Pergamon

Pharmacology Biochemistry and Behavior, Vol. 47, No. 4, pp. 795–802, 1994

0091-3057/94 $6.00 + .00

Ethanol Self-Administration Patterns and Taste Aversion Learning Across Inbred Rat Strains

DALE S. CANNON,*[1] JAY K. LEEKA† AND ANDREW K. BLOCK‡

*Department of Veterans Affairs Medical Center, Dallas, TX
*Department of Psychiatry, The University of Texas Southwestern Medical Center, Dallas, TX
†Department of Veterans Affairs Medical Center, Dallas, TX
‡Department of Veterans Affairs Medical Center, Dallas, TX

Received 8 March 1993

CANNON, D. S., J. K. LEEKA AND A. K. BLOCK. *Ethanol self-administration patterns and taste aversion learning across inbred rat strains.* PHARMACOL BIOCHEM BEHAV 47(4) 795–802, 1994. – Initial self-administration of high doses of EtOH is shown to be associated in some inbred rat strains with the eventual development of a low preference for EtOH, presumably as a consequence of taste aversion learning occurring during initial intake. Only modest support was obtained for the hypothesis that strain differences in the aversiveness of EtOH affects taste aversion learning. The instrinsic palatability of EtOH and the salience of EtOH as a conditioned stimulus may also affect EtOH preference, but there do not appear to be differences among strains in their general ability to form taste-toxicosis associations.

Ethanol Self-administration Preference Taste aversion learning Rats

A BEHAVIORAL process that may affect ethanol (EtOH) self-administration in rats is taste aversion learning (1). Taste aversion learning is defined as the avoidance of a flavor after it has been paired with a noxious event (2,4). It is known that EtOH can function as both an unconditioned stimulus (US) (3,5,14) and conditioned stimulus (CS) (15) in taste aversion learning. Moreover, it has been demonstrated that EtOH aversion is produced under some conditions by oral EtOH self-administration (6,7,9). To assess a rat strain's preference for EtOH, intake is usually measured after several weeks of ad lib experience with an EtOH solution. Thus, preference measures would be affected by any taste aversion acquired during initial EtOH ingestion.

A recent series of studies that compared a low EtOH preference rat strain [Wistar Kyoto (WKY)] and a high-preference strain [Marshall (M520)] found that only the low-preference strain developed a conditioned aversion to the taste of an EtOH solution after 2–3 days of EtOH self-administration (7). Further, a low EtOH dose (i.e., 1.0 g/kg) administered IP was more effective in conditioning an aversion to saccharin in the low-preference strain. These strain differences in EtOH-induced taste aversions did not appear to be the result of a general inability of the high-preference strain to learn taste-toxicosis associations, as the strains did not differ in saccharin aversion following LiCl injections. Finally, the high-preference strain consumed novel flavors, including both EtOH and non-EtOH solutions, less readily on initial presentation, which could have protected it from developing a conditioned aversion to the taste of the EtOH solution. Initial intakes of small amounts of the solution would attenuate taste aversion learning by reducing the associability of the taste of the EtOH solution, reducing the associability of the pharmacological effects of the drug, and increasing EtOH tolerance prior to ingestion of high levels of the drug (1).

Because genotypes are fixed by chance (16), the generalizability of our previous findings (7) to other high- and low-preference strains is not known. That initial acceptance and EtOH preference are not always inversely related is indicated by the finding that C57BL mice, a high-preference strain, drank more EtOH on initial presentation than BALB/c mice, a low-preference strain (15). In the present research, the behavioral variables investigated in our earlier studies with

[1] Requests for reprints should be addressed to Dale S. Cannon, Ph.D., Chief, Psychology Service (116B), Department of Veterans Affairs Medical Center, 4500 S. Lancaster Road, Dallas, TX 75216.

WKYs and M520s were studied in additional low- and high-preference rat strains to assess the generality of our earlier findings. Seven of the inbred strains [viz., ACI, Brown Norway (BN), Buffalo (BUF), Fischer 344 (F344), Maudsley Reactive (MR), M520, and WKY strains] that were studied by Spuhler and Deitrich (17) were used in this series of experiments. These strains have been shown to have a wide range of interstrain variability in EtOH preference (14). An additional study investigated whether EtOH is differentially effective as a CS across strains.

All rats except the M520s and MRs were obtained from Harlan Sprague-Dawley. The M520s were bred in our lab from stock obtained from the National Cancer Institute. The MRs were obtained from Research Services, Winston Salem, NC. To attempt to equate strains on mean body weight (8), animals of the same size were requested from suppliers and, to eliminate gender differences in body weight, only males were used. For each experiment, the number of rats per strain as well as the mean and standard deviation body weight per strain are shown in Table 1. In each experiment in this series, small but statistically reliable differences were obtained in mean body weight per strain (cf. Table 1), $ps < 0.001$ [To assess the effect of strain differences in body weight, between-strain comparisons were computed with and without body weight as a covariate in every experiment. In no case did use of body weight as a covariate alter the findings; so, for the sake of simplicity and brevity, only the analyses not using weight as a covariate are reported. It should be noted that there was not a consistent difference across studies in the mean weights of particular strains. Using the experiment means as the dependent variable, there was not a significant difference between strains across studies (cf. Table 1).]

