in

Figure 8. Effects of the D2 antagonist, raclopride, on activity of replicate 1 (a) and replicate 2 (b) FAST and SLOW mice given saline or ethanol. Data are presented as delta distance (cm). Raclopride (0.25 or 0.5 mg/kg) or saline was injected 30 minutes prior to saline or ethanol (2.0 g/kg). Duration of the activity test was 15 minutes. Each data point represents 8-10 mice; SEM larger than symbol size are shown. $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$ vs saline-treated, zero-dose group; ** $p < 0.01$ vs ethanol-treated, zero-dose animals; $^{++}$ $p < 0.01$ vs ethanol-treated mice given 0.25 mg/kg raclopride.

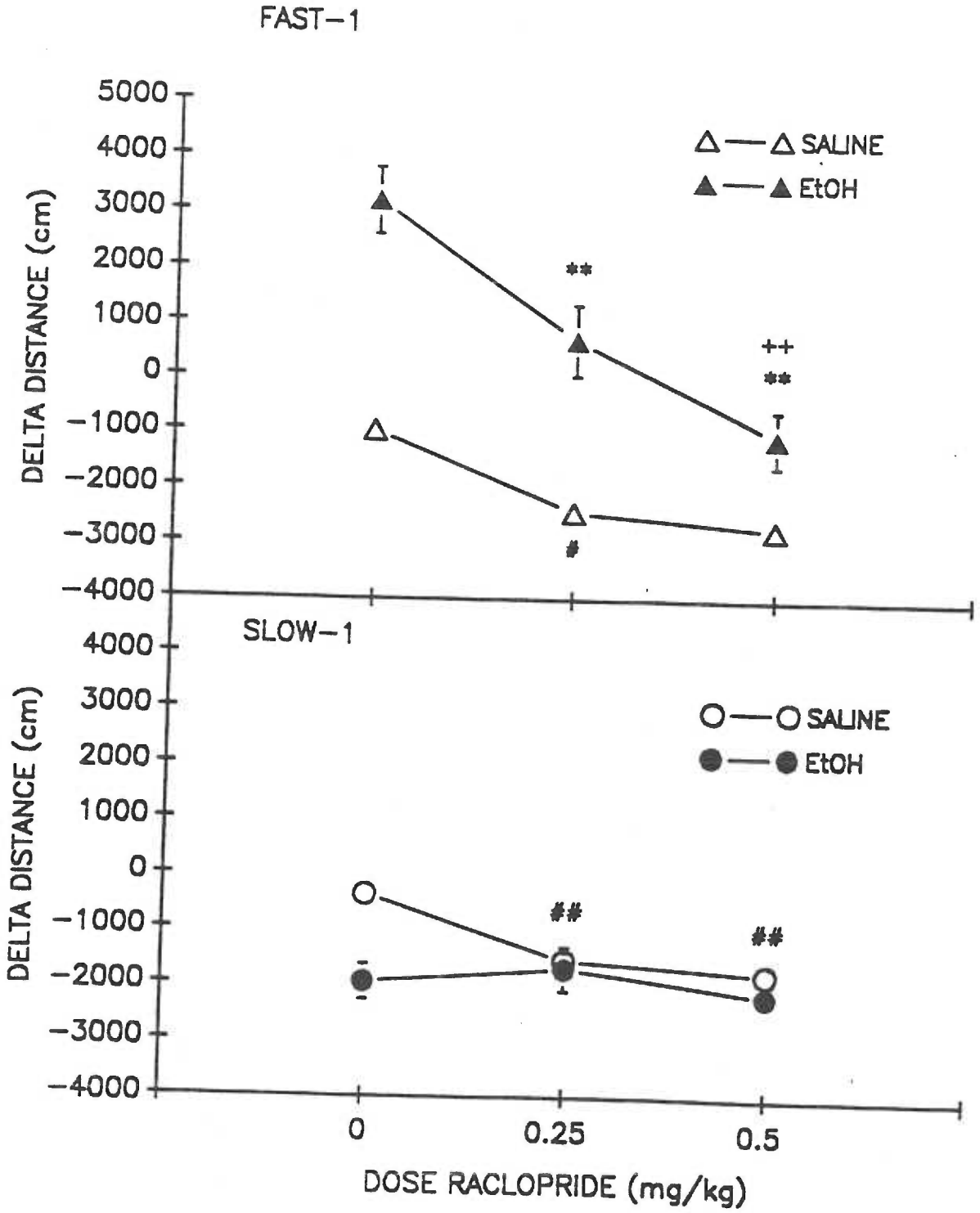
FAST–1
5000
4000
3000
2000
1000
0
−1000
−2000
−3000
−4000
DELTA DISTANCE (cm)
SALINE
EtOH
**
++
**
#
SLOW–1
DELTA DISTANCE (cm)
SALINE
EtOH
##
##
0
0.25
0.5
DOSE RACLOPRIDE (mg/kg)

Figure 8a

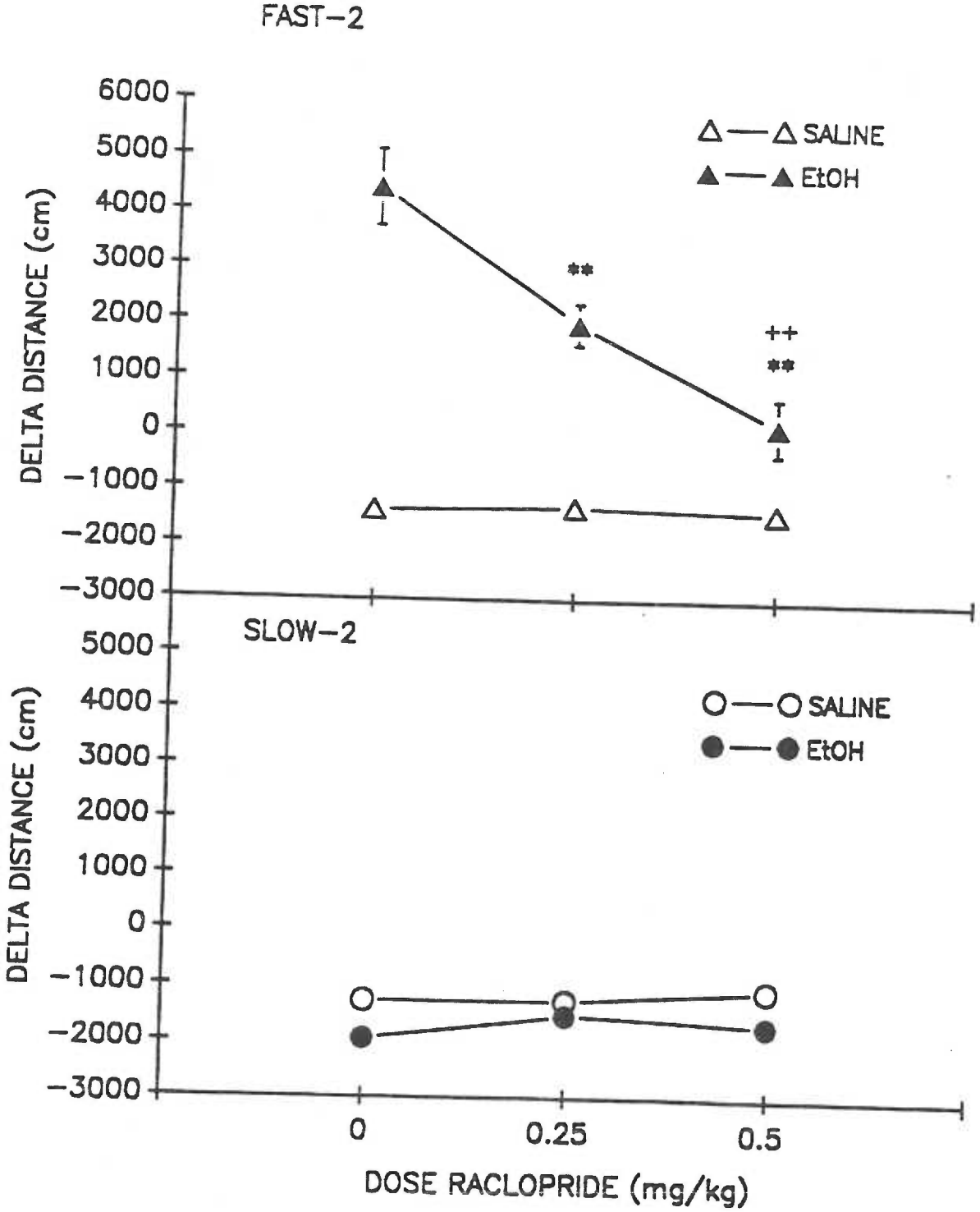
FAST–2
6000
5000
4000
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
SALINE
EtOH
**
++
**
SLOW–2
5000
4000
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
SALINE
EtOH
0
0.25
0.5
DOSE RACLOPRIDE (mg/kg)

Figure 8b

saline-treated animals.

SLOW-1 mice were significantly depressed by ethanol (main effect of ethanol; $F[1,54]=9.7$, $p < 0.01$), and by raclopride ($F[2,54]=4.2$, $p< 0.05$). A simple main effects analysis of a significant raclopride by ethanol condition interaction ($F[2,54]=3.8$, $p < 0.05$) showed a significant effect of raclopride on the activity of saline-treated animals ($F[2,54]=7.3$, $p < 0.01$), but no parallel decreases in ethanol-induced activity. A subsequent Newman-Keuls test indicated that both doses of raclopride administered with saline reduced activity relative to the saline control group ($p < 0.01$ for both comparisons; 0.25 vs 0.50, NS). It is possible that the lack of effect of raclopride on ethanol-treated animals was due to floor effects. However, examination of data from Day 2 indicated that ethanol-treated animals were engaging in forward locomotion. Another possible explanation is that the magnitude of depression of non-drug ethanol-treated animals appeared to be greater than usual due to slightly higher basal activity on day 1, compared to the other groups on day 1, resulting in a more negative difference score than normal. However, examination of day 2 data do not support this explanation, since non-drug ethanol-treated animals also had higher day 2 scores than the other ethanol-treated groups. Finally, the magnitude of the activity depression of SLOW-1 mice produced by ethanol in this study was greater than the depression of SLOW-1 mice seen in other studies. It may be that, for reasons unrelated to ethanol or dopamine drug effects, these animals were less active than usual. Replication of this study might produce results in which saline-treated and ethanol-treated SLOW-1 mice were affected by raclopride in a

parallel fashion.

Data from replicate 2 mice also support the hypothesis of dopamine system involvement in ethanol-stimulated activity. Analysis of variance including data from FAST-2 mice revealed that, in addition to significant main effects of raclopride and ethanol ($F[2,50]=13.8$ and $F[1,50]=113.8$ respectively, $p < 0.001$ for both tests), there was a significant raclopride by ethanol interaction ($F[2,50]=13.6$, $p < 0.001$). The activity of saline-treated FAST-2 mice was not altered by raclopride administration, however, ethanol-stimulated activity was significantly decreased by raclopride (simple main effects, $F[2,50]=28.1$, $p < 0.001$). Decreases in activity of raclopride-treated mice compared to ethanol controls were dose-dependent (control > 0.25 mg/kg > 0.5 mg/kg, $p < 0.01$ for all comparisons, using the Newman-Keuls test). Analysis of variance on data from SLOW-2 mice revealed that ethanol significantly depressed the locomotor activity of these animals ($F[1,43]=8.2$; $p < 0.01$). However, raclopride did not alter saline activity, nor did it affect the activity of ethanol-treated SLOW-2 mice.

Because the basal locomotor activity of replicate 1 mice was decreased by doses of raclopride that had no effect on replicate 2 mice, it was hypothesized that FAST-1 and SLOW-1 mice were more sensitive to raclopride's effects than were FAST-2 and SLOW-2 mice. The effect of raclopride alone on activity clouds interpretation of the results, thus, an additional experiment was performed to assess the effects of a lower dose of raclopride on saline- and ethanol-treated FAST-1 mice. Mice were treated and tested as in the initial study except that a dose of 0.125 mg/kg raclopride was used. Data are presented in figure 9, as the

Figure 9. Effects of raclopride (0.125 mg/kg) on activity of saline- and ethanol-treated FAST-1 mice. Data are presented as delta distance (cm). Saline or raclopride was administered 30 minutes prior to saline or ethanol (2.0 g/kg), followed by a 15-minute activity test. Each data point represents 8 animals, SEM larger than symbol size are shown. * $p < 0.05$ vs ethanol-treated, no-drug animals.

