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Voluntary consumption of ethanol in 15 inbred mouse strains

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Abstract. To determine genetic differences in ethanol consumption, 15 commonly used inbred strains of mice were given ad libitum two-bottle choice between ethanol, 0.2% saccharin, or ethanol plus saccharin in one bottle versus tap water in the other bottle. Three different concentrations of ethanol were used: 3%, 6% and 10% (v/v). Of the 15 strains, the C57BL/6J, C57BR/cdJ and C57L/J strains showed the most consistent higher intake of ethanol either with or without 0.2% saccharin. In marked contrast, the DBA/1J and DBA/2J strains consistently showed the lowest intake. Consumption of 3% ethanol without saccharin was highly genetically correlated with saccharin consumption ($r=0.77$), suggesting that low concentrations of ethanol may have a sweet taste that affects voluntary consumption. Most strains showed very different patterns of response to ethanol with or without saccharin. Three patterns of strain responses were identified. Some strains avoided higher concentrations of ethanol whether in water or saccharin; some appeared to be sensitive to the ability of saccharin to mask the odor of ethanol; and some may have reduced consumption only when ethanol concentrations were high enough to produce aversive postingestional effects. Whereas earlier studies generally attempted to explain strain differences in consumption by invoking a single mechanism, our results demonstrate that more than one mechanism is necessary to explain the preferential ethanol intake of all strains studied.

Key words: Ethanol – Self-administration – Alcohol preference – Pharmacogenetics – Strain differences – Inbred strains

When presented with a choice between a 10% (v/v) ethanol solution and water (two-bottle choice), large inbred strain differences in voluntary ethanol consumption have been observed (Fuller 1964; McClearn 1968; Rodgers 1972). In the most comprehensive sampling of inbred mouse strains reported in the literature, Rodgers (1972) studied 14 strains for voluntary consumption of 10% ethanol versus tap water over a 2-week period. The largest differences observed were between DBA/2J mice, which strongly avoided the ethanol solution, and C57BL/Crgl and C57BL/6J mice, which consumed more of the 10% ethanol solution than water. A/Crgl, BALB/cCrgl and C3H/HeJ mice tended to be ethanol avoiders, while C57BL/10J, C57BR/cd, C57L/J and C58/J mice were ethanol preferrers (Rodgers 1972). Under these conditions, C57BL/6J mice voluntarily consume more than 10 g/kg per day of ethanol, while DBA/2J mice usually consume 0.1–2 g/kg per day (Fuller 1964; McClearn 1968; Rodgers 1972).

Since the C57BL/6J and DBA/2J strains appear to be among the extremes in voluntary ethanol consumption, virtually all work concerning possible mechanisms has focused on one or both of these strains, as reviewed by Phillips and Crabbe (1991). Proposed mechanisms for which there is some experimental support include rates of metabolism and associated enzyme activities (Rodgers et al. 1963; Schlesinger 1966; Thiessen et al. 1967), neural sensitivity to the intoxicating effects of ethanol (Schneider et al. 1973; Belknap et al. 1977; Belknap and Deutsch 1982; Gentry 1985), brain monoamine levels (Yoshimoto and Komura 1987), acetaldehyde accumulation (Schlesinger et al. 1966; Sheppard et al. 1970), emotionality (Whitney 1972), stimulus saliency of ethanol solutions involving taste or odor (Nachman et al. 1971; Belknap et al. 1977, 1978), sweet taste (Gentry and Dole 1987) and caloric utility (Rodgers 1972; Gentry and Dole, 1987). Almost all of this work has focused on only two inbred strains and only one drug (ethanol); thus, it is largely unknown how these proposed mechanisms might generalize to other genotypes, or to other drugs voluntarily self-administered. Furthermore, most of these studies have attempted to assess whether one mechanism can account for genetic differences in consumption. Studies with limited numbers of strains cannot easily detect the influence of multiple mechanisms controlling the trait ex-

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amined. It is also difficult to identify important genetic mechanisms if only two strains are studied, for a strain difference in a hypothesized mechanism is very likely to arise by chance (Crabbe et al. 1990).

The goal of this study was to characterize 15 strains for their consumption of ethanol solutions at different concentrations when water was freely available as an alternative. This included eight strains not previously studied by Rodgers (1972). Since some strains are thought to avoid the flavor of ethanol, we also studied intake of ethanol solutions to which saccharin had been added.