EXPERIMENT 1

In the first experiment, EtOH preference was determined after 3 weeks of EtOH self-administration. To increase EtOH consumption to levels more likely to result in measurable taste aversion learning, EtOH was presented as an EtOH-cola solution rather than the usual EtOH-water solution. Water was the alternative fluid offered rats.

The use of cola rather than water to dilute EtOH raises questions about the comparability of the preference measures obtained using the two diluents. It is expected, of course, that the absolute level of EtOH intake would be greater when EtOH is presented in a cola solution. Indeed, that is the reason a cola solution was used in this experiment. Consequently, the preference ratios (i.e., EtOH solution intake as a proportion of total fluid intake) will be higher than those reported in experiments using EtOH-water solutions. The important issue for this series of experiments, however, is not the absolute level of EtOH intake but the relative level of intake across strains. If the effect of cola is relatively constant across strains, the preference measures obtained in this experiment should correlate with measures observed when EtOH is presented in a water solution. If this correlation is high, then it can be assumed that conclusions drawn in this series of experiments about strain differences in taste aversion learning are not confounded by differential effects of cola across strains. The effect of this procedural change was evaluated by comparing the present results with those of Li and Lumeng (14).

Method

Animals were housed individually in 18 × 18 × 24 cm stainless steel cages in a room with a 12 L : 12 D cycle, and Tekland rodent chow was available ad lib throughout the study. Intakes were determined by weighing fluid bottles daily. EtOH was presented as an EtOH-cola solution (10% EtOH, w/v). Decarbonated, nondiet cola was used. All strains were given a two-bottle choice between EtOH-cola and water for 24 days.

EtOH preference ratio was defined as the ratio of EtOH-cola intake to total fluid intake over the last 3 days of the experiment. The mean daily dose (g/kg/day) was also computed over the last 3 days.

TABLE 1

BODY WEIGHT IN GRAMS PER STRAIN PER EXPERIMENT

Experiment		Strain						
		WKY	ACI	F344	BUF	M520	MR	BN
1	Mean	279	234	257	319	276	307	279
	SD	19	15	12	29	11	12	18
	n	10	10	10	10	10	14	10
2	Mean	233	229	232	246	257	230	216
	SD	21	12	18	16	18	20	19
	n	30	29	30	30	28	28	30
3	Mean	255	213	240	268	208	231	230
	SD	15	8	11	16	35	22	18
	n	30	30	30	30	30	30	27
4	Mean	287	247	256	303	240	246	268
	SD	11	44	17	27	17	36	18
	n	50	50	50	50	40	50	50
5	Mean	292	251	271	318	273	281	300
	SD	37	16	36	53	45	28	52
	n	9	10	10	10	12	9	10
	Mean*	269	235	251	291	251	259	259

*Mean of the means of Experiments 1-5.

Results

Mean EtOH consumption (g/kg/day) and EtOH preference ratio per strain over days 22-24 are shown in Table 2. One-way analyses of variance (ANOVAs) indicated significant strain differences on both variables, $Fs(6, 67) > 22.7$, $ps < 0.001$. The results of Tukey post hoc tests are shown in Table 2.

Comparison of the mean g/kg/day per strain in Table 2 with the data published for the same strains by Li and Lumeng (14) indicates the EtOH-cola solution used in this study resulted in greater intake than the EtOH-water solution used in their study but did not alter relative EtOH preference across strains. The mean EtOH intake (g/kg/day) per strain for males in the Li and Lumeng (14) study is shown in Table 2. A repeated measures ANOVA demonstrates the mean daily EtOH intake across strains was greater in the present study (mean = 5.70, SD = 2.25) than in the Li and Lumeng study (mean = 1.83, SD = 1.42), $F(1, 6) = 59.1$, $p < 0.001$. The correlation between the mean g/kg/day per strain across the two studies is $r(5) = 0.83$, $p = 0.02$.

Inspection of the means of the g/kg/day and preference ratios in Table 2 shows that these two measures covary. In fact, the correlation between the two variables across all 74 rats is $r(72) = 0.93$, $p < 0.001$. Given the statistical redundancy of these two measures, only the g/kg/day variable was used as the measure of EtOH preference in subsequent studies.

Discussion

The results of Experiment 1 confirm the expectation that these rat strains vary widely in their EtOH preference and, thus, are appropriate strains to use to investigate correlates of EtOH preference. Relative to the other strains in this study, WKYs, ACIs, and F344s have low EtOH preference; BUFs have moderate preference; and M520s, MRs, and BNs have high preference. Further, the results indicate the use of an EtOH-cola solution, rather than an EtOH-water solution, increases EtOH consumption without altering relative strain preference for EtOH.

EXPERIMENT 2

Previous research found that a low-preference strain (i.e., WKY) developed a conditioned aversion to the taste of an EtOH solution following EtOH self-administration but a high-preference strain (i.e., M520) did not (7). Experiment 2 investigates the relation between EtOH preference (as assessed in Experiment 1) and conditioned taste aversion following initial EtOH self-administration.

In the self-administration procedure used in our previous studies (6,7), the only fluid available to rats was the EtOH solution. Because most EtOH preference studies employ a two-bottle procedure with both an EtOH solution and a non-EtOH fluid available simultaneously, both a one-bottle and two-bottle procedure were used in this experiment to determine the effect on taste aversion learning of this procedural variation.