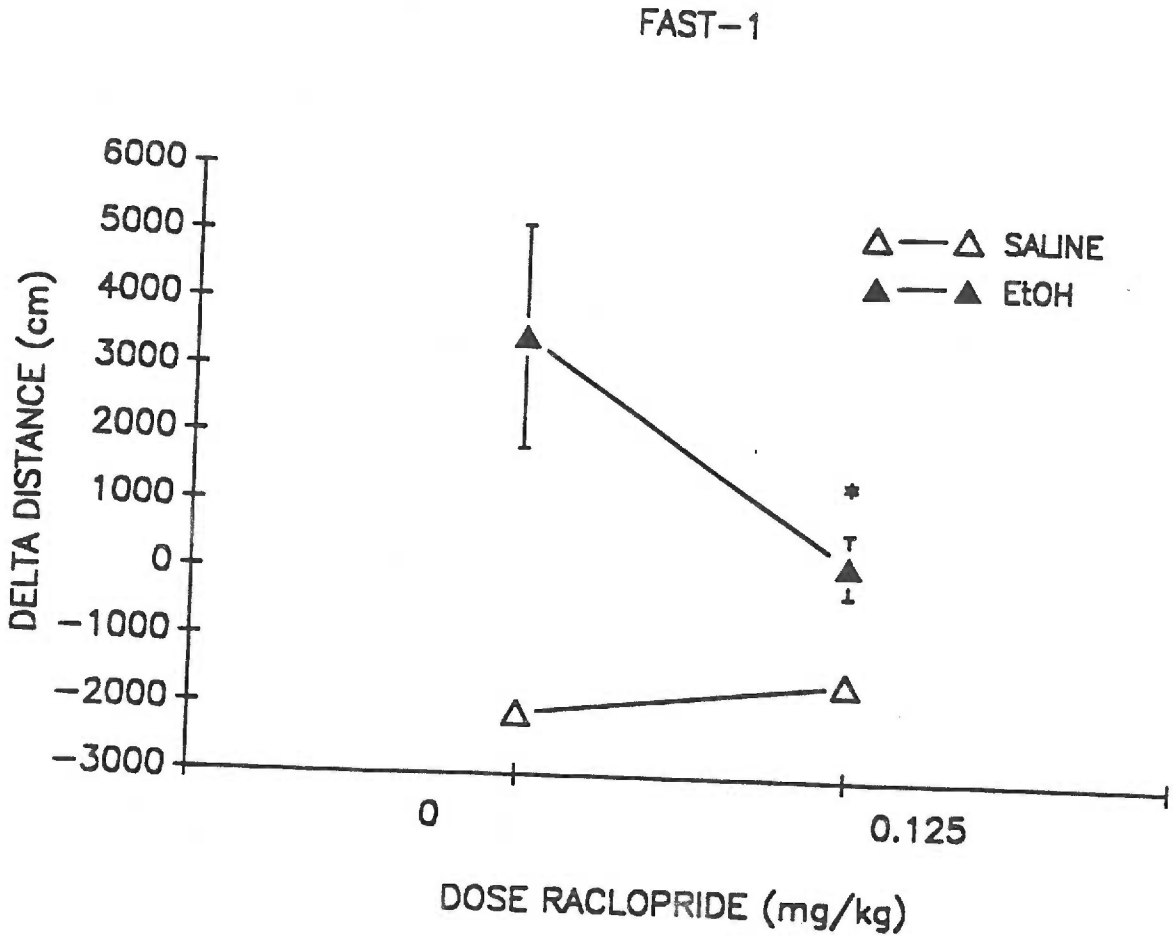
FAST–1
6000
5000
4000
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
SALINE
EtOH
*
0
0.125
DOSE RACLOPRIDE (mg/kg)

Figure 9

change in distance traveled. Analysis of variance grouped on drug condition and ethanol condition revealed a significant stimulant effect of ethanol ($F[1,28]=17.4$, $p < 0.001$), and a significant raclopride by ethanol interaction ($F[1,28]=4.7$, $p < 0.05$). This interaction was due to a significant antagonistic effect of raclopride on ethanol-stimulated activity ($F[1,28]=7.0$, $p < 0.01$), whereas the activity of saline-treated animals was not affected by this dose. In addition, there was no significant difference between saline-treated and ethanol-treated animals given 0.125 mg/kg, indicating that this dose of raclopride completely blocked ethanol-stimulated activity without affecting motor ability by itself.

In summary, data from these two studies suggested that the replicates differed in their sensitivities to the effects of raclopride. However, when appropriate doses were used, raclopride blocked ethanol-stimulated activity in both replicates of FAST mice without affecting motor ability. These data provide strong support for a role of dopamine systems in general, and the D2 receptor subtype specifically, in activation of FAST mice in response to ethanol.

Experiment 5: SCH-23390 and Raclopride

This experiment assessed the effects of administration of SCH-23390 maleate, raclopride, or the combination of these two dopamine antagonists on saline activity and ethanol-induced activity. Given that D1 and D2 receptors appear to act additively or synergistically in their mediation of motor behaviors, it was hypothesized that, barring any floor effects, coadministration of a D1 and a D2 antagonist would result

in greater antagonism of ethanol-stimulated activity than administration of either drug alone. Since replicate 1 mice appeared to be more sensitive than replicate 2 mice to these dopaminergic agents, they were administered slightly lower doses of drug. FAST-1 and SLOW-1 mice were administered 0.015 mg/kg of SCH-23390 and 0.1 mg/kg of raclopride, whereas FAST-2 and SLOW-2 mice were administered 0.03 mg/kg and 0.25 mg/kg of SCH-23390 and raclopride, respectively. The antagonist combinations were mixed in a single solution for each replicate. These doses were chosen on the basis of their ability to decrease but not completely block, ethanol-stimulated activity in each replicate of the FAST lines, and can be considered to be approximately equipotent between the two replicates. Thus, detection of additive or synergistic effects of D1 and D2 receptor blockade should be possible. One of the four drug conditions was administered 30 minutes prior to a second injection of saline or ethanol, followed by a 15-minute activity test.

Data are presented in figures 10a-10d. Although there was a significant main effect of ethanol ($F[1,285]=64.2$, $p < 0.001$) and a significant line by ethanol interaction ($F[1,285]=164.5$, $p < 0.001$), examination of the data showed that, while three of the lines behaved according to their selection response, FAST-1 mice were not stimulated by ethanol (figure 10a). However, analysis of variance including data for only FAST-1 mice showed a significant main effect of ethanol ($F[1,72]=35.1$, $p < 0.001$), due to the result that saline-treated animals exhibited habituation, while ethanol-treated animals had activity scores similar to their saline treatment scores on day 1. Thus, while FAST-1 mice were not stimulated by ethanol relative to their own baseline

Figure 10. Effects of SCH-23390 maleate and raclopride, alone or in combination, on activity of FAST-1 (a), FAST-2 (b), SLOW-1 (c), and SLOW-2 (d) mice given saline or ethanol. Data are presented as delta distance (cm). Replicate 1 animals received 0.015 mg/kg SCH-23390, 0.1 mg/kg raclopride, or the same respective doses combined in one solution. Replicate 2 animals received 0.03 mg/kg SCH-23390, 0.25 mg/kg raclopride, or the same respective doses combined in one solution. These doses are assumed to be equipotent between the replicates. Drug administration occurred 30 minutes prior to an injection of saline or ethanol (2.0 g/kg), which was immediately followed by a 15-minute activity test. Vertical lines are SEM. Each bar represents 9-11 animals. ** $p < 0.01$ vs ethanol-treated, no-drug group; $^{++}$ $p < 0.01$ vs ethanol-treated animals given SCH-23390, or raclopride.