Materials and methods

Animals and housing. Male mice from the following inbred strains were purchased from the Jackson Laboratory, Bar Harbor, ME at 5–6 weeks of age: A/HeJ, AKR/J, BALB/cJ, CBA/J, CE/J, C3H/HeJ, DBA/1J, DBA/2J, C57BL/6J, C57BR/cdJ, C57L/J, PL/J, SJL/J, SWR/J, and 129/J. Brief descriptions of their origin and characteristics have been given by Festing (1989). Mice were housed in groups of four with others of the same strain until a few days before testing began.

Preference testing. Two-bottle choice testing began at 9–11 weeks of age. Ten mice per strain (except DBA/2J, C57BL/6J, $N=16$; A/HeJ, AKR/J, C57L/J, $N=9$; DBA/1J, $N=8$) were housed singly in standard shoebox cages and provided with two bottles (inverted 25 ml graduated cylinders) in each home cage. Daily fluid consumption was recorded at 2–3 h after light onset on a 12:12 L: D cycle. Body weights were monitored each week. The average weight was 22.0 g at the beginning of this study. The position of the two bottles was alternated at each concentration change, and fresh fluids provided. Multiple pairs of ethanol and water tubes were monitored on empty cages to control for evaporation and accidental spillage. Ambient temperature was maintained at 21–22°C. One bottle always contained tap water, while the other contained ethanol, saccharin, or ethanol plus saccharin prepared in tap water according to the following schedule:

Phase 1: (no saccharin)
Days 1–3:3% ethanol
Days 4–6:6% ethanol
Days 7–10:10% ethanol

Phase 2: (saccharin)
Days 11–14:0.2% saccharin alone
Days 15–17:0.2% saccharin plus 3% ethanol
Days 18–20:0.2% saccharin plus 6% ethanol
Days 21–24:0.2% saccharin plus 10% ethanol

Because of the expense of these animals, we felt it necessary to subject each mouse to multiple concentrations rather than using independent groups for each concentration. The 0.2% saccharin concentration was chosen for phase 2 because it is the maximally preferred concentration for both the C57BL/6J and DBA/2J strains (Fuller 1974).

Data analysis. For data analysis, only the last 2 days at each concentration were used, since this allowed each mouse the most time to stabilize its consumption after a concentration and position change. Examination of control tubes revealed a small (average less than 0.2 ml/day), nearly constant loss of fluid due to evaporation. Daily consumption was corrected separately for drug and vehicle bottles by subtracting control-tube values. All ethanol concentrations were expressed by volume (v/v). Consumption, the primary measure of intake, was expressed in g/kg per day (ethanol) or mg/kg per day (saccharin). Statistical analyses of consumption data were made by separate, mixed ANOVAs (Strain by Concentration) for phase 1, saccharin alone, and days 15–24 of phase 2. Post-hoc comparisons used simple main effects. The ethanol data were also expressed as preference ratios, which represent the percent of the total fluid intake from both bottles taken from the drug-containing bottle. Preference ratios were included for comparison to previously published values. Statistical tests of preference or avoidance for each strain were based on t tests comparing consumption in ml/day from the ethanol or ethanol/saccharin bottle with the tap water alternative. This approach was adopted to avoid statistical analyses of ratio scores. The degree of genetic determination was calculated as R^2, the between-strains sum of squares divided by the total sum of squares from a one-way ANOVA. When two measures from the same strain were compared, significance was assessed by a t test; all were planned comparisons. Statistical and graphics work was carried out using SYSTAT and CRUNCH software for the IBM compatible microcomputer (Wilkinson 1990). Correlations were assessed for significance with two-tailed tests.

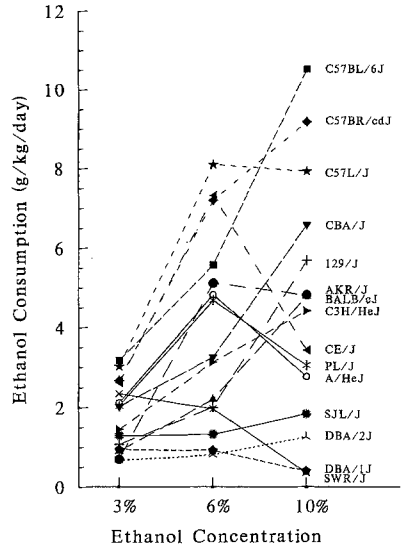

Fig. 1. Ethanol consumption in g/kg per day for three ethanol concentrations, 3%, 6% and 10% (v/v), without saccharin (phase 1) for 15 inbred strains. The standard errors, pooled across strains, were 0.50 for 3%, 1.23 for 6%, and 1.85 for 10%. The g/kg per day values can be converted to ml/day per 25 g mouse by dividing by 0.96 (3%), 1.92 (6%), or 3.2 (10%), respectively