Method

Within each strain, rats were randomly assigned to a forced access (group FA), choice access (group CA), or control (group CON) condition ($ns = 10$ per group, with the exceptions of M520 group FA, $n = 8$; ACI group CA, $n = 9$; and MR group CON, $n = 8$). To adapt animals to the fluid deprivation schedule to be employed during the aversion test, all rats were given water 20 min/day at approximately 1400 h daily for the first 14 days of the study. Then, on 5 consecutive days, group CON rats were given 5 ml of 2.5% (w/v) EtOH-cola at 1000 h to familiarize them with the taste of the solution without giving them experience with the pharmacological effects of EtOH. [Five milliliters of 2.5% (w/v) EtOH-cola in rats the size used in this experiment equates to an approximate EtOH dose of 0.5 g/kg, which has been shown to be an ineffective dose for conditioning taste aversions (7) (cf. Experiment 3 in this series).] Groups FA and CA were given 5 ml of water at 1000 h on these 5 days. Water was given ad lib to all groups on the next 3 days. Then, on 3 conditioning days, group FA was given 10% (w/v) EtOH-cola ad lib and group CON was given water ad lib. Group CA was given two bottles ad lib, one containing water and the other the EtOH-cola solution. To compensate for anticipated differences in total fluid consumption during conditioning, all animals then were given water ad lib for 1 day before being placed on a 20-min/day water drinking schedule for 3 days. [Across strains and conditioning days, animals in the CA groups drank more total fluid (mean = 49.1 ml/day, SD = 16.0) than did those in the FA groups (mean = 40.0 ml/day, SD = 13.4), $F(1, 409) =$

TABLE 2

MEAN EtOH INTAKE (g/kg/day) AND EtOH PREFERENCE RATIO (i.e., EtOH-COLA PROPORTION OF TOTAL FLUID INTAKE) PER STRAIN OVER DAYS 22-24 OF EXPERIMENT 1

	Experiment 1				Li and Lumeng (12)	
	Preference Ratio		g/kg/day		g/kg/day	
Strain	Mean	SD	Mean	SD	Mean	SD
WKY	0.24[a]	0.17	2.2[a]	1.6	0.3	0.4
ACI	0.50[b]	0.22	4.3[b]	2.0	0.2	0.2
F344	0.53[b]	0.14	4.5[b]	1.3	0.8	0.4
BUF	0.52[b]	0.11	5.8[bc]	1.4	2.0	1.0
M520	0.67[bc]	0.07	6.6[cd]	1.1	3.4	1.5
MR	0.71[c]	0.08	7.7[de]	0.8	3.6	2.5
BN	0.84[c]	0.07	8.8[e]	0.7	2.5	1.5

[a-e]Strains with different letter superscripts are significantly different, Tukey post hoc comparisons, ps < 0.05.

TABLE 3

MEAN EtOH INTAKE (g/kg/day) PER STRAIN OVER CONDITIONING DAYS OF EXPERIMENT 2

	Group FA		Group CA	
Strain	Mean	SD	Mean	SD
WKY	10.2	2.4	7.9	2.1
ACI	12.8	1.7	9.2	1.9
F344	12.4	3.0	8.5	0.9
BUF	13.0	2.5	8.4	2.1
M520	13.8	3.3	11.7	3.0
MR	13.9	7.9	10.3	6.5
BN	13.4	2.7	10.4	1.7

39.8, $p < 0.001$. On the ad lib water consumption day following conditioning, there was no difference across strains in the amount of water consumed by Group CA (mean = 35.0 ml, SD = 10.0) and group FA (mean = 36.6 ml, SD = 11.1). Thus, there should have been no difference in fluid deprivation between these two groups on the posttest.] Finally, all groups were given 10% (w/v) EtOH-cola for 20 min to test for taste aversion.

Results

Conditioning day intakes (g/kg/day), averaged over the 3 conditioning days, are shown in Table 3 for groups FA and CA. As can be seen, across strains group FA consistently ingested more EtOH than did group CA, $F(1, 121) = 29.5$, $p < 0.001$. There was also a significant strain difference, $F(6, 121) = 2.41$, $p = 0.031$, but there was no strain by condition interaction.

Test day intakes are shown in Fig. 1. A strain by experimental condition ANOVA was significant only for the interaction effect, $F(12, 184) = 2.98$, $p < 0.001$. One-way ANOVAs for each strain were significant only for WKYs, $F(2, 27) = 10.95$, $p < 0.001$, and F344s $F(2, 27) = 3.61$, $p = 0.041$. Tukey post hoc tests indicate that, for the WKY rats, both groups FA and CA drank less during the aversion test than did group CON, $ps < 0.001$; for the F344 rats, the only significant difference was between group CON and group CA, $p = 0.033$.

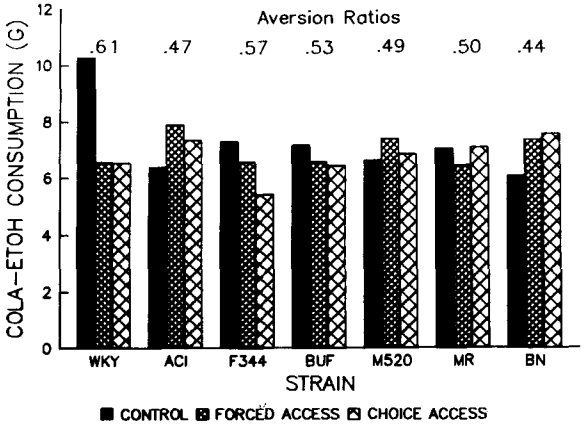

FIG. 1. Mean posttest consumption (g) by strain of a 10% (w/v) EtOH-cola solution in Experiment 2. During conditioning, control groups were familiarized with the flavor of the solution but had no ad lib access to it; forced access groups had ad lib access to the solution as their only fluid, and choice access groups had ad lib access to both the solution and water. See text for explanation of aversion ratios.