FAST–1

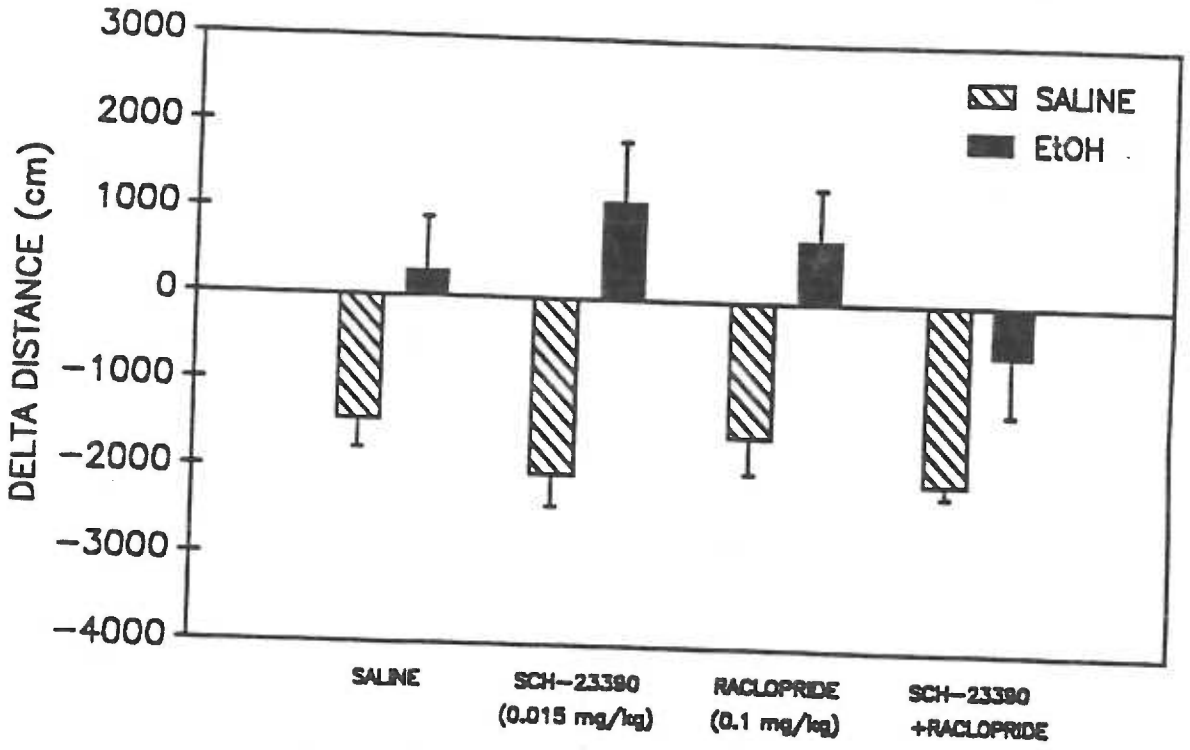

Figure 10a

FAST–2

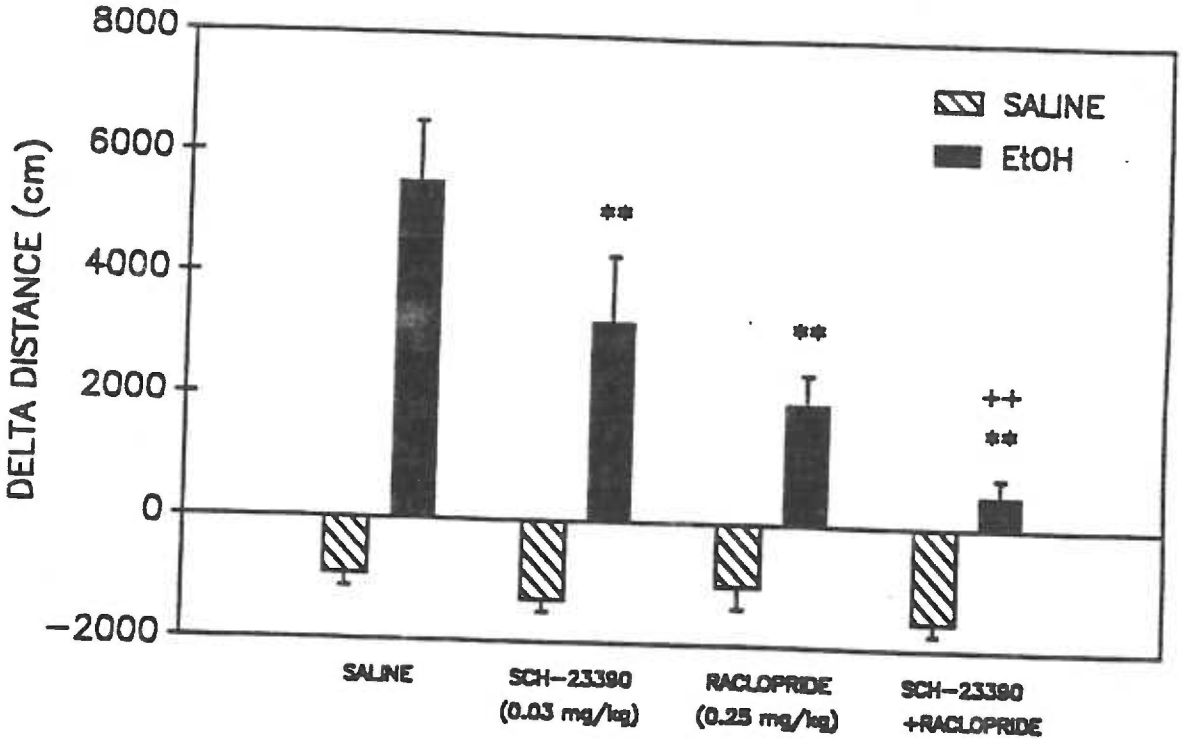

Figure 10b

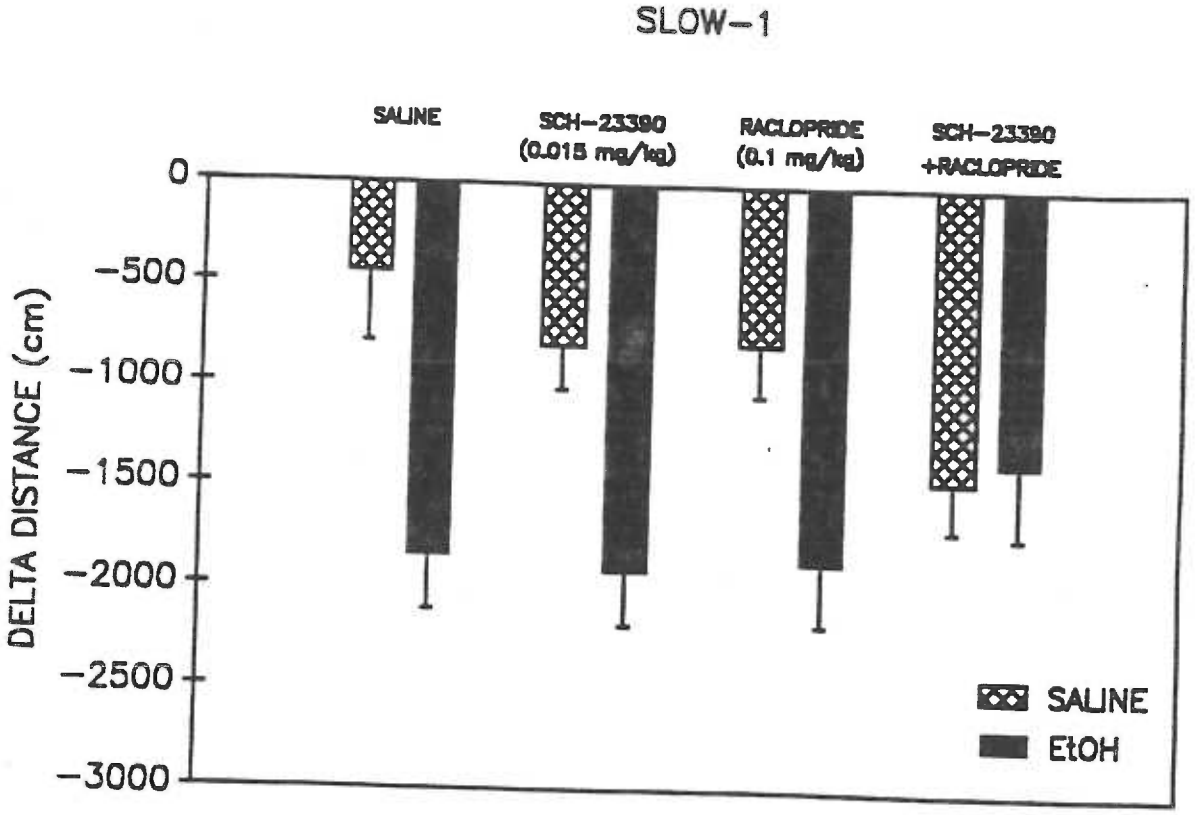
SLOW–1
SALINE
SCH–23390 (0.015 mg/kg)
RACLOPRIDE (0.1 mg/kg)
SCH–23390 +RACLOPRIDE
0
–500
–1000
–1500
–2000
–2500
–3000
DELTA DISTANCE (cm)
SALINE
EtOH

Figure 10c

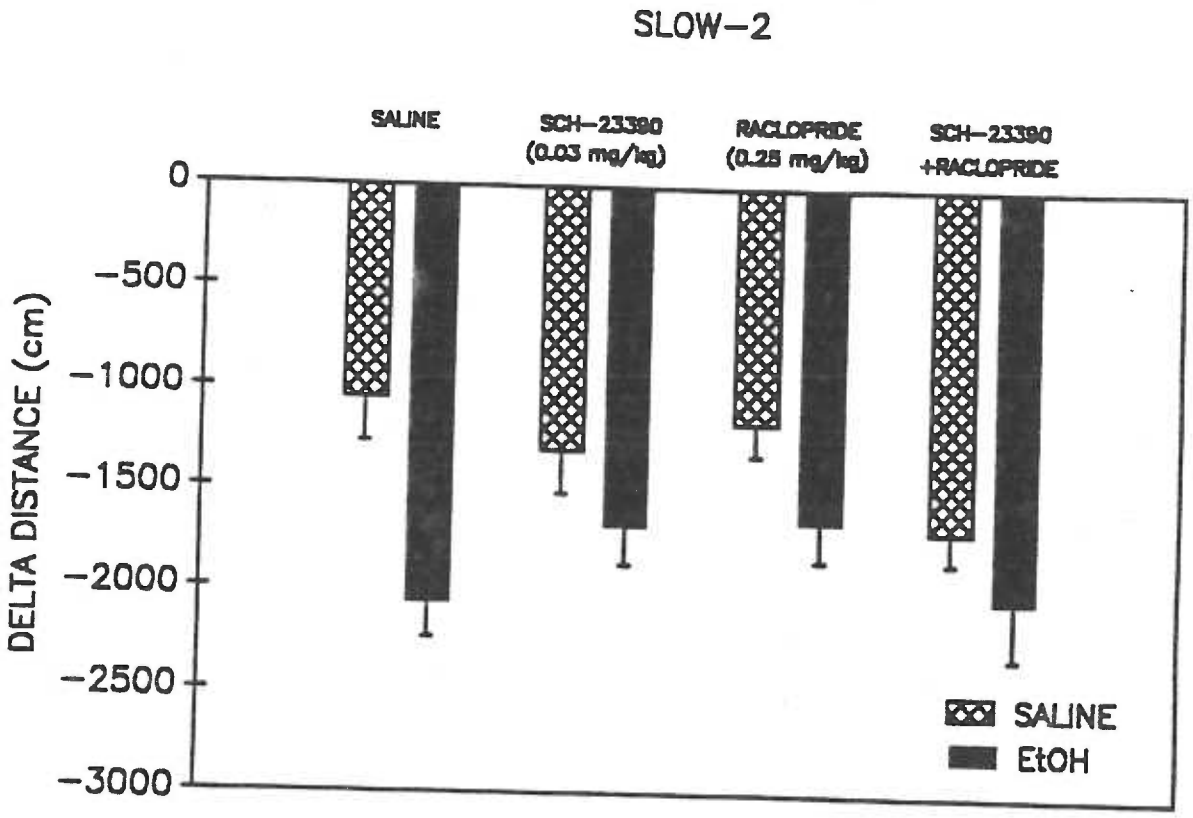
SLOW–2
SALINE
SCH–23390 (0.03 mg/kg)
RACLOPRIDE (0.25 mg/kg)
SCH–23390 +RACLOPRIDE
0
–500
–1000
–1500
–2000
–2500
–3000
DELTA DISTANCE (cm)
SALINE
EtOH

Figure 10d

activities (delta distance), ethanol-treated animals were more active than their saline controls. There were no significant effects of dopaminergic drug administration on the activity of FAST-1 animals, nor was there any significant interaction between drug condition and ethanol condition.

Data for FAST-2 mice are presented in figure 10b. Ethanol produced significant stimulation of the activity of FAST-2 mice ($F[1,69]=98.3$, $p < 0.001$), which was significantly reduced by drug administration ($F[3,69]=8.6$, $p < 0.001$). The significant interaction between drug condition and ethanol condition ($F[3,69]=5.7$, $p < 0.01$) was examined by a simple main effects analysis and Newman-Keuls mean comparisons, which revealed significant effects of drug administration on ethanol-stimulated activity ($F[3,69]=14.1$, $p < 0.001$), but not on saline activity ($F[3,69]=0.3$, $p=0.8623$). All ethanol-treated animals given dopaminergic drugs exhibited reductions in activity relative to saline plus ethanol-treated controls ($p < 0.01$, in all cases). In addition, the locomotor activity of animals given both antagonists was significantly lower than activity of animals given either SCH-23390 or raclopride alone ($p < 0.01$ in both cases). While all ethanol-treated animals were significantly different from their respective saline controls given the same drug treatment ($p < 0.001$ for saline, SCH-23390, and raclopride treated animals, $p < 0.05$ for SCH-23390 and raclopride coadministration groups), FAST-2 mice given both antagonists exhibited very slight, if any, stimulant response to ethanol.

The response profiles of SLOW-1 and SLOW-2 mice were quite comparable to each other despite the use of different doses between the

replicates, lending support to the contention that these doses are equipotent (see figures 10c-10d). In general, SLOW mice were depressed by ethanol; however, the magnitude of depression in SLOW-1 mice in response to ethanol appeared to be greater than in SLOW-2 mice due to the higher activities of saline-treated SLOW-1 mice across all drug conditions. While SCH-23390 alone or raclopride alone had no appreciable effect, the combination of the antagonists appeared to cause slight decreases in saline activity of both replicates of SLOW mice. Analysis of variance on each replicate of the SLOW mice largely supported the above characterizations, revealing significant main effects of ethanol for both replicates ($F[1,72]=18.9$, $p < 0.001$; $F[1,72]=15.4$, $p < 0.001$, for replicate 1 and replicate 2, respectively), but no main effects of drug, and no drug by ethanol interaction. However, the interaction between drug and ethanol condition approached significance in SLOW-1 mice ($F[3,72]=2.5$, $p=0.056$), due to the slight decrease in saline activity, and the slight increase in ethanol-induced activity produced by coadministration of SCH-23390 and raclopride.

In summary, consistent with the hypothesis and with the results of the previous studies with these antagonists, ethanol-induced activity in SLOW mice was not sensitive to manipulation of the dopamine system. The responses of FAST-2 mice in this study also confirmed the earlier observations that dopamine receptor blockade produced decreases in the ethanol-stimulated activity of FAST mice, with the added observation that D1 and D2 antagonist coadministration resulted in even greater decreases in ethanol-stimulated activity. However, FAST-1 mice were not stimulated by ethanol, and were also not affected by dopamine antagonism

in this study.

Experiment 6: Apomorphine

The effect of apomorphine, a mixed agonist, was assessed in this experiment. A dose-response curve for apomorphine effects on locomotor activity in a different set of selected mouse lines suggested that 2 μmol/kg has effects typical of doses acting presynaptically, while 8 μmol/kg acts post-synaptically (Dudek et al., 1984). Based on those data, animals were administered 2 μmol/kg or 8 μmol/kg apomorphine three minutes before saline or ethanol injection, and tested for 15 minutes in activity monitors (2 μmol/kg = 0.6076 mg/kg). Analysis of variance grouped on line, replicate, apomorphine condition, and ethanol condition revealed significant effects of ethanol ($F[1,211]=53.6$, $p < 0.001$), and a significant interaction between line and ethanol ($F[1,211]=70.2$, $p < 0.001$). Examination of the data showed that while FAST-2 mice were significantly stimulated by ethanol, FAST-1 mice treated with apomorphine vehicle and ethanol did not show the expected stimulant response (see figure 11a). In addition, SLOW-1 mice were depressed by ethanol while SLOW-2 mice showed little change in activity after ethanol administration. Because of the different patterns of response among the four lines, further statistical analyses were performed separately for each line and replicate.

Although ethanol-treated FAST-1 mice given no drug did not show a stimulant response to ethanol, the effects of apomorphine on the activity of these mice resulted in a significant main effect of ethanol ($F[1,54]=22.6$, $p < 0.001$). There was no significant main effect of

Figure 11. Effects of apomorphine, a mixed dopamine agonist, on activity of saline- and ethanol-treated replicate 1 (a) and replicate 2 (b) FAST and SLOW mice. Data are presented as the mean change in distance traveled (cm). Apomorphine (2.0 or 8.0 μmol/kg; 2.0 μmol/kg = 0.6076 mg/kg) or its vehicle (0.1 % sodium metabisulfite in saline) was injected 3 minutes prior to saline or ethanol (2.0 g/kg) injection. Duration of the activity test was 15 minutes. Each data point represents 9-10 animals; SEM larger than symbol size are shown. * $p < 0.05$ vs ethanol-treated, zero-dose animals; + $p < 0.05$ vs ethanol-treated given 2 μmol/kg.