Results

Fluid consumption and preference ratios

All of the consumption measures were highly significantly genetically determined ($P<0.001$), with coefficients of genetic determination (R^2) of 24%, 30% and 23% for the 3%, 6% and 10% ethanol (without saccharin) concentrations, respectively. The corresponding values in the presence of saccharin were 44%, 54% and 32%, respectively. For saccharin alone, this coefficient was 39%. These values give the proportion of the total variation resulting from between-strain (predominantly genetic) sources.

The results for the voluntary consumption of ethanol alone (no saccharin) are given in Fig. 1 in terms of g/kg per day for each of the 15 strains. The strains are listed in rank order from the highest to the lowest in terms of their 10% ethanol consumption (g/kg per day). On this measure, an over 25-fold range in ethanol consumption was seen among the strain means, which differed significantly

Table 1. Ethanol preference ratios for three ethanol concentrations, 3%, 6% and 10% v/v without saccharin (phase 1). An asterisk (*) or hache symbol (#), respectively, denotes a significant preference for or avoidance of the ethanol bottle. The strains are listed in rank order from the highest to the lowest in 10% ethanol preference

Strain	EtOH 3%	EtOH 6%	EtOH 10%
C57BL/6J	65± 7*	59±10	68± 9*
C57BR/cdJ	44±11	60±13	47±12
C57L/J	48± 7	72±13	45±14
CBA/J	45±16	40±16	40±16
AKR/J	16± 8 #	58±15	38±14
BALB/cJ	15± 7 #	24±12 #	29±14
C3H/HeJ	42±12	46±15	29±12
129/J	36±11	24±12 #	25±12
CE/J	66±10	73±12*	24±13 #
PL/J	42±10	44±14	21±12 #
DBA/2J	17± 8 #	13± 6 #	20± 9 #
A/HeJ	50±12	41±15	13±11 #
SJL/J	25± 9 #	13± 9 #	13± 8 #
DBA/1J	22±11 #	10± 5 #	4± 1 #
SWR/J	48±13	30±14	3± 1 #

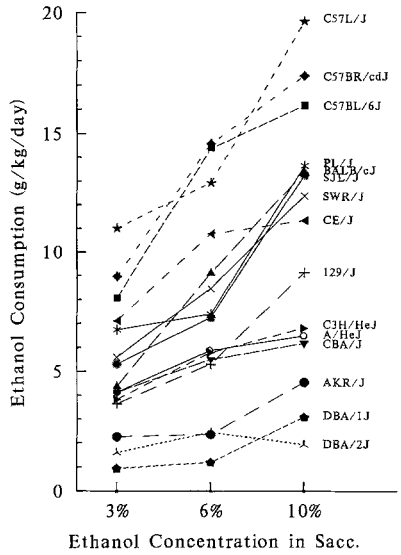

Fig. 2. Ethanol consumption in g/kg per day for three ethanol concentrations, 3%, 6% and 10% (v/v), in the presence of 0.2% saccharin (phase 2) for 15 inbred strains. The standard errors, pooled across strains, were 1.02 for 3%, 2.17 for 6%, and 2.54 for 10%. The g/kg per day values can be converted to ml/day per 25 g mouse by dividing by 0.96 (3%), 1.92 (6%), or 3.2 (10%), respectively. Data for the consumption of saccharin alone are given in Fig. 4

[$F(14,142)=4.6$, $P<0.0001$]. Drinking was concentration-dependent [$F(2,284)=21.5$, $P<0.0001$], and there was a significant interaction of strain and concentration [$F(28,284)=2.2$, $P<0.001$]. Post-hoc analyses revealed that strains differed significantly in consumption of 6% and 10% EtOH ($P<0.0001$), but not 3% EtOH ($P>0.10$). About half of the strains showed significantly different consumption as a function of concentration ($P\leq 0.01$); for some strains, this effect was weaker (AKR/J, $P<0.05$; BALB/cJ, $P=0.06$), and for some, there was no significant effect of concentration on consumption (A/J, C3H/HeJ, DBA/1J, DBA/2J, PL/J, SJL/J, and SWR/J). The three members of the C57 strain family (C57BL/6J, C57BR/cdJ and C57L/J) were the highest consumers in terms of both 10% ethanol consumption and preference ratios, while the DBA/1J, DBA/2J and SWR/J strains were the lowest. Strains CE/J, CBA/J, AKR/J, PL/J, C3H/HeJ, 129/J and BALB/cJ can be described as intermediate consumers of 10% ethanol.