To assess the relation between conditioned taste aversion and EtOH preference, an aversion ratio was computed for each strain to be equal to 1 minus the quotient of the mean of group CA divided by the sum of the means of group CON and group CA. The ratio of group CA to the sum of group CON plus group CA is more normally distributed than the simple ratio of group CA to group CON. The ratio is subtracted from 1.0 so that higher values indicate greater aversion for a strain relative to within-strain controls naive to the pharmacological effects of EtOH. A ratio of 0.50 or less indicates no aversion. The aversion ratio for each strain is shown in Fig. 1. The correlation between this aversion ratio and EtOH preference (i.e., the mean g/kg/day per strain over the last 3 days of Experiment 1) was $r(5) = -0.77$, $p = 0.05$.

Discussion

Although the choice access procedure resulted in less EtOH intake than did the forced access procedure, the choice access procedure did produce conditioned taste aversion in two of the low EtOH preference strains (i.e., WKY and F344) relative to within-strain control groups naive to the pharmacological effect of EtOH. That conditioned taste aversions were obtained using the choice access procedure is significant because rats in EtOH preference studies are usually given a two-bottle choice procedure. The failure to obtain an aversion in the F344 Group FA suggests the aversive effect of initial EtOH exposure may not be as great in this strain as it is for the WKY strain.

The negative correlation between the aversion ratio of this experiment and final EtOH consumption in Experiment 1 suggests that conditioned aversion acquired by a strain during the first 3 days of EtOH ingestion is inversely related to EtOH preference following 3 weeks of EtOH self-administration. This finding is consistent with the hypothesis that aversion acquired during initial intake suppresses subsequent EtOH preference. However, this general relation was not true of all strains. The ACIs, a low-preference strain in Experiment 1, did not acquire a conditioned aversion in this study.

EXPERIMENT 3

Experiment 3 explored the possibility that strain differences in EtOH self-administration are the result of differences in the aversiveness of EtOH as an US. This hypothesis is suggested by the reported positive association between EtOH preference and EtOH metabolism and tolerance (17). If the acute physiological effects of EtOH are diminished in high EtOH preference strains, one would expect the aversiveness of those effects also would be decreased.

The hypothesis that there are strain differences in the aversiveness of EtOH as an US that are associated with EtOH preference leads to three predictions tested in this experiment: a) the slope of the dose-response curve differs across strains (i.e., there is an interaction effect between strain and conditioning dose on the aversion posttest), b) the slope of the dose-response curve is correlated with EtOH preference (i.e., the steeper the slope, the lower the EtOH preference as assessed in Experiment 1), and c) the slope of the dose-response curve is correlated with degree of aversion produced by EtOH

self-administration (i.e., the steeper the slope, the greater the aversion ratio found in Experiment 2).

Method

Subjects were individually housed and fed as in Experiment 1. They were given water 20 min/day at 1400 h for 14 days prior to the start of the study and were maintained on that schedule throughout the experiment. Subjects within each strain were randomly assigned to four EtOH dosage groups (0.0, 0.5, 1.0, and 1.5 g/kg). For most strains, there were seven animals in both of the lower two dosage conditions and eight in both of the higher two dosage conditions. For BNs, there were five and six rats, respectively, in the 0.0 and 0.5 g/kg groups. For M520s, there were eight rats in the 0.0 g/kg group and seven rats in the 1.5 g/kg group.

At 1000 h on the conditioning day, all rats were given a 0.1% (w/v) saccharin-water solution for 20 min and then were given an IP injection within 1 min of removal of the bottle. Rats in the 0.0 g/kg groups were given 3 ml of 0.9% saline, and rats in the other groups were given appropriate amounts of a 22.5% (w/v) EtOH-water solution. Two days later, all animals were given the saccharin solution for 20 min at 1000 h.

Results

Posttest saccharin intake is shown in Fig. 2. A strain by conditioning dosage ANOVA was significant for both the strain effect, $F(6, 179) = 14.6$, $p < 0.001$, and the dosage effect, $F(3, 179) = 73.0$, $p < 0.001$. The interaction effect was not significant. Because the interaction effect was the effect of primary interest, a power analysis was conducted (10). If the population effect size were small (i.e., f = 0.10), power would be 0.09; if the population effect size were medium (i.e., f = 0.25), power would be 0.49; and if the population effect size were large (i.e., f = 0.40), power would be 0.95. Thus, with the sample size used in this experiment, the probability of detecting a small interaction effect is low but the probability of detecting a large interaction effect is quite high.

The slope of the dose-response curve for each strain was computed as the correlation coefficient between conditioning dose and posttest saccharin intake. The correlations were as follows: WKY, −0.85; ACI, −0.69; F344, −0.88; BUF, −0.74; BN, −0.63; M520, −0.50; and MR, −0.75. The correlation between this dose-response curve measure and the Experiment 1 EtOH preference measure (g/kg/day) was 0.59. This relation was in the predicted direction, i.e., the steeper the curve, the lower the EtOH preference; but the correlation failed to reach statistical significance. However, with only seven pairs of observations, the power of this test is quite low. If the population correlation were low (i.e., $r = 0.10$), power would be 0.04; if the population correlation were medium (i.e., $r = 0.30$), power would be 0.10; if the population correlation were large (i.e., $r = 0.50$), power would be 0.22; and if the population correlation were equal to the 0.59 observed in this study, power still would be only 0.31 (10).