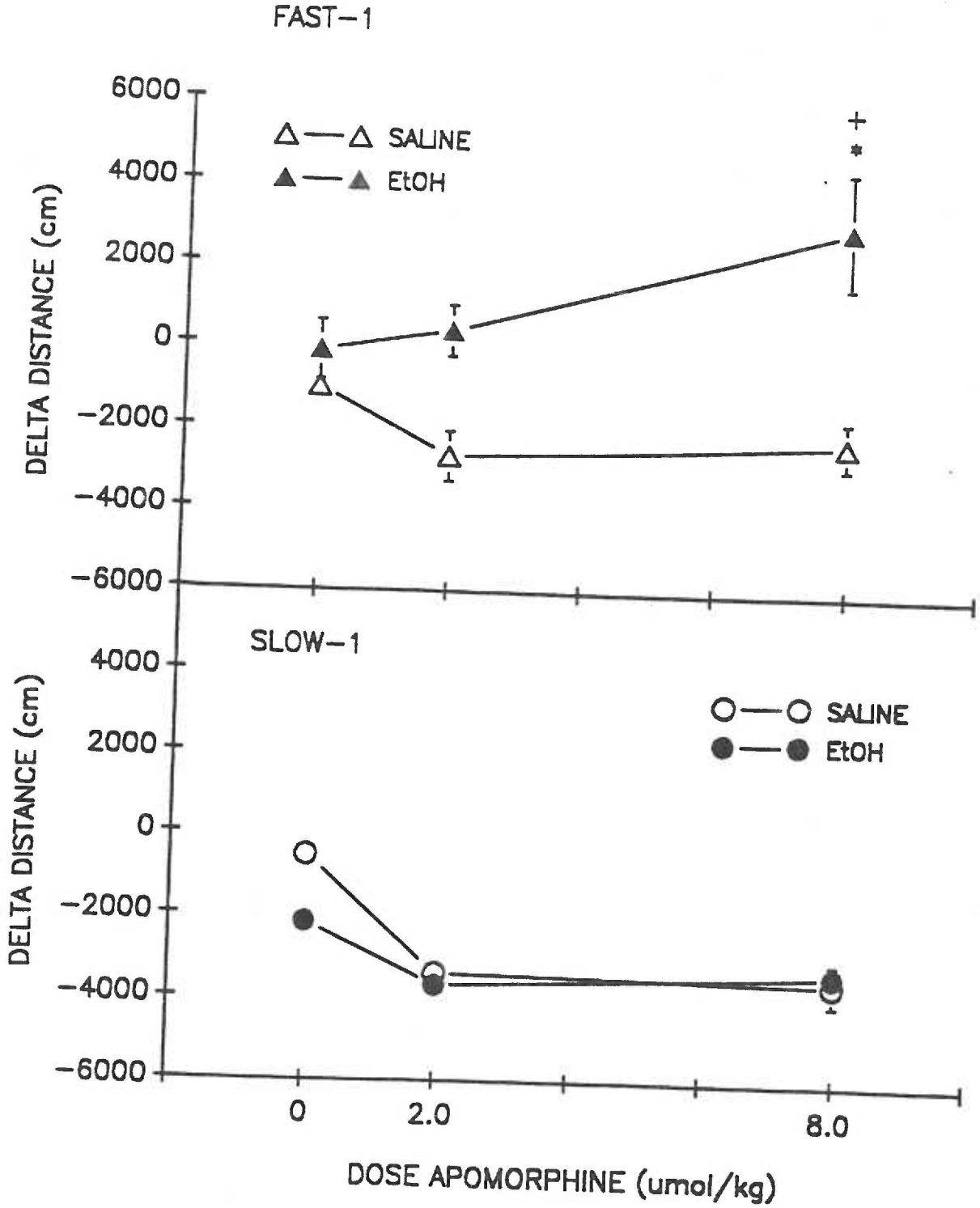
FAST–1
6000
4000
2000
0
−2000
−4000
−6000
DELTA DISTANCE (cm)
SALINE
EtOH
+
*
SLOW–1
4000
2000
0
−2000
−4000
−6000
DELTA DISTANCE (cm)
SALINE
EtOH
0
2.0
8.0
DOSE APOMORPHINE (umol/kg)

Figure 11a

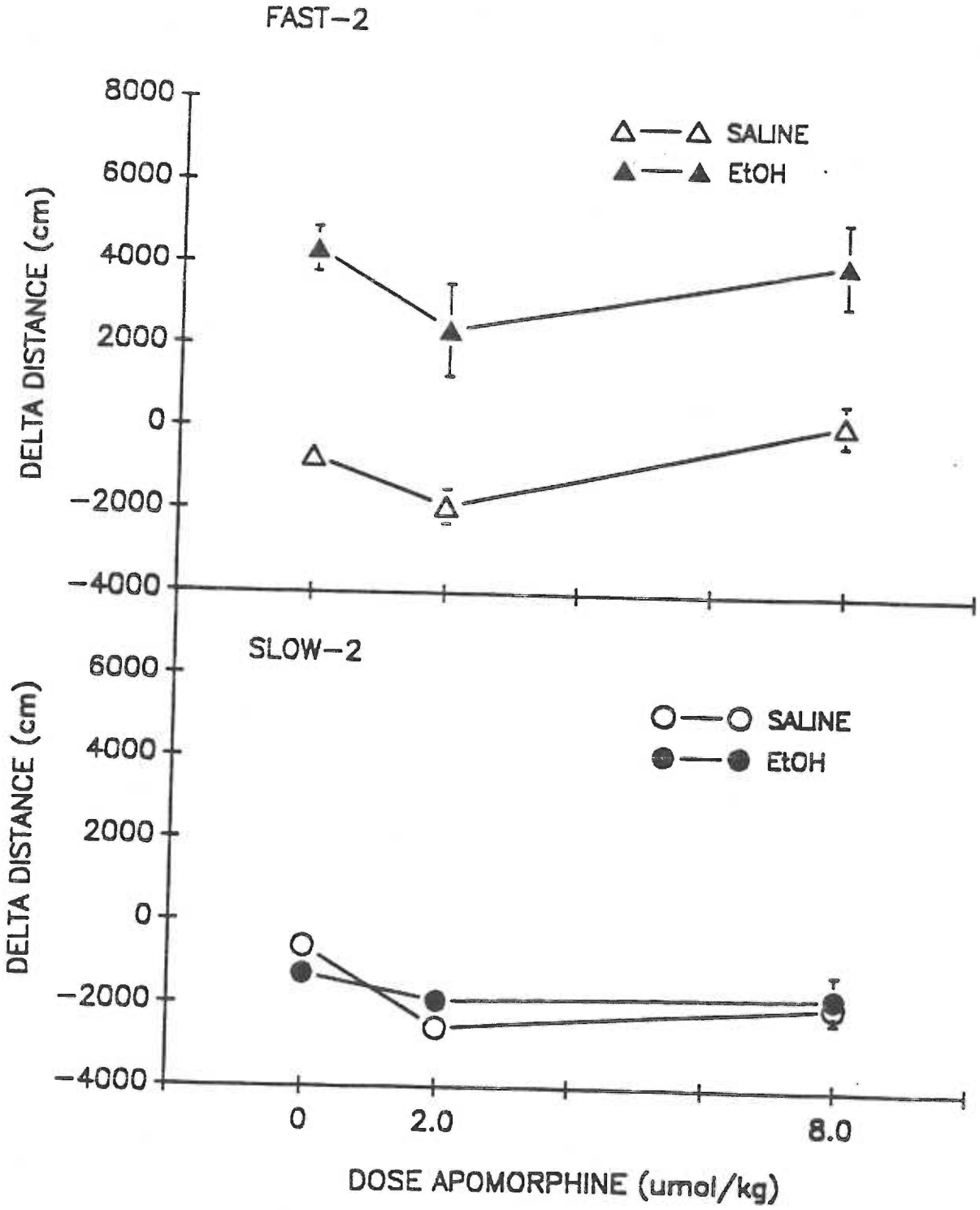
FAST–2
8000
6000
4000
2000
0
−2000
−4000
DELTA DISTANCE (cm)
SALINE
EtOH
SLOW–2
6000
4000
2000
0
−2000
−4000
DELTA DISTANCE (cm)
SALINE
EtOH
0
2.0
8.0
DOSE APOMORPHINE (umol/kg)

Figure 11b

apomorphine because responses of saline- and ethanol-treated animals were largely opposite in direction, which resulted in a significant interaction between apomorphine and ethanol condition ($F[2,54]=3.8$, $p < 0.05$). The activating effects of apomorphine on ethanol-treated mice were significant ($F[2,54]=4.5$, $p < 0.05$). Although visually apparent in figure 11a, no significant effects of apomorphine on the activity of saline-treated mice were found ($F[2,54]=1.8$, $p = 0.313$). Mean comparisons between ethanol-treated FAST-1 mice using the Newman-Keuls test revealed that mice pretreated with 8 μmol/kg apomorphine were significantly more activated than were mice given 2 μmol/kg or vehicle ($p < 0.05$ for each comparison).

Analysis of variance including data from SLOW-1 mice showed that their activity was significantly decreased by ethanol ($F[1,54]=4.4$, $p < 0.05$), and by apomorphine ($F[2,54]=27.3$, $p < 0.001$). Simple main effects analysis of a significant apomorphine by ethanol interaction ($F[2,54]=4.1$, $p < 0.05$) followed by Newman-Keuls mean comparisons revealed that apomorphine significantly affected the activity of both saline- and ethanol-treated animals ($F[2,54]= 25.9$, $p < 0.001$; $F[2,54]=5.6$, $p < 0.01$, respectively). Both doses of apomorphine significantly decreased the activity of saline-treated animals ($p < 0.01$ for both comparisons), and significantly decreased the activity of ethanol-treated animals ($p < 0.01$, $p < 0.05$ for 2 and 8 μmol/kg, respectively) relative to their respective vehicle controls. However, the magnitude of the decrease in saline-treated animals produced by 2 μmol/kg apomorphine was greater than that seen in ethanol-treated

animals. While the activity levels of saline- and ethanol-treated mice given no apomorphine were significantly different ($p < 0.001$), activity levels of both saline- and ethanol-treated animals pretreated with 2 μmol/kg apomorphine were approximately the same, and there was no difference between the activity of animals pretreated with 2 μmol/kg compared to those given 8 μmol/kg, in either the saline or the ethanol condition. Examination of the data from day 2 only, revealed that SLOW-1 mice given apomorphine and ethanol or saline exhibited very little, if any, forward locomotion. Thus, though it appeared that ethanol-treated animals were less sensitive to apomorphine than saline-treated animals, it may be that detection of further decreases in activity caused by apomorphine was not possible.

As seen in figure 11b, FAST-2 mice were highly stimulated by ethanol. An ANOVA including data for FAST-2 mice revealed significant effects of apomorphine and ethanol ($F[2,49]=3.9$, $p < 0.05$; $F[1,49]=57.0$, $p < 0.001$, respectively), however, the interaction between apomorphine and ethanol was not significant. Further statistical analysis of apomorphine effects on each ethanol condition was precluded by the lack of a significant interaction. A Newman-Keuls comparison of the means of drug groups collapsed on ethanol condition revealed no significant effects of 8 μmol/kg on activity relative to non-drug treated animals, while the effects of 2 μmol/kg apomorphine approached, but did not reach, significance. However, the activity of animals given 8 μmol/kg was significantly higher than the activity of animals administered 2 μmol/kg apomorphine ($p < 0.05$).

Ethanol treatment did not produce activity changes in SLOW-2 mice,

however, these animals were significantly affected by apomorphine administration ($F[2,54]=6.3$, $p < 0.01$). Responses to apomorphine were similar between saline- and ethanol-treated mice, as confirmed by the lack of a significant interaction between apomorphine and ethanol condition. Newman-Keuls mean comparisons of apomorphine treatment groups collapsed on ethanol treatment condition revealed that both 2 μmol/kg and 8 μmol/kg apomorphine significantly decreased activity relative to non-drug treated animals ($p < 0.01$ and $p < 0.05$, respectively). It should be noted that some SLOW-2 mice, like SLOW-1 mice, exhibited no forward locomotion when treated with apomorphine at either dose. Thus floor effects confounded detection of further decreases in activity that apomorphine might have produced.