The ethanol preference ratios without saccharin are shown in Table 1. Avoidance of ethanol was defined as a significantly greater consumption (ml) from the water bottle compared to the ethanol-containing bottle (i.e., a preference ratio significantly less than 0.5), while preference for ethanol was defined as the opposite outcome.

Figure 2 gives phase 2 results, when 0.2% saccharin was added to the ethanol bottle. Strains differed significantly in consumption of saccharin plus ethanol [$F(14,142)=11.4$, $P<0.0001$], and drinking was concentration-dependent [$F(2,284)=39.1$, $P<0.0001$]. Strains consumed more ethanol when it was offered in higher concentrations, but there was no significant interaction of strain and concentration [$F(28,284)=1.3$, $P>0.10$]. Another way of viewing these data is to compare strain- and concentration-specific consumption of ethanol when saccharin was added (i.e., compare Figs 1 and 2). For the 3%, 6% and 10% ethanol concentrations, the average increase in ethanol consumption due to saccharin was 3.0-fold, 2.0-fold and 2.4-fold, respectively, over all strains. However, the increase was highly strain specific. For example, 10% ethanol consumption was not significantly greater in the presence of saccharin compared to its absence for mice of strains DBA/1J, DBA/2J, AKR/J, C3H/HeJ, CBA/J and 129/J. In contrast, the largest proportionate increases due to saccharin were seen in strains PL/J, SJL/J and SWR/J, who all showed more than a 3-fold increase ($P<0.005$) in ethanol consumption due to saccharin.

Other measures of avidity

Two other measures of the response to ethanol were derived. One was the percent reduction in fluid consumption (ml/day) when 10% ethanol was added to the saccharin bottle in phase 2 (Y axis of Fig. 3). For comparison purposes, we also calculated the percent reduction in fluid consumption (ml/day) when 10% ethanol was added without saccharin in phase 1 (X axis of Fig. 3). The reduction in fluid consumption resulting from the addition of 10% ethanol to either 0.2% saccharin (phase 2) or to water (phase 1) differed widely among the 15 strains (Fig. 3).

Interestingly, some strains reacted very differently to the addition of 10% ethanol depending on whether saccharin was also present in the same bottle. Three groups of strains could be broadly discerned; those that significantly reduced intake only when ethanol was given alone, and not when given with saccharin (BALB/cJ, PL/J and SJL/J); those that significantly reduced intake due to 10% ethanol under both phase 1 and phase 2 conditions (DBA/2J, DBA/1J, SWR/J and A/HeJ); and those that

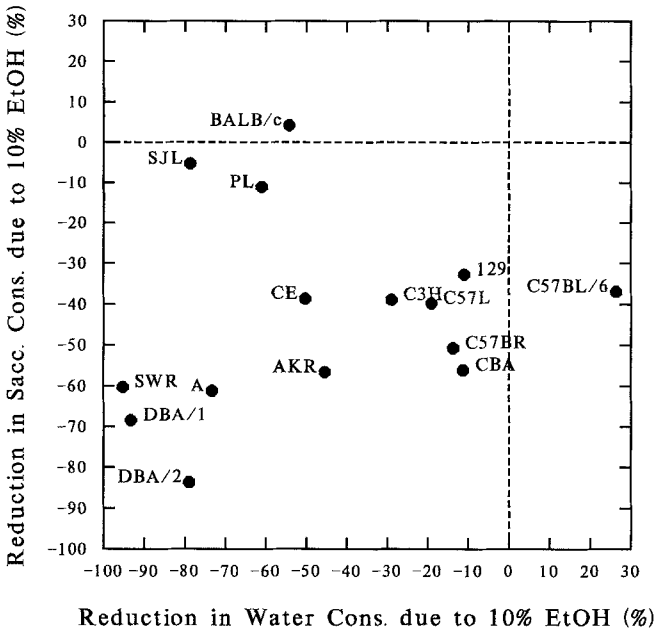

Fig. 3. The percent reduction in fluid consumption due to 10% ethanol based on a comparison of 10% ethanol in saccharin vs. saccharin alone from phase 2 (Y axis), and the comparison of 10% ethanol without saccharin vs. water alone from phase 1 (X axis). The dotted lines indicate no change (0%) due to the addition of 10% ethanol