The slope of the dose-response curve was associated with the degree of aversion following self-administration as determined in Experiment 2. The aversion ratio computed in Experiment 2 was correlated across strains with the slope measure in this study, $r(5) = -0.76$, $p = 0.05$. Thus, the steeper the dose-response curve in this study, the greater the aversion ratio in Experiment 2.

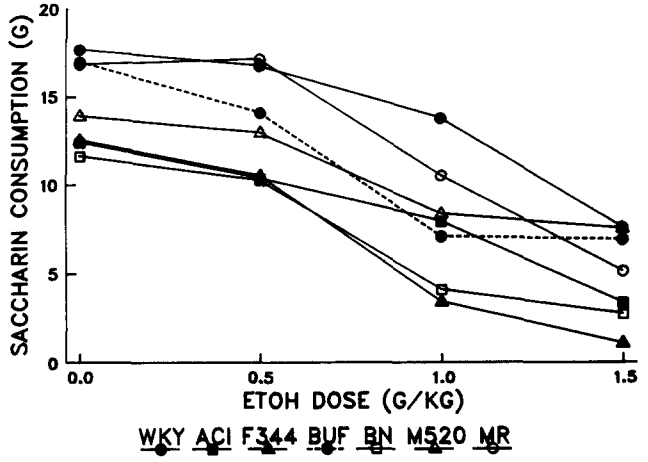

FIG. 2. Mean posttest consumption (g) by strain of a 0.1% saccharin solution following conditioning with an IP injection of either a 0.0, 0.5, 1.0 or 1.5 g/kg dose of 22.5% (w/v) EtOH in Experiment 3.

Discussion

The results provide only weak support for the hypothesis that EtOH is a more aversive US for strains with low EtOH preference. As predicted by the hypothesis, the slope of the dose-response curve was correlated with the Experiment 2 aversion ratio, suggesting the aversiveness of an acute dose of EtOH is associated with the degree of taste aversion learning that results from self-administration. However, two other predictions derived from the hypothesis were not supported by the results: the slope of the dose-response curve did not differ across strains nor was it correlated with EtOH preference. The interaction effect used to test for strain differences in the dose-response curve had adequate power to detect a large population effect but well could have failed to detect a smaller population effect. Thus, it can be concluded that it is not very likely that there is a large difference between strains in the slope of the dose-response curve, at least under the experimental conditions employed in this experiment. The effect of altering experimental conditions (e.g., fluid deprivation level, range of conditioning doses, duration of the posttest, number of conditioning trials) is not known. The test of the association between the dose-response curve and EtOH preference had very low power, so little weight should be attached to this negative finding.

This experiment does not address the issue of whether there are differences in the aversiveness of EtOH following chronic administration. It is possible that differences in acquired tolerance might result in differential taste aversion learning.

An alternative interpretation of the results of Experiment 3 would be that saccharin aversions were conditioned by aversive effects on the peritoneum of the high EtOH concentration rather than the systemic effects of EtOH. This interpretation cannot be ruled out conclusively, but it should be noted that the cue-to-consequence specificity of taste aversion learning (11,12) would favor an interpretation based on the association of taste cues and the systemic effects of EtOH.

EXPERIMENT 4

Experiment 4 investigates the possibility that strains differ in their ability to learn aversions to the taste of an EtOH

solution. A difference in the conditionability of EtOH as a CS, if found, could be due to either of two factors. It could be that EtOH solutions may be a less salient CS for some strains, or it is possible that strains differ in their ability to form taste-toxicosis associations regardless of the CS. Neither of these possibilities has been assessed in these rat strains, but a difference between high and low EtOH preference mice in the effectiveness of EtOH as a CS in a taste aversion learning paradigm has been reported (15). A high-preference mouse strain, C57BL, did not acquire a LiCl-induced aversion to the taste of EtOH as readily as did the low-preference mouse strain, BALB/c.

In Experiment 4, an EtOH-water solution was employed as the CS across a range of conditioning doses using lithium chloride as the US. A relatively weak EtOH solution was used to reduce the likelihood of aversive pharmacological effects of EtOH intake. The critical test of the hypothesis that there are strain differences in the conditionability of EtOH as a CS is the strain by conditioning dosage interaction effect.

Method

In unspecified regards, the procedure was the same as that of Experiment 3. On the conditioning day, subjects were presented 7 ml of a 2.5% (w/v) EtOH-water solution for 20 min at 1000 h. Immediately after the CS presentation, rats were given LiCl according to group assignment. Subjects within strains were randomly assigned to one of five LiCl dosage groups (n = 10 per group except for the M520s, for which group size was eight). The dosages were 1.2% of body weight of 0.0375, 0.075, 0.1125, and 0.15 M LiCl administered IP. Control subjects (0.00 M LiCl) were injected with 3 ml of normal saline. Two days later, subjects were given 2.5% (w/v) EtOH-water for 20 min at 1000 h as a posttest.