In summary, the biphasic nature of the effects of apomorphine on activity was demonstrated in replicate 2 mice. In contrast, FAST-1 and SLOW-1 mice did not show biphasic activity responses to apomorphine. The activity of SLOW-1 mice was decreased by apomorphine at both doses while FAST-1 mice were not significantly affected by either dose. It may be that FAST-1 mice were more sensitive to apomorphine, and that the doses used were sufficient to act post-synaptically. The differences in response to apomorphine between the replicates (particularly in the FAST lines), suggest that, as is the case with dopamine antagonists, the replicates may be differentially sensitive to this dopamine agonist. Interpretation of these data were complicated by floor effects in both replicates of the SLOW lines and by effects of apomorphine itself on locomotor activity. Apomorphine increased the activity of ethanol-treated FAST-1 mice; however, the activity of FAST-1 mice treated with

saline was not increased, and if anything, was slightly reduced by apomorphine.

Experiment 7: SKF-38393

The effects of the D1 dopamine agonist, SKF-38393, were assessed in this experiment. Mice were injected with saline or SKF-38393 two minutes prior to saline or ethanol injection, and placed in activity monitors for 30 minutes. Cumulative data for the first 15 minutes are presented in figure 12, for comparison with the other experiments described here. At the time this experiment was done, there were insufficient numbers of animals available to test representative groups of both replicates, thus data were analyzed collapsed on replicate. Analysis of variance grouped on line, drug condition, and ethanol condition revealed significant effects of line ($F[1,134]=17.7$, $p < 0.001$), SKF-39393 ($F[2,134]=7.4$, $p < 0.001$), and ethanol ($F[1,134]=12.6$, $p < 0.001$), as well as a significant line by ethanol interaction ($F[1,134]=13.6$, $p < 0.001$). As expected, FAST mice were stimulated by ethanol. A separate ANOVA including data for FAST mice revealed significant effects of ethanol ($F[1,71]=16.8$, $p < 0.001$) and of drug ($F[2,71]=4.0$, $p < 0.05$), in which 10 mg/kg SKF-38393 increased activity ($p < 0.05$ as assessed by Newman-Keuls mean comparisons of drug groups collapsed on ethanol condition), while 40 mg/kg produced no change. The interaction between SKF-38393 condition and ethanol was not significant, indicating that the agonist had similar effects on both ethanol- and saline-treated mice.

SLOW mice were also affected by administration of SKF-38393, but

Figure 12. Effects of the D1 agonist, SKF-38393, on saline- and ethanol-treated FAST and SLOW mice. Data are presented collapsed on replicate as delta distance (cm). Saline or SKF-38393 (10 or 40 mg/kg) was injected 2 minutes prior to a saline or ethanol (2.0 g/kg) injection. Duration of the activity test was 30 minutes, however, cumulative data for the first 15 minutes are presented here for comparison with other experiments. Each data point represents 10-14 animals; SEM larger than symbol size are shown. * $p < 0.05$, ** $p < 0.01$ vs ethanol-treated, no-drug group; $^{++}$ $p < 0.01$ vs saline-treated animals given 10 mg/kg SKF-38393.

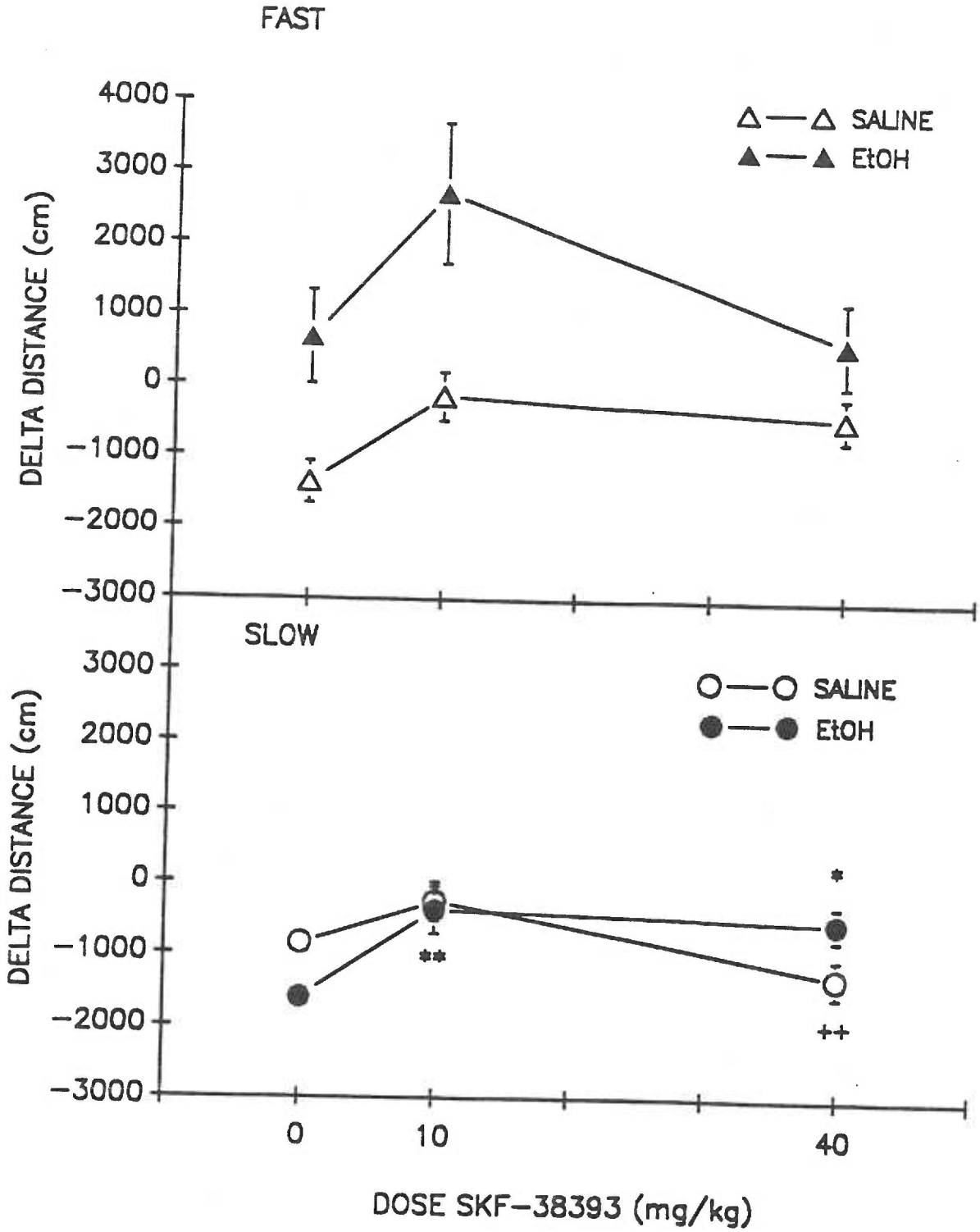
FAST
4000
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
SALINE
EtOH
SLOW
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
SALINE
EtOH
*
**
++
0
10
40
DOSE SKF−38393 (mg/kg)

Figure 12

showed a dose-response profile slightly different from that seen in FAST mice. Analysis of variance on SLOW mice only, showed that while there was a significant effect of SKF-38393 ($F[2,63]=6.4$, $p < 0.01$), there was no significant effect of ethanol on activity, although there appeared to be a small depressant effect in ethanol-treated mice that did not receive SKF-38393. Simple main effects analysis of an interaction between agonist and ethanol condition ($F[2,63]=4.6$, $p < 0.05$) revealed significant effects of SKF-38393 on both activity of saline-treated animals and activity after ethanol administration ($F[2,63]=4.6$, $p < 0.05$; $F[2,63]=6.7$, $p < 0.01$, respectively). However, these changes in activity were not parallel. The activities of ethanol-treated mice given 10 mg/kg or 40 mg/kg SKF-38393 were significantly greater than their saline controls ($p < 0.01$, $p < 0.05$, respectively), but were no different from each other. In contrast, while the activities of SLOW mice given 10 mg/kg or 40 mg/kg SKF-3839, but no ethanol, were different from each other (10 mg/kg > 40 mg/kg, $p < 0.01$), neither dose caused significant changes in activity when compared to their saline controls. In addition, comparison of saline- and ethanol-treated animals at each dose of SKF-38393 revealed that the activity of ethanol-treated animals was significantly lower than saline-treated animals when no agonist was present (the small depressant effect of ethanol), and that the activity of ethanol-treated SLOW mice given 40 mg/kg SKF-38393 was significantly higher than that of saline-treated animals given the same dose. However, there was no difference between the mice given 10 mg/kg and saline or ethanol. Thus, it appeared that while neither dose of SKF-38393 alone produced significant changes in activity of SLOW mice

relative to saline treated animals, 10 mg/kg of the agonist significantly reversed the ethanol-induced locomotor depression of SLOW mice.

In summary, SKF-38393 affected activity in both lines. While saline-treated and ethanol-treated FAST mice did not appear to respond differentially to pretreatment with this D1 agonist, pretreatment with SKF-38393 appeared to reverse the slight depression of activity of ethanol-treated SLOW mice, with no effect on saline-treated SLOW mice. On the other hand, if the activity of saline-treated SLOW mice given the zero dose of dopaminergic drug had been slightly lower, the patterns of response would have been parallel in all four lines.

Experiment 8: Quinpirole

The effect of the D2-specific agonist, quinpirole, was assessed in this experiment. Mice were injected with saline or quinpirole 3 minutes prior to an injection of saline or ethanol, and tested for 15 minutes in activity monitors. Analysis of variance grouped on line, replicate, quinpirole condition, and ethanol condition revealed significant effects of line ($F[1,198]=97.9$, $p < 0.001$), ethanol ($F[1,198]=49.6$, $p < 0.001$), and a significant interaction between line and ethanol ($F[1,198]=98.9$, $p < 0.001$), likely due to stimulation of FAST mice by ethanol, and a small depressant response in SLOW mice (see figure 13). Although there were significant differences between the replicates ($F[1,198]=25.3$, $p < 0.001$), as well as significant interactions between line and replicate, and between line, replicate, and ethanol condition ($F[1,198]=12.0$, $p < 0.001$; $F[1,198]=6.2$, $p < 0.05$, respectively), these differences appeared

Figure 13. Effects of the D2 agonist, quinpirole, on activity of FAST and SLOW mice given saline or ethanol. Data are presented collapsed on replicate as delta distance (cm). Quinpirole was administered 3 minutes prior to saline or ethanol (2.0 g/kg) injection, followed by a 15-minute activity test. Each data point represents 17-19 animals; SEM larger than symbol size are shown.

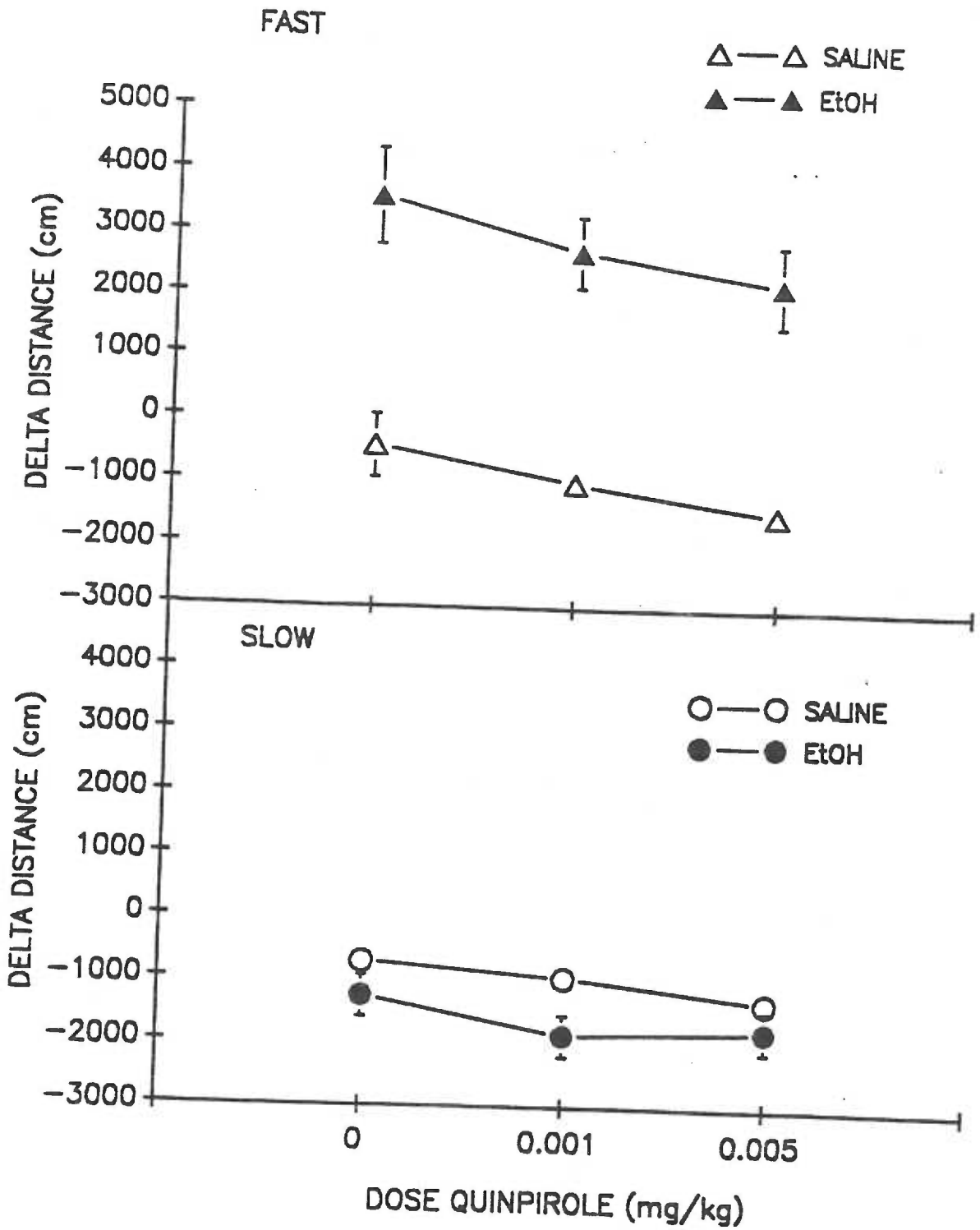
FAST
SALINE
EtOH
5000
4000
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
SLOW
SALINE
EtOH
4000
3000
2000
1000
0
−1000
−2000
−3000
DELTA DISTANCE (cm)
0
0.001
0.005
DOSE QUINPIROLE (mg/kg)