significantly reduced intake only when ethanol was given in saccharin, but not when ethanol was given alone (C57BL/6J, C57BR/cdJ and CBA/J).

Correlations among variables

Table 2 shows the genetic correlations among all ethanol consumption measures taken in this study. Of interest is the high genetic correlation ($r = 0.77$, $P < 0.001$) between the consumption of saccharin alone and the consumption of 3% ethanol without saccharin. The correlations for the 6% and 10% concentrations were 0.51 ($P = 0.052$) and 0.46 ($P < 0.08$), respectively. The results for 3% and 10% ethanol are shown in Fig. 4. Influence analysis (Wilkinson 1990) revealed that the lower genetic correlations seen at the higher ethanol concentrations were almost entirely due to one strain, SWR. With this strain omitted, the correlations with saccharin consumption became 0.78 ($P < 0.001$), 0.70 ($P < 0.005$) and 0.75 ($P < 0.002$), respectively, for the 3%, 6% and 10% ethanol concentrations in phase 1. However, this strain was included in all data analyses to follow unless explicitly noted otherwise.

In addition to genetic correlations, which were calculated between strains (Table 2), environmental correlations, calculated within strains, and phenotypic correlations, calculated across all subjects without regard to strain, were also determined (Crabbe et al. 1990; Falconer 1981). For ethanol consumption and saccharin consumption, the phenotypic correlations were 0.34 ($P < 0.001$), 0.24 ($P < 0.002$) and 0.17 ($P < 0.04$) for the 3%, 6% and 10% concentrations, respectively ($df = 155$), while the corresponding environmental correlations were 0.15, 0.11, and 0.05 (all n.s., $df = 127$). Thus, the positive relationship between ethanol consumption and saccharin consumption was predominantly genetic in origin.

Discussion

Studies involving a sizable number of inbred strains offer a number of advantages compared to studying only one or two strains. First, they allow a more accurate assessment of the coefficient of genetic determination (heritability), which estimates the proportion of the observed variability that is genetically determined. Second, they allow an assessment of genetic correlations, or the degree to which two traits show common genetic influences (Crabbe et al. 1990); for example, it is of interest to know

Table 2. Correlation coefficients among the consumption measures taken in this study. All variables except the last two were expressed in g/kg per day or mg/kg per day. Correlations above 0.51 are significantly greater than zero ($P < 0.05$, two-tailed, $df = 13$)

	3% Et	6% Et	10% Et	SACC	3% Et in SACC	6% Et in SACC	10% Et in SACC	% Reduction EtOH only
1. 3% Et	1.00							
2. 6% Et	0.74	1.00						
3. 10% Et	0.54	0.63	1.00					
4. SACC	0.77	0.51	0.46	1.00				
5. 3% Et in SACC	0.88	0.78	0.62	0.77	1.00			
6. 6% Et in SACC	0.84	0.69	0.68	0.78	0.93	1.00		
7. 10% Et in SACC	0.72	0.58	0.56	0.71	0.93	0.92	1.00	
8. % Red Et only	0.47	0.52	0.95	0.29	0.48	0.54	0.41	1.00
9. % Red Et in SACC	0.05	0.07	0.15	−0.19	0.35	0.33	0.55	0.12