Results

Intake of the EtOH solution on the posttest is shown in Fig. 3. A strain by dosage ANOVA was significant for both the strain effect, $F(6, 304) = 43.07$, $p < 0.001$, and the dosage effect, $F(4, 304) = 16.6$, $p < 0.001$. There was no strain by dosage interaction. Power analyses of the test of the interaction effect indicate adequate power. If the population effect size were small (i.e., f = 0.10), power would be 0.12; if the population effect size were medium (i.e., f = 0.25), power would be 0.72; and if the population effect size were large (i.e., f = 0.40), power would be 1.00. Thus, with the sample size used in this experiment, the probability of detecting all but a very small interaction effect is quite high.

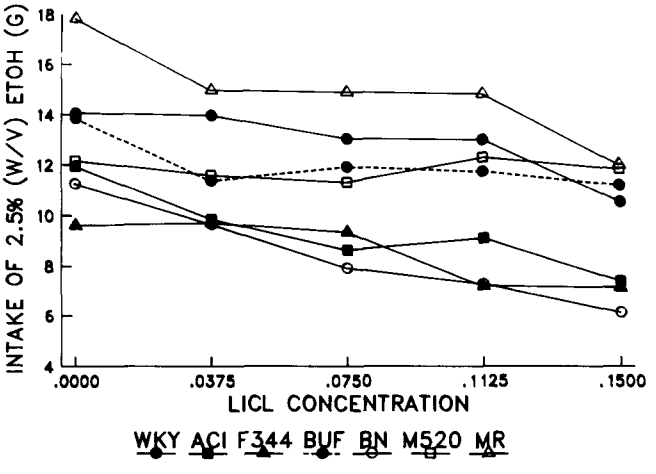

FIG. 3. Mean posttest consumption (g) by strain of a 2.5% EtOH-water solution following conditioning with a 1.2% body weight IP injection of either 0.0, 0.0375, 0.075, 0.1125, or 0.15 M LiCl in Experiment 4.

Even though the strain by dosage interaction effect was not significant, it should be noted that the M520s were the only strain not to acquire any aversion to the EtOH-water solution even at the highest LiCl dose. Within this strain, there was no LiCl dosage effect, $F(4, 35) = 0.39$, $p = 0.81$.

Discussion

The results indicate no overall difference between strains in their ability to form EtOH-LiCl associations. Although conclusions must be drawn cautiously from negative results, the sample size was large enough to provide adequate power for the test of the interaction effect. Moreover, the conclusion that strains do not differ in their general ability to form taste-toxicosis associations also is supported by an unpublished study in which the procedure of Experiment 4 was repeated except saccharin was the CS. In that study, there were no differences between strains in ability to learn LiCl-induced saccharin aversions.

It is possible that the taste of EtOH is a less salient CS for the high-preference M520s. The M520s did not manifest a conditioned aversion to the EtOH-water solution even when a relatively high LiCl dose was employed as the US. This failure to learn an aversion cannot be attributed to an inability to form taste-LiCl associations because, in the unpublished study, M520s acquired strong aversions to a saccharin CS following injections of these same LiCl dosages.

EXPERIMENT 5

In Experiment 5, we examined the possibility that the strain differences in taste aversion learning observed in Experiment 2 are a function of differences in patterns of EtOH self-administration. Specifically, it was predicted that initial intake is inversely related to eventual preference because high initial intake would enhance taste aversion learning (1). Our previous research found that WKYs consumed more EtOH than did M520s during the first 2 h of EtOH exposure (7).

Method

Apparatus. Drinking was measured in a recording cage the same size as the home cage using a Coulbourn lickometer that detects licks by means of a photoelectric beam broken by the rat's tongue. The number of licks was recorded online by a microcomputer at 2-min intervals.

Procedure. Animals were housed in a room with a 12 L : 12 D cycle, and onset of the dark phase of the cycle occurred at 1800 h. Fluids and food were available ad lib throughout the study. Rats were given 2 days to habituate to the recording cage, and then continuous recordings of 10% (w/v) EtOH-cola intake were made on days 1-3 of the study. Water was also available, but water intake was not recorded online. Rats were then returned to the home cage with both water and EtOH-cola available ad lib. On days 11 and 19 of the study, they were returned to the recording cage for 24 h with both fluids still available, and ingestion of the EtOH solution again was measured continuously.

Bottles were refilled and rats were weighed between 1400–1430 h daily, and fluid intake for each 24 h period was determined by weighing the fluid bottles. The amount of EtOH consumed during each 2-min interval was computed to be proportional to the number of licks that occurred during that interval.

Results

Mean EtOH intake (g/kg/day) on days 1-3, 11, and 19 are shown in Fig. 4. The correlation between mean strain intake on day 19 of this study and the strain means at the end of Experiment 1 was $r(5) = 0.87$, $p = 0.011$. Thus, the relative EtOH preference of these strains was stable from sample to sample.

A strain by day repeated measures ANOVA of EtOH intake (g/kg/day) over days 1-3, 11, and 19 indicated a significant interaction effect, $F(24, 252) = 3.50$, $p < 0.001$. Repeated measures ANOVAs computed separately for each strain indicated WKYs, ACIs, and F344s significantly decreased their intake over days and MRs significantly increased their intake, $Fs(4, 32) > 2.97$, $ps < 0.032$. Repeated measures ANOVAs computed over just days 1-3 revealed the same pattern of findings. [Similar results were obtained in equivalent groups in Experiments 1 and 2. Over the first 3 days of Experiment 1, three strains decreased daily EtOH intake (i.e., WKY, F344, and M520), three remained constant (i.e., ACI, BUF, and BN), and MRs increased daily intake. In Experiment 2, Group CA of the lower preference strains (i.e., WKY, ACI, F344, and BUF) decreased EtOH daily intake over the 3 conditioning days, but the higher preference strains (i.e., M520, BN, and MR) did not change EtOH intake across days.] Further, there was no difference between strains in total EtOH intake on day 1; but the strains did differ on days 2, 3, 11, and 19, $Fs(6, 63) > 3.39$, $ps < 0.006$. Tukey post hoc tests indicate that by day 3, the intake of the MRs was greater than that of the WKYs, BUFs, and ACIs, $ps < 0.001$, and that of the M520s was greater than that of the WKYs, $p = 0.03$.