Figure 13

to be due to differences in the magnitude of response to ethanol between the replicates. Combining data from the two replicates for each line did not alter interpretation of the results, thus data are presented collapsed on replicate.

Analysis of variance was performed separately for each line, and confirmed that FAST mice were significantly stimulated by ethanol ($F[1,102]=77.7$, $p < 0.001$), while the activity of SLOW mice was significantly decreased by ethanol ($F[1,108]=8.4$, $p < 0.01$). Although quinpirole appeared to slightly decrease activity in both saline- and ethanol-treated mice, there were no significant effects of quinpirole, nor were there any significant interactions between quinpirole and ethanol, in either line.

In summary, the D2 agonist, quinpirole, had little effect on activity in either line in this study. The doses used in this study were quite low, but dose-response studies performed to characterize the responses of FAST and SLOW lines to quinpirole had indicated that doses only slightly higher than the ones used here were sufficient to cause large decreases in the activity of both lines. It appeared that additional manipulation of the dopamine system with a D2 agonist had little effect on locomotor responses to ethanol. However, because of the uncertainties associated with the doses used, no strong conclusions can be made with regard to the ability of D2 agonists to change locomotor responses to ethanol.

Experiment 9: SKF-38393 and Quinpirole

The effects of coadministration of SKF-38393 and quinpirole were

assessed in this study. Animals received an injection of saline, SKF-38393 (10 mg/kg), quinpirole (0.005 mg/kg), or a combination of the agonists (using the same doses used for each agonist separately contained in one solution), 3 minutes prior to saline or ethanol injection, and were then placed in activity monitors for 15 minutes. An ANOVA grouped on line, replicate, drug treatment condition, and ethanol condition revealed significant effects of line ($F[1,285]=145.2$, $p < 0.001$), and ethanol ($F[1,285]=44.8$, $p < 0.001$), as well as a significant interaction between line and ethanol ($F[1,285]=159.6$, $p < 0.001$), due to locomotor stimulation produced by ethanol in FAST mice, and a depressant effect of ethanol in SLOW mice. In addition, there were significant effects of replicate ($F[1,285]=44.9$, $p < 0.001$), which interacted with line ($F[1,285]=24.8$, $p < 0.001$), and ethanol ($F[1,285]=5.7$, $p < 0.05$). Examination of the data indicated that, consistent with the results of the ANOVA, the patterns of response were dissimilar among the lines and replicates (see figures 14a-14d), so data for each line and replicate were analyzed separately.

An ANOVA on data from FAST-1 mice revealed significant effects of drug ($F[3,72]=12.0$, $p < 0.001$) and ethanol ($F[1,72]=63.6$, $p < 0.001$), as well as a significant interaction between drug and ethanol condition ($F[3,72]=2.8$, $p < 0.05$). Simple main effects analysis of this interaction revealed a significant effect of agonist administration on activity of saline-treated mice ($F[3,72]=3.9$, $p < 0.05$), and comparison of means with the Newman-Keuls test revealed that the differences between the treatment groups were due to significant differences between mice given SKF-38393 alone and quinpirole alone ($p < 0.05$). Although it

Figure 14. Effects of SKF-38393 and quinpirole, alone or in combination, on saline- and ethanol-treated FAST-1 (a), FAST-2 (b), SLOW-1 (c), and SLOW-2 (d) mice. Data are presented as delta distance (cm). SKF-38393 (10 mg/kg), quinpirole (0.005 mg/kg), or the same respective doses contained in one solution, was injected 3 minutes prior to injection of saline or ethanol (2.0 g/kg). Test duration was 15 minutes. Each vertical bar represents 9-10 animals; vertical lines are SEM. * $p < 0.05$, ** $p < 0.01$ vs ethanol-treated, no-drug animals; $^{+}$ $p < 0.05$ vs ethanol-treated animals given SKF-38393.