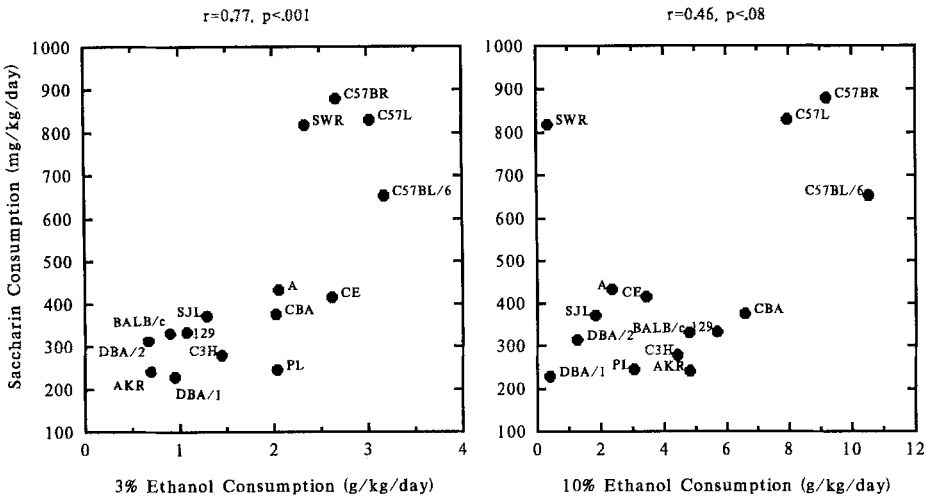

Fig. 4. Scatterplots of strain means for saccharin consumption alone (mg/kg/day) plotted against 3% ethanol consumption (*left panel*) and 10% ethanol consumption (*right panel*). The genetic correlations ($df = 13$) were 0.77 ($P < 0.001$) and 0.46 ($P < 0.08$), respectively, while the phenotypic correlations ($df = 155$) were 0.34 ($P < 0.001$) and 0.17 ($P < 0.04$). The consumption of saccharin alone in ml/day per 25 g mouse can be calculated by dividing the mg/kg per day values by 80

whether the genetic determinants of oral ethanol self-administration are the same or different from those known to characterize other responses to ethanol and other drugs of abuse (Crabbe and Belknap 1992). Third, they allow the identification of genotypes (strains) that exhibit very high or very low drug-seeking behavior, which may serve to model some genetic determinants of high and low abuse risk in humans. Fourth, they can be useful for gene mapping studies (Belknap et al. 1992), which could identify specific markers of genetic susceptibility to drug self-administration.

The DBA/1J, DBA/2J and SJL/J strains showed consistent avoidance over all three ethanol concentrations in phase 1. These strains developed an avoidance relatively early in this experiment, and at a relatively low ethanol consumption of less than 1.3 g/kg per day. Another strain, SWR/J, ranked above the median in consumption and preference for 3% ethanol, but showed the strongest avoidance of all 15 strains at the 10% ethanol concentration. This suggests that a relatively strong aversion developed in this strain to ethanol, but only at the higher concentrations. This also appeared to be the case for the A/HeJ, CE/J and PL/J strains, who showed no avoidance of 3% ethanol, but a significant avoidance of 10% ethanol. Several strains showed no significant avoidance or preference at any concentration – C57L/J, C57BR/cdJ, CBA/J and C3H/HeJ. Those strains showing a significant preference for ethanol without saccharin, i.e., greater consumption (ml) from the ethanol-containing bottle than the water bottle, were the CE/J strain at 6% ethanol, and the C57BL/6J strain at the 3% and 10% ethanol concentrations.

Different factors may be postulated to affect the variables we have used to index avidity for ethanol. For percent reduction of fluid intake due to 10% ethanol (phase 1), the factors may be presumed to be: (1) taste and/or odor of ethanol, which may be termed "preingestional" effects; and (2) pharmacological, "postingestional" effects of ethanol. Either may serve to increase consumption, or to induce aversion. For percent reduction in saccharin due to 10% ethanol (phase 2), the properties presumably reflected by this measure are: (1) reduced taste of ethanol, on the presumption that the strong sweet taste of saccharin can at least partially mask the milder taste of ethanol; (2) increased consumption of ethanol (g/kg per day), leading to enhanced aversion; and (3) preingestional palatability of saccharin, which has a strong sweet taste. This last possibility is probably not a major contributor to strain differences, since the percent reduction measure corrects for differences in saccharin palatability, and was genetically uncorrelated with saccharin consumption alone ($r = -0.15$, n.s.).