Mean cumulative hourly intake per strain over the first 12 h of day 1 is shown in Fig. 5. These data suggest greater initial intake by two low-preference strains, i.e., WKYs and F344s, than by the high-preference MR strain. One-way ANOVAs

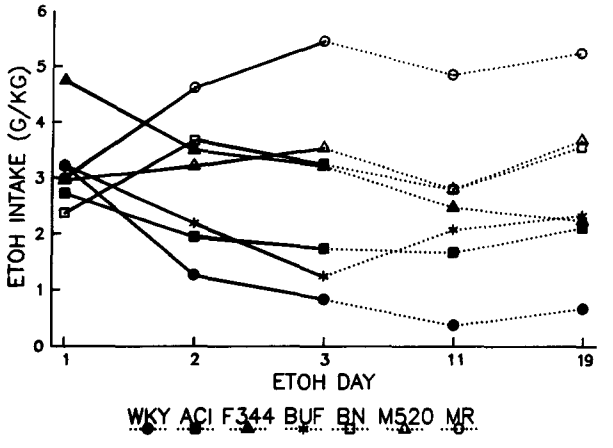

FIG. 4. Mean EtOH consumption (g/kg/day) by strain of a 10% (w/v) EtOH-cola solution on days 1, 2, 3, 11, and 19 of Experiment 5.

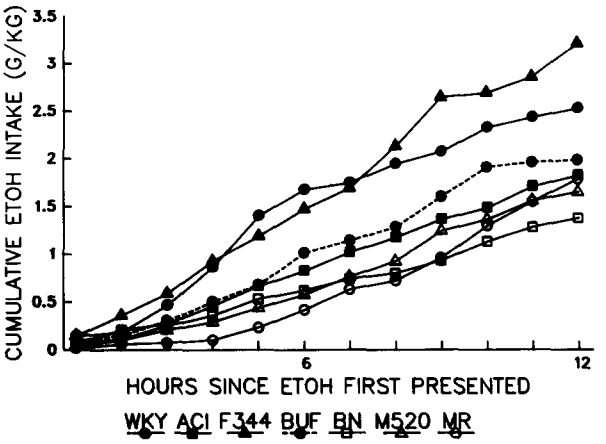

FIG. 5. Mean cumulative EtOH consumption (g/kg) by strain of a 10% (w/v) EtOH-cola solution during the first 12 h of Experiment 5.

indicate significant group differences in cumulative intake over hours 3-10, $Fs(6, 63) > 2.42$, $ps < 0.05$. Tukey multiple comparison tests indicate the cumulative total intakes of the F344s were greater than those of the MRs from the second through the fifth hours of EtOH exposure, $ps < 0.04$, and the cumulative intakes of the WKYs were greater than those of the MRs from the fourth through the sixth hours, $ps < 0.02$.

Discussion

These results strongly support the hypothesis that initial pattern of EtOH self-administration affects taste aversion learning. Two low-preference strains (i.e., WKY and F344) drank EtOH on day 1 in a manner that would be expected to result in more taste aversion learning than that produced by the self-administration pattern of the high-preference MR strain. Although there was not a strain difference in total EtOH intake on day 1, the WKYs and F344s consumed a greater proportion of their day 1 intake during the first few hours of EtOH exposure than did the MRs. Evidence that this pattern of self-administration did condition taste aversions is found in the fact that EtOH intake decreased in these two strains over days 1-3.

The MRs, on the other hand, began with low levels of EtOH consumption, which then increased over days 1-3. These data do not explain why the intake of MRs increased, i.e., what was reinforcing about EtOH, but they do suggest that one reason no aversion developed in this strain may be that the pattern of initial EtOH self-administration minimized taste aversion learning (1).

Our previous research found that WKYs also accept other novel flavors more rapidly than do M520s (7), which suggests that the difference in pattern of initial EtOH self-administration found in this study may be a function of a phenotype not specific to EtOH.

GENERAL DISCUSSION

Differences in taste aversion learning resulting from differences in the initial pattern of EtOH self-administration of high EtOH doses appear to be an important determinant of EtOH preference for some rat strains. The two strains (WKY and F344) that initially ingested relatively large amounts of EtOH

decreased their consumption markedly over the first 72 h of self-administration, suggesting the taste of EtOH was not aversive to them at first but became so with experience. Further, they were the only two strains to acquire significant aversions to the taste of EtOH, relative to same-strain controls, following EtOH self-administration. By contrast, the high-preference MRs initially drank little EtOH. Initial ingestion of a high EtOH dose would enhance taste aversion learning by maximizing the associability of both the taste and the pharmacological effects of EtOH at the time those pharmacological effects would be expected to be most aversive (i.e., before tolerance develops) (1).