FAST–1

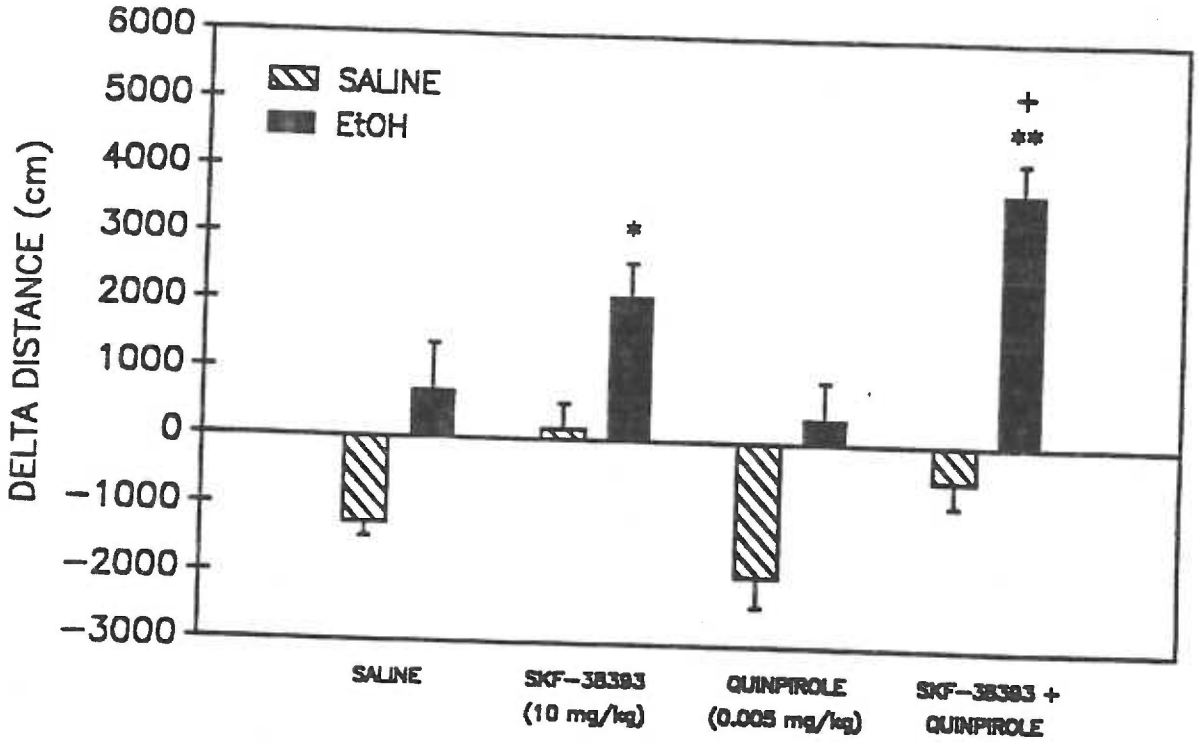

Figure 14a

FAST–2

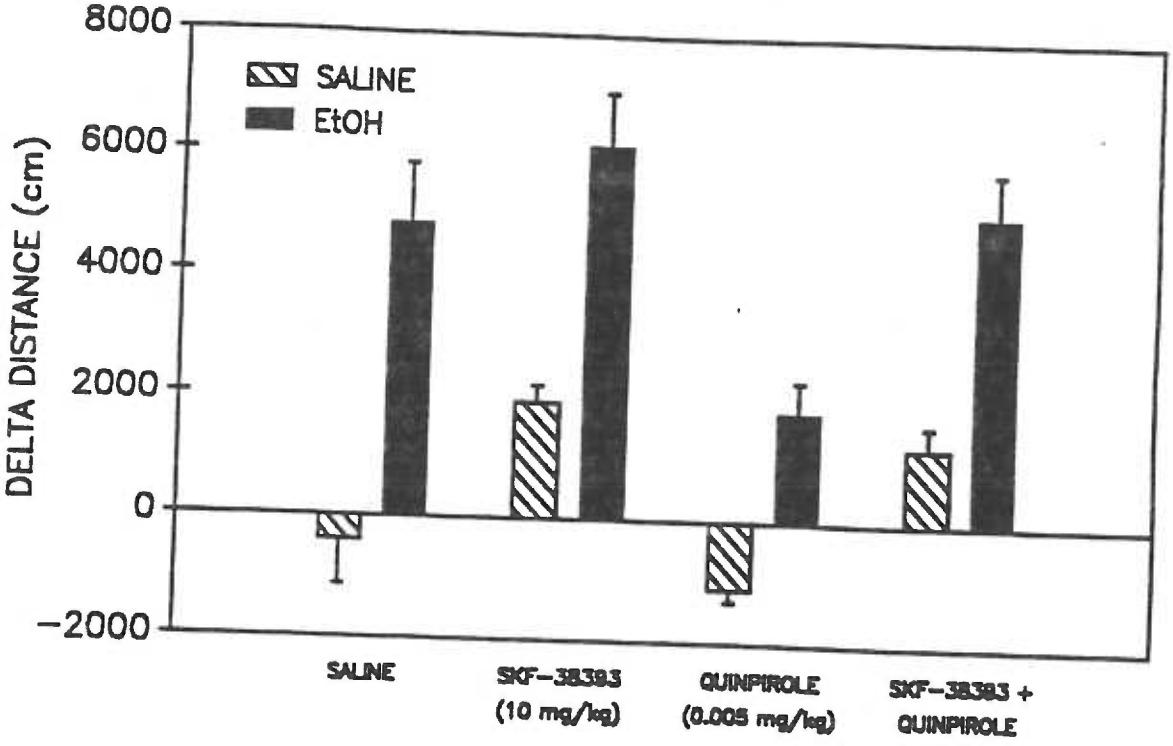

Figure 14b

SLOW–1

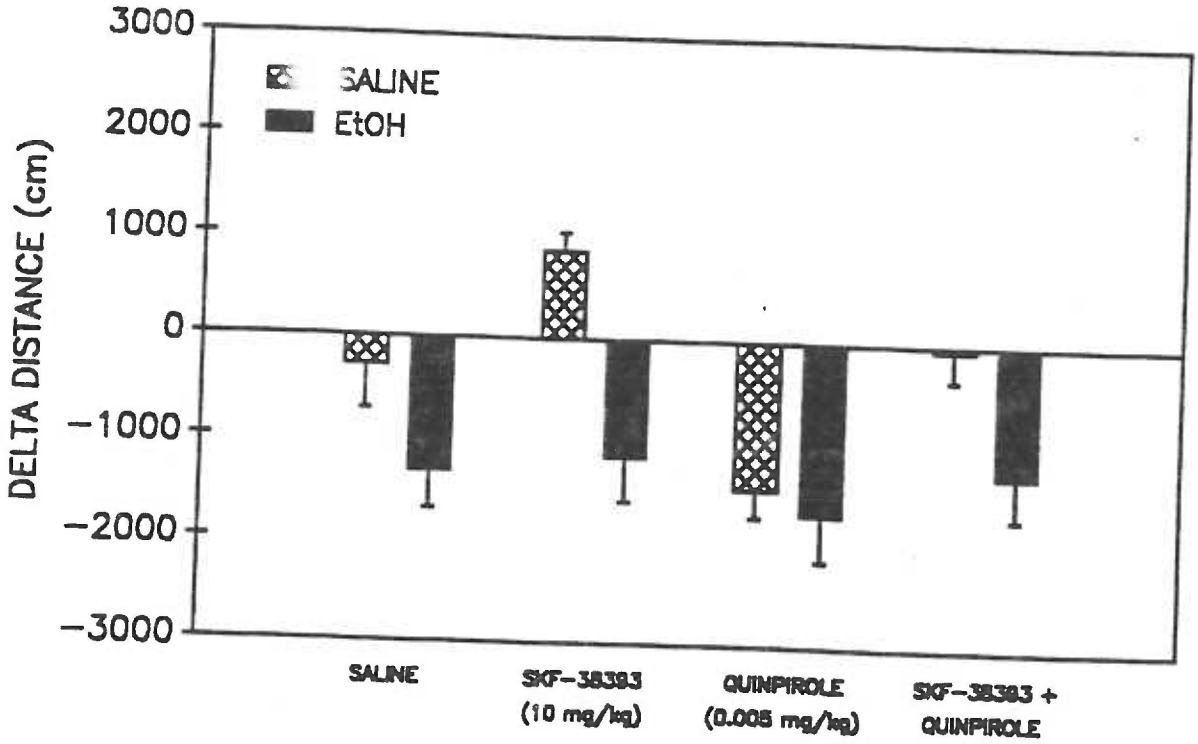

Figure 14c

SLOW–2

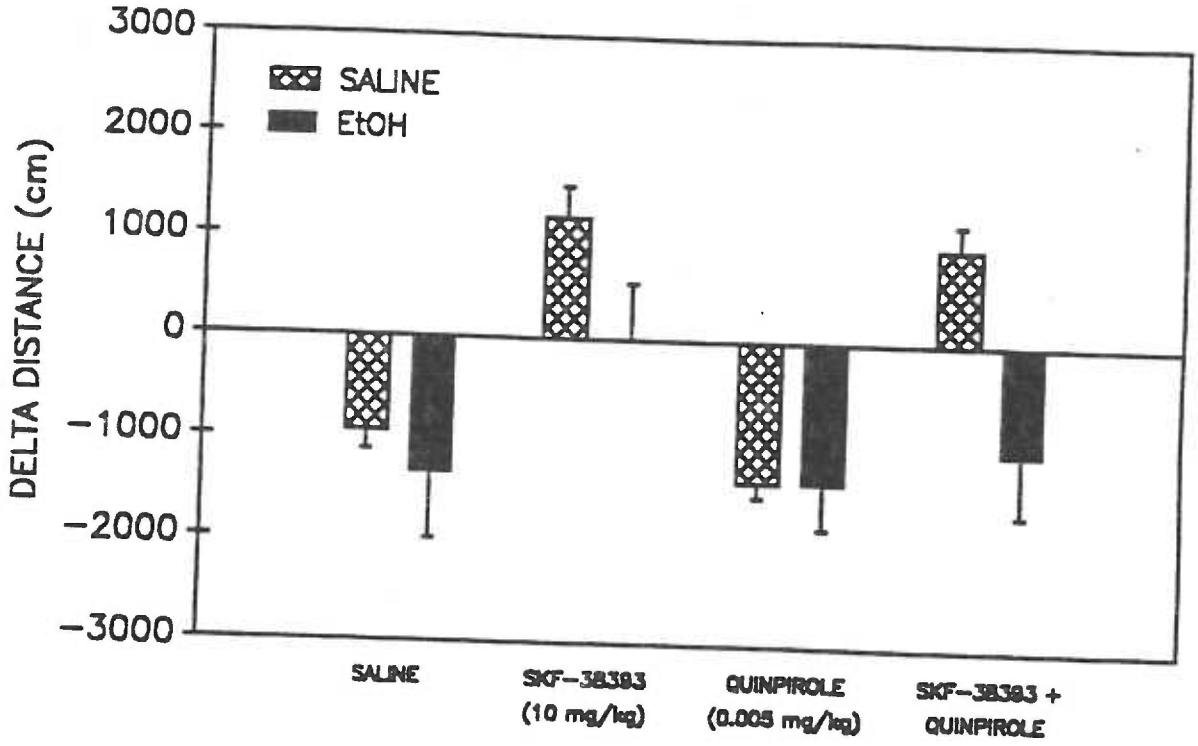

Figure14d

appeared that saline activity was increased by SKF-38393, and slightly decreased by quinpirole, none of the agonist treatment conditions significantly altered saline activity relative to the activity of saline controls. Interestingly, ethanol-treated animals exhibited a different pattern of results (see figure 14a). Agonist administration produced significant effects on the activity of ethanol-treated animals ($F[3,72]=10.9$, $p < 0.001$). Ethanol administration without agonist pretreatment produced little stimulation in FAST-1 mice, however, pretreatment with SKF-38393 alone, or in combination with quinpirole, significantly enhanced locomotor stimulation in response to ethanol ($p < 0.05$ and $p < 0.01$, respectively, as assessed by Newman-Keuls mean comparisons). In addition, the enhancement of ethanol-stimulated activity produced by coadministration of the agonists was significantly greater than that produced by SKF-38393 alone ($p < 0.05$), a result made even more interesting by the lack of effect of quinpirole administration on the activity of ethanol-treated mice. The responses of FAST-1 mice to administration of each agonist in this study were consistent with results from each of the previous experiments using SKF-38393 or quinpirole.

In contrast to FAST-1 mice, FAST-2 mice were highly stimulated by ethanol administration (figure 14b). Analysis of variance including data for FAST-2 mice confirmed that ethanol produced significant changes in activity ($F[1,72]=79.5$, $p < 0.001$), as did drug administration ($F[3,72]=12.1$, $p < 0.001$). However, the interaction between drug and ethanol condition was not significant, suggesting that saline- and ethanol-treated mice did not differ in their responses to agonist

administration. In general, informal examination of the data led to interpretations that were in agreement with statistical results. Specifically, it appeared that SKF-38393 produced increases in the activity of both saline- and ethanol-treated animals ($p < 0.01$, as assessed by Newman-Keuls mean comparisons of animals given SKF-38393, collapsed on ethanol condition, relative to saline controls). Coadministration of SKF-38393 and quinpirole did not change ethanol-stimulated activity, and produced very slight, if any, changes in activity after saline injection. On the other hand, quinpirole produced a slight decrease in the activity of saline-treated FAST-2 mice, and produced a large decrease in the ethanol-stimulated activity of FAST-2 mice (quinpirole group, collapsed on ethanol-condition < saline control, $p < 0.01$). These results are inconsistent with data from the previous quinpirole experiment, in which quinpirole had no effect on either saline- or ethanol-treated animals. The previous data were presented collapsed on replicate, and separate analysis of each line and replicate revealed no significant effects of quinpirole in any line, though there is perhaps a hint of greater sensitivity of replicate 2 mice to quinpirole.

Informal examination of the data of SLOW-1 mice, shown in figure 14c, indicated that SKF-38393 administration slightly increased, while quinpirole decreased saline activity. In contrast, coadministration of the agonists had no effect on saline activity. Ethanol decreased the locomotor activity of SLOW-1 mice, and administration of dopamine agonists did not appear to alter this response. An ANOVA on data from SLOW-1 mice confirmed that there were significant effects of drug

condition ($F[3,72]=5.3$, $p < 0.01$), due to significantly lower scores of quinpirole-treated animals relative to saline-, SKF-38393-, and SKF-38393 plus quinpirole-treated mice ($p < 0.05$, $p < 0.01$, $p < 0.05$, respectively, with data collapsed on ethanol condition). There was also a significant effect of ethanol ($F[1,72]=19.8$, $p < 0.001$) on activity, however, the interaction between drug and ethanol condition was not significant, so that further analyses of the effects of drug administration on each ethanol condition were not warranted.

The response profile of SLOW-2 mice to the various agonist and ethanol treatments, seen in figure 14d, was similar, but not identical, to that of SLOW-1 mice. Ethanol significantly decreased the locomotor activity of SLOW-2 mice, as confirmed by the results of an ANOVA ($F[1,69]=8.8$, $p < 0.01$). The effects of agonist administration were also significant ($F[3,69]=9.3$, $p < 0.001$), however, the interaction between drug and ethanol condition was not significant. Further statistical analyses of the effects of agonist administration on each ethanol treatment condition was not warranted in the absence of a significant interaction. However, Newman-Keuls mean comparisons of drug groups collapsed on ethanol treatment condition revealed that, while quinpirole had little effect on activity, the activity of mice treated with SKF-38393 was enhanced relative to non-drug treated animals ($p < 0.01$). Coadministration of the agonists also resulted in enhancement of activity relative to non-drug treated animals ($p < 0.05$), however, the effects of these drugs appeared to be dependent on ethanol treatment. The activity of saline-treated mice was slightly increased by the agonist combination, but the response of the ethanol-treated group was

not altered.

In summary, coadministration of D1 and D2 receptor agonists significantly enhanced the activity of ethanol-treated FAST-1 mice to a greater degree than administration of SKF-38393 alone, and in contrast to quinpirole alone, which did not alter the effects of ethanol on locomotor activity. The responses of FAST-1 mice in this study were consistent with results from the previous agonist studies. Enhancement of ethanol-stimulated activity was not observed in FAST-2 mice, however, it may be that maximal stimulation was produced by ethanol administration in this line, thereby preventing detection of enhanced locomotor activation by SKF-38393, or SKF-38393 in combination with quinpirole. Visual inspection of data from SLOW mice led to the impression that the activity of saline-treated SLOW mice was sensitive to additional stimulation of dopamine systems, whereas ethanol-treated animals were generally insensitive. However, statistical analyses of the data did not confirm these observations.

DISCUSSION

The hypothesis for the experiments described here was based on the rationale that differences in dopaminergic systems produced by genetic selection of the FAST and SLOW lines would result in differences in response to manipulation of dopamine function in the presence of ethanol. However, a more critical analysis of the experimental design utilized in these studies has led to the realization that it was not well-suited for detection of genetic differences between the lines. Doses of dopaminergic drugs were chosen so that the effects of these

drugs on ethanol-induced locomotor activity changes would not be confounded by disruption or enhancement of motor activity by the drugs themselves. This particular design was based on previous studies aimed at determining neurochemical mechanisms underlying ethanol-stimulated activity, and is more suitable for that purpose than for identification of genetic differences produced during selection. The guidelines for interpretation of experiments using selectively bred lines outlined by Crabbe et al. (1990) are perhaps not as directly applicable to the results of these experiments.

There are several experimental approaches that would be better suited for addressing correlated responses and genetic differences produced by selection. For example, as discussed in Phillips et al. (1992), drugs that act via substrates altered during selection of the FAST and SLOW lines would likely produce locomotor activity differences similar to line differences seen in response to low doses of ethanol. Such differences would be regarded as correlated responses to selection. On the other hand, the lack of differences in locomotor activity responses of FAST and SLOW lines to a particular class of drugs would imply that the substrates upon which those drugs acted were different from those altered during selection. If dopaminergic systems were altered by selection, drugs that acted via dopamine systems, such as amphetamine, cocaine, or dopamine receptor agonists and antagonists, would produce different locomotor activity responses in the FAST and SLOW lines. The locomotor responses of FAST and SLOW mice to some of the dopamine agonists and antagonists used in the experiments described here were characterized in order to select doses appropriate for these

experiments. In general, the FAST and SLOW lines did not consistently differ in their responses to these drugs, suggesting that dopamine receptor-mediated functions were not differentially altered during selection.

In addition to characterization of drug responses, studies of neurochemical or pharmacological characteristics may provide information regarding differences between selected lines. For the FAST and SLOW lines, this might include measurement of dopamine levels, dopamine turnover, dopamine receptor density, and dopamine receptor affinity. No data have yet been published assessing these aspects of dopamine system functioning in the FAST and SLOW lines. As discussed previously, it may be that selection altered only one aspect of dopaminergic systems, but not others, so it will be important to test several aspects of dopamine function before making strong conclusions with regard to whether or not dopamine systems were altered during selection. Additionally, even if there are no basal differences in dopaminergic characteristics between the lines, it is possible that the presence of ethanol is necessary for detection of functional dopamine differences between FAST and SLOW mice. One approach might be to find equipotent stimulating doses of ethanol for the two lines and assess the effects of dopamine drugs on stimulated activity. Differences in sensitivity to dopamine drugs between equally stimulated FAST and SLOW mice may imply genetic differences in dopamine system function in the presence of ethanol.