When strain differences in the effects of 10% ethanol to reduce fluid intake were considered, three different patterns could be discerned, and different factors must be hypothesized to explain them. The first pattern is exemplified by the BALB/cJ, PL/J and SJL/J strains. They showed no significant reduction in fluid consumption due to the addition of 10% ethanol to 0.2% saccharin, even at ethanol intakes of 13.3–13.6 g/kg per day (phase 2), but showed a significant and marked reduction of fluid intake when 10% ethanol was added in the absence of saccharin, at intakes of only 1.8–4.8 g/kg per day (phase 1). These strains are shown in the upper left of Fig. 3. Their reduced intake of ethanol in phase 1 cannot easily be ascribed to postingestional effects of ethanol, since this factor had no effect at the much higher ethanol intakes seen in phase 2. It is possible that mice of these three strains have such a strong relative avidity for saccharin ("sweet tooth") that they are willing to tolerate ethanol's aversive effects more than the other strains, even at ethanol intakes above the median. This seems unlikely, since these three strains were all below the median in consumption of saccharin alone (Fig. 4). Thus, the reduced taste of ethanol given in saccharin is the most plausible explanation for the pattern of results seen in these three strains.

Another group of strains (DBA/2J, DBA/1J, SWR/J and A/HeJ) showed significant and marked reductions in fluid intake with 10% ethanol added, both in the presence (phase 2) and absence (phase 1) of saccharin. These strains are shown in the lower left of Fig. 3. A combination of factors would appear to be necessary to explain this pattern of results. In other studies, the DBA/2J strain

has been reported to be more sensitive to the aversive taste or odor of ethanol alone compared to C57BL/6J mice (Belknap et al. 1978). The DBA/2J strain has also been reported to develop a significant avoidance of either 2% or 10% ethanol within 10 min of introduction of the two-bottle choice, while C57BL/6J mice do not. Blood ethanol levels in the DBA/2J mice at these times averaged 0.0012%, and no mouse showed values over 0.006% (Belknap et al. 1977). These values are so low as to be pharmacologically almost negligible. Thus, preingestional stimuli seem likely as the basis for the initial development of ethanol avoidance, though protracted avoidance may also involve other mechanisms. However, the addition of saccharin did not increase ethanol consumption appreciably in the DBA/1J and DBA/2J strains, although it presumably masked the taste of ethanol. Therefore, these strains may be reacting primarily to the odor of ethanol in maintaining their marked avoidance.

Yet a third group of strains were those that significantly reduced fluid intake with ethanol in saccharin (phase 2), but showed no significant reduction for ethanol given alone (phase 1). These were the C57BL/6J, C57BR/cdJ and CBA/J strains (Fig. 3). For this group of strains, in contrast to most other strains, it appears that 10% ethanol without saccharin has few or no aversive properties in the amounts self-administered, although these amounts were among the highest seen among all strains studied without saccharin (Fig. 1). No avoidance was seen at any concentration in phase 1. Fluid consumption (ml/day) was about the same for 3% and 10% ethanol, even though the ethanol intake tripled to 7–12 g/kg per day. However, the amount of ethanol (g/kg per day) self-administered was even greater in saccharin than without saccharin for two of these strains (52% in C57BL/6J, and 40% in C57BR/cdJ). Evidence from other studies shows the C57BL/6J strain to be less sensitive to the development of lithium chloride-induced taste aversions to ethanol than are the DBA/2J or BALB/cJ strains (Nachman et al. 1971; Belknap et al. 1978), suggesting that the stimulus saliency of ethanol taste or odor is much reduced in C57BL/6J mice. The C57BL/6J strain has also been described as showing little avoidance of the intoxicating effects of 10% ethanol consumption induced by the inhibition of ethanol metabolism (Gentry 1985) or thirst motivation (Belknap et al. 1978), and this may also apply to the other closely-related strains in this group. However, at the very high levels of ethanol intake seen in phase 2 (16.2–17.3 g/kg per day), dose-dependent, postingestional aversive effects most plausibly explain the reduction in fluid intake seen in the presence of saccharin in these strains.

Since each mouse was subject to multiple concentrations of ethanol with and without saccharin, there is a risk that experiential effects, e.g., learning or habituation, may alter the strain differences observed from those which would be seen when exposing each mouse to only a single choice situation. One clue can be gained by comparing the present results with the two-bottle choice data reported by Rodgers (1972) using a single concentration of ethanol, 10%, for a 2-week period. Seven of the strains he used were the same as in the present study, although the subline was different (Crgl versus J) for two of them. The genetic correlation between preference ratios in the two studies was 0.92 ($P < 0.003$, $df = 5$), thus demonstrating remarkable agreement despite the over 25-year interval between them, and differences in procedure. This high correlation also suggests that the use of 10% ethanol for 2 weeks leads to very similar strain differences compared to the present protocol. Similarly, in our concurrent work with morphine and saccharin drinking in these same 15 strains, mean strain saccharin consumption was highly genetically correlated ($r = 0.89$) with the saccharin values reported here, even though in the morphine study, saccharin was presented first (Belknap et al. submitted), and in the current study, after ethanol.