Differential taste aversion learning resulting from differences in initial EtOH consumption patterns may not be an important determinant of subsequent EtOH self-administration in other strains, though. The low-preference ACI strain, for example, did not acquire an EtOH-cola aversion during self-administration of the solution in Experiment 2, nor was their initial consumption very great in Experiment 5. It is possible that the taste of EtOH intrinsically is less palatable for ACIs than for other strains. The high-preference M520s, on the other hand, may fail to acquire EtOH taste aversions during self-administration because the taste of EtOH is a less salient CS for them than for other strains. Further research is obviously needed to confirm these possibilities, but it is clear that initial self-administration patterns and consequent taste aversion learning differences do not account for all the variance in EtOH self-administration across inbred rat strains.

There is minimal support in our results for the hypothesis that strain differences in taste aversion learning are a function of differences in the aversiveness of EtOH across strains. The slope of the EtOH dose-response aversion learning curve was correlated with a measure of the degree of EtOH aversion acquired during self-administration, but other predictions derived from this hypothesis were not confirmed. Nonetheless, the hypothesis has intuitive appeal and is consistent with the reported relation between EtOH preference and EtOH tolerance (17). Therefore, further analyses of the relation between EtOH tolerance and the aversiveness of EtOH as an US in taste aversion learning are encouraged.

No evidence was obtained that suggests that strains differ in their general ability to form taste-toxicosis associations.

The finding that there are strain differences in the pattern of initial EtOH self-administration may have implications for the use of these strains to develop general models of the determinants of EtOH preference. If, as suggested by our previous research (7), it were established that rat strains with low EtOH preference generally drink novel solutions, including but not limited to EtOH solutions, more readily than do high-preference strains, then one either would have to demonstrate that similar effects obtain in other species to which one wants to generalize (e.g., humans) or would have to conclude that the rat model fails in this regard.

The procedural significance of these results for future studies of EtOH preference is that the pattern of initial EtOH self-administration needs to be assessed if not directly controlled. These findings do not negate the associations between EtOH preference and EtOH metabolism and tolerance reported previously (17), but they do suggest that EtOH self-administration patterns also need to be considered in studies of EtOH preference.

ACKNOWLEDGEMENTS

This research was supported by a National Institute on Alcohol Abuse and Alcoholism grant and by the Medical Research Service of the Department of Veterans Affairs.

REFERENCES

1. Baker, T. B.; Cannon, D. S. Alcohol and taste-mediated learning. Addict. Behav. 7:211–230, 1982.
2. Barker, L. M.; Best, M. R.; Domjan, M. (eds.). Learning mechanisms in food selection. Waco, Texas: Baylor University Press; 1977.
3. Berman, R. F.; Cannon, D. S. The effect of prior ethanol experience on ethanol-induced saccharin aversions. Physiol. Behav. 12: 1041–1044; 1974.
4. Braveman, N. S.; Bronstein, P. (eds.). Experimental assessments and clinical applications of conditioned food aversions. New York: The New York Academy of Sciences; 1985.
5. Cannon, D. S.; Berman, R. F.; Baker, T. B.; Atkinson, C. A. Effects of preconditioning unconditioned stimulus experience on learned taste aversions. J. Exp. Psychol. Anim. Behav. 1:270–284; 1975.
6. Cannon, D. S.; Carrell, L. E. Effect of taste aversion learning on ethanol self-administration. Pharmacol. Biochem. Behav. 28:53–56; 1987.
7. Cannon, D. S.; Carrell, L. E. Rat strain differences in ethanol self-administration and taste aversion learning. Pharmacol. Biochem. Behav. 28:57–63; 1987.
8. Cannon, D. S.; Leeka, L. K. Effect of body weight on ethanol-induced taste aversion learning. Pharmacol. Biochem. Behav. 37: 379–381; 1990.
9. Carey, N. K. A decrease in ethanol preference in rats resulting from forced ethanol drinking under fluid deprivation. Physiol. Behav. 8:373–375; 1971.
10. Cohen, J. Statistical power analysis for the behavioral sciences (second edition). Hillsdale: Erlbaum; 1988.
11. Garcia, J.; Hankins, W. G.; Rusiniak, K. W. Behavioral regulation of the milieu interne in man and rat. Science 185:824–831; 1974.
12. Garcia, J.; Koelling, R. A. Relation of cue to consequence in avoidance learning. Psychon. Sci. 4:123–124; 1966.
13. Lester, D.; Nachman, M.; LeMagnen, J. Aversive conditioning by ethanol in the rat. Q. J. Stud. Alcohol 31:578–584; 1970.
14. Li, T.-K.; Lumeng, L. Alcohol preference and voluntary alcohol intakes of inbred rat strains and the National Institutes of Health heterogeneous stock of rats. Alcohol.: Clin. Exp. Res. 8:485–486; 1984.
15. Nachman, M.; Larue, C.; LeMagnen, J. The role of olfactory and orosensory factors in the alcohol preference of inbred strains of mice. Physiol. Behav. 6:53–95; 1971.
16. Petersen, D. R. Pharmacogenetic approaches to the neuropharmacology of ethanol. In: Galanter, M., ed. Recent developments in alcoholism, vol. 1. New York: Plenum Press; 1985: 49–69.
17. Spuhler, K.; Deitrich, R. A. Correlative analysis of ethanol-related phenotypes in rat inbred strains. Alcohol.: Clin. Exp. Res. 8:480–484; 1984.