The experimental design used in these studies provided information regarding neurochemical mechanisms underlying ethanol-stimulated activity. The data presented here provide support for the involvement

of dopaminergic systems in mediating the locomotor stimulant response to an acute low dose of ethanol in the selectively bred FAST lines. Although the depressant effect of ethanol was blocked by pretreatment with the D1 agonist, SKF-38393, this was an inconsistent result. Thus, there was no general support for dopamine involvement in mediation of the locomotor depressant effects of ethanol in the SLOW lines.

In general, pretreatment with the dopamine antagonists, haloperidol, SCH-23390, and raclopride, reduced ethanol-stimulated activity in FAST mice at doses that had no effect on the activity of saline-treated animals. Sulpiride had no effect on either saline- or ethanol-treated FAST mice, possibly due to its inability to penetrate the blood-brain barrier. Effective antagonist doses differed between the replicates; FAST-1 mice were affected by lower doses of SCH-23390 and raclopride than were FAST-2 mice. Saline- and ethanol-treated SLOW mice were not differentially affected by dopamine antagonist administration, suggesting that the response of SLOW mice to ethanol administration is independent of dopamine systems. Differential sensitivity to dopamine antagonists between the replicates was also observed in the SLOW lines. The activity of SLOW-1 mice was decreased by doses of SCH-23390 and raclopride that had no effect on SLOW-2 mice.

Pretreatment with the agonists, apomorphine, SKF-38393, and quinpirole, produced changes in locomotor activity specific to receptor subtype. Apomorphine administration produced a biphasic response in both saline- and ethanol-treated FAST-2 mice, in which the lower dose decreased, and the higher dose increased activity. This dose-dependent response to apomorphine is consistent with previously published

literature (e.g. DiChiara et al., 1978). In contrast, FAST-1 mice showed slight but insignificant decreases in saline activity in response to both apomorphine doses, and significant increases in activity after ethanol treatment. Apomorphine appeared to produce greater decreases in the activity of saline-treated SLOW mice compared to ethanol-treated mice, however, interpretation of these data were confounded by a possible "floor effect", in which ethanol- and apomorphine-treated animals exhibited no forward locomotion. The magnitude of the activity decrease produced by apomorphine in SLOW-1 mice was greater than that observed in SLOW-2 mice. Thus, it may be that, in addition to the antagonists, the replicates differ in sensitivity to the effects of this dopamine agonist.

SKF-38393, a D1 agonist, also produced what appears to be a biphasic response to the two doses tested in FAST mice. The lower dose of SKF-38393 increased the locomotor activity of FAST mice, but the higher dose produced no change in activity. The activity of SLOW mice was equally increased by both doses of SKF-38393. Interestingly, while the effects of SKF-38393 on saline- and ethanol-treated FAST mice were parallel, the depressant effects of ethanol on the activity of SLOW mice appeared to be reversed by SKF-38393, while saline-treated animals were unaffected by administration of the agonist. However, as already mentioned, this result in SLOW mice was not replicated in the agonist coadministration study. Finally, the D2-specific agonist, quinpirole, had little effect on the activity of saline- and ethanol-treated animals in either line.

The coadministration studies involved administration of each

dopamine-receptor-subtype-specific drug alone, as well as in combination, and were thus independent replications of the individual agonist and antagonist experiments. In general, the results of the coadministration studies were consistent with the individual experiments, with a few exceptions. For example, in experiment 2, a 0.045 mg/kg dose of SCH-23390 had no effect on the activity of FAST-2 mice, while in the antagonist coadministration study (experiment 5), 0.03 mg/kg of this D1 antagonist significantly decreased ethanol-stimulated activity in these mice. Data from the individual SKF-38393 experiment (experiment 7) indicated that this D1 agonist increased the activity of ethanol-treated SLOW mice, without changing the activity of saline-treated mice. The activities of saline- and ethanol-treated FAST mice increased in a parallel fashion. In the agonist coadministration study (experiment 9), the effect of SKF-38393 administration on the activity of FAST-2 mice was consistent with data from the previous experiment. However, the activity of ethanol-treated FAST-1 mice was enhanced by SKF-38393 administration, while saline-treated animals were unaffected. In addition, as previously mentioned, depression of locomotor activity by ethanol in SLOW mice was not reversed. It may be that the inconsistent results of SKF-38393 administration are due to the lack of representative numbers of both replicates in the SKF-38393 experiment, and that a replication of the experiment with a full complement of animals would provide a more consistent picture. Finally, quinpirole, a D2 agonist, decreased activity in SLOW-1 and FAST-2 mice in the coadministration studies, but had no effect on any line when tested previously (experiment 8).

The agonist and antagonist coadministration experiments were intended to assess the relative roles of D1 and D2 receptors in expression of activity after ethanol treatment. In general, the results of these studies were consistent with the notion that concomitant stimulation of D1 and D2 receptors produces more robust behavioral responses than either one alone. For example, in the antagonist coadministration study (experiment 5), both SCH-23390 and raclopride, administered alone, decreased the ethanol-stimulated activity of FAST-2 mice. The combination of these antagonists produced even greater decreases in ethanol-stimulated activity, suggesting either additive or synergistic interactions between D1 and D2 receptors for expression of ethanol-stimulated activity. In addition, enhancement of the activity of ethanol-treated FAST-1 mice was produced by administration of SKF-38393 alone, but a significantly greater enhancement was produced by the combination of SKF-38393 and quinpirole, a result made even more interesting by the result that quinpirole by itself had no effect on ethanol-treated FAST-1 mice. Since FAST-1 mice were not significantly stimulated by ethanol and FAST-2 mice were highly stimulated in these studies, these data also suggest that enhancement of ethanol-stimulated activity by agonists is difficult to demonstrate in the presence of maximal stimulation by ethanol, while decreases in activity of ethanol-treated mice require a stimulant response. A dose-response study performed to assess the effects of several doses of ethanol on locomotor activity of the FAST and SLOW lines using the selection protocol showed that while 1.5, 2.0, and 2.5 g/kg ethanol produced optimal stimulation, 1.0 g/kg ethanol produced less stimulation (Phillips, T. J., personal

communication). Since locomotor activation by ethanol has been demonstrated to be dose-dependent, it may be possible to demonstrate enhancement of ethanol-stimulated activity by dopamine agonists in FAST-2 mice at a submaximal dose, e.g. 0.5 or 1.0 g/kg, of ethanol.

The magnitude of the difference in delta distance scores between FAST and SLOW mice varies across these experiments. Magnitude differences range from approximately 1000 to 5000 cm in FAST-1 and SLOW-1 mice, from about 3000 to 6500 cm in FAST-2 and SLOW-2 mice, and from 2000 to 6500 cm in experiments in which data are collapsed on replicate. Examination of these magnitude differences fails to reveal any obvious systematic differences, such as selection generation, age, or sex, that might contribute to this variability. Instead, this variability may be due to sensitivity of locomotor activity to environmental factors. Examination of the response to selection of FAST and SLOW mice (Figure 1) reveals parallel fluctuations in the activity of all the lines from generation to generation, suggesting the influence of undefined environmental factors (e.g. seasonal) on all genotypes. In addition, it has been demonstrated that differences in lighting conditions can affect the magnitude of the difference between the lines, and even produce greater line differences than those produced by conditions used during selection (Crabbe et al., 1987). While the lighting conditions were consistent throughout the studies discussed in this thesis, undefined environmental differences in environmental factors may have produced variable line differences from experiment to experiment.

In these experiments, locomotor activity differences between FAST-1 and SLOW-1 mice were generally not as great as the differences between

FAST-2 and SLOW-2 mice. These results are consistent with selection responses of replicate 1 and replicate 2 mice; SLOW-1 mice tend to be slightly more depressed by ethanol than SLOW-2 mice, whereas FAST-1 mice are generally less stimulated by ethanol than FAST-2 mice. In some of the studies described here, FAST-1 mice were not stimulated by ethanol when compared to their own baseline activity. The lack of stimulation by ethanol of FAST-1 mice may be a result of the experimental protocol used in these experiments, in which animals were habituated to the activity monitors on day 1, and subsequently tested with drug administration on day 2. Crabbe et al. (1988) have demonstrated that this order of drug administration is not as effective as the reverse order, in which ethanol is administered on the first test session, in eliciting stimulation in response to ethanol in FAST mice. In addition, there is evidence that ethanol has anticonflict effects, demonstrated by increases in punished responding for food or water after administration of ethanol (Glowa et al., 1989, Koob et al., 1989, McCloskey et al., 1987). As discussed in Phillips et al. (1992), it is possible that locomotor activation by ethanol may be more easily elicited in a stressful novel environment than in an environment to which the animals have already been exposed. FAST-2 mice may be less sensitive to the anticonflict effects of ethanol than are FAST-1 mice so that previous exposure to the testing environment does little to alter their stimulant response to ethanol. Additional pharmacologic studies to demonstrate ethanol-induced stimulation in FAST-1 mice, and subsequent decreases in this response by dopamine receptor blockade, will likely involve drug and ethanol exposure on day 1, followed by saline on day 2, or will

exclude the saline day altogether.

Peripheral dopaminergic systems have been shown to mediate renal, hepatic, and mesenteric vasculature (Angehrn et al., 1980, Chapman et al., 1980, Goldberg, 1978), and heart rate and cardiac output (Nagahama et al., 1986), all of which may effect drug elimination rates. Thus, the effects of systemic administration of dopamine agonists and antagonists on ethanol-stimulated activity described in these experiments may have been due to altered bioavailability of ethanol to the central nervous system (CNS). Increases or decreases in the amount of ethanol reaching the CNS will change locomotor responses to ethanol. The direction of change depends upon whether the new effective ethanol concentration is on the ascending or descending limb of the ethanol dose-response curve. In addition to peripheral dopamine effects on ethanol bioavailability, it may be that dopamine drug pharmacokinetics differ between the lines. The effects of systemic administration of dopamine drugs on ethanol bioavailability can be ascertained fairly readily by administration of ethanol with or without a dopamine drug, and subsequently measuring brain ethanol concentrations. Although this information was not obtained during the course of these experiments, future studies will likely include consideration of pharmacokinetic and bioavailability factors.

CONCLUSIONS

The FAST lines of mice, selectively bred for high sensitivity to the stimulating effects of ethanol, were demonstrated to be good tools for the study of the possible involvement of dopamine systems in

mediating activation produced by ethanol. The oppositely selected SLOW mice consistently showed no activation in response to ethanol, and did not exhibit the responses to dopamine manipulation that were seen in FAST mice. Thus, while further characterization of SLOW mice is warranted, these lines appear to be less useful for studying the substrates mediating ethanol-stimulated activity.

The pharmacological approach used in these studies was an effective method of determining neurochemical mechanisms underlying ethanol-stimulated activity, but was ineffective in addressing genetic differences produced by selection. Results of the agonist and antagonist experiments were generally consistent with the hypothesis that the activating effects of ethanol on FAST mice could be altered by dopaminergic agents at doses that had no effect on motor activity on their own. In addition, as predicted, SLOW mice were generally unaffected by dopaminergic manipulation. Results of the coadministration studies indicated that, consistent with published literature concerning the relative roles of D1 and D2 receptors on expression of locomotor behavior, maximal expression of ethanol-stimulated activity also requires concomitant D1 and D2 receptor stimulation.

Future Directions

Since dopamine systems appear to be important for expression of ethanol-stimulated activity in FAST mice which are bred specifically for this response, it follows that dopamine systems may be important in mediating ethanol's stimulant effects in all mice that exhibit this

response. It would be of interest to identify a number of inbred strains that are highly stimulated by ethanol, and, using a pharmacologic approach, determine whether dopaminergic systems mediate their locomotor stimulant responses. However, pharmacologic studies do not provide information concerning the specific dopamine pathways or the specific brain structures that are involved. Thus, future experiments using FAST mice, and the identified inbred strains, will likely involve chemical lesioning of dopamine pathways, as well as injection of dopamine drugs directly into brain. Additionally, in using these techniques, the possible peripheral effects of dopamine drugs would be circumvented.

Finally, while dopaminergic pathways are important in mediating this response, it is unlikely that they are the sole substrates for locomotor activation produced by ethanol. For example, in addition to the possible role for GABA, as previously discussed, roles for noradrenergic (Koechling et al., 1990), and nicotinic acetylcholine receptors (Blomqvist et al., 1992) have recently been demonstrated. Thus, systematic identification of all the neurochemical substrates involved, and analysis of their interconnections, are necessary for a full understanding of the mechanisms underlying ethanol-stimulated activity.

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