Experiential effects cannot, however, be ruled out. Some strains (C57L/J, C57BL/6J, BALB/cJ, and C57BR/cdJ) which ingested relatively large amounts of ethanol in phase 1 (over 4 g/kg per day) went on to ingest more than 13 g/kg per day by the end of phase 2. It is possible that tolerance to an aversive effect of ethanol could support such a pattern of results. These strains have been reported to develop tolerance to ethanol-induced hypothermia with repeated injections (Crabbe et al. 1982). It could similarly be postulated that strains which showed a tendency to avoid higher concentrations of ethanol alone near the end of phase 1 (e.g., PL/J, CE/J, A/HeJ) had developed a toxic reaction. However, none of these three strains were particularly strong avoiders at the end of phase 2. More generally, all strains continued to gain weight normally throughout the study, indicating that no severe toxicity had developed (data not shown). On the whole, we believe that experiential effects on ethanol consumption are not of primary importance compared to the genetic effects.

The apparent genetic correlation between the consumption of saccharin alone and the consumption of 3% ethanol without saccharin suggests that ethanol may have a sweet taste to mice at the lower concentrations; this is apparently the case in humans (Richter 1941; Wilson 1972). Modest phenotypic correlations between ethanol and saccharin preference have been reported to occur under restricted conditions in rats (Kampov-Polevy et al. 1990). However, it has also been reported that rats generalize the taste of ethanol to mixtures of sweet and bitter solutions, but not to sweet solutions alone (Kiefer et al. 1990). Gentry and Dole (1987) have postulated that C57BL/6J mice are motivated to drink 10% ethanol by the taste and caloric properties it shares with sucrose. Positive correlations between 10% ethanol and 0.1% saccharin consumption have recently been reported in seven selectively-bred lines or strains of rats, and in an F_2 cross between two of the lines (Overstreet et al. 1993). These studies and the present work suggest that strain differences in the palatability for sweet tasting substances may be one determinant of ethanol consumption, especially at the lower concentrations. However, there are also likely to be other mechanisms.

Lush (1981, 1984, 1989) and Lush and Holland (1988) determined the two-bottle choice behavior of a large number of inbred mouse strains for a single concentration of a number of tastants, including sucrose, saccharin,

glycine, phenylthiourea (PTC), cycloheximide and quinine. The results were reported as preference ratios. Twelve of these strains were the same as in the present study, although the sublines were different for most strains. Only sucrose (50 mM or 1.7%) and saccharin (3.2 mM or 0.066%) from Lush's studies were significantly correlated with 3% ethanol from the present study, with genetic correlations of 0.57 and 0.59, respectively ($P<0.05$, $df=10$). The correlations with the higher ethanol concentrations were not significant. However, with the SWR strain removed from the analysis, significance was obtained for both 3% and 10% ethanol, but not 6%.

The use of 15 inbred mouse strains allowed a wider sampling from available strains than have ethanol consumption studies in the past. As a result, some interesting patterns of ethanol two-bottle choice behavior were seen in some of these strains, e.g., SWR/J, PL/J and SJL/J. Wider sampling also allowed a more accurate assessment of genetic correlations. In addition, use of a large battery of strains allowed us to detect the positive genetic relationship between saccharin consumption and ethanol consumption. The pattern of strain responses to ethanol alone and to ethanol in saccharin suggests that the predominant controlling mechanisms vary from strain to strain rather than being unitary across all strains. Earlier studies, using many fewer strains, had tended to ascribe a particular, unitary mechanism to explain the genetic control of ethanol drinking.

Finally, the data presented here describing the voluntary consumption of ethanol will be compared with morphine, etonitazene and diazepam data in these same strains generated using a similar protocol. When examined in conjunction with similar data gathered in the BXD recombinant inbred strains derived from a cross between C57BL/6J and DBA/2J mice, and in F_2 crosses between these and other strains, the present data should be of value in gene mapping efforts due to the substantial number of strains tested (Belknap and Crabbe 1992; Belknap et al. 1992). We plan to pursue this work in the near future.

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