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Long-Lasting Reduction in Ethanol Selection After Involuntary Intake of Ethanol/Chlordiazepoxide

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CHAN, A. W. K., D. L. SCHANLEY AND F. W. LEONG. *Long-lasting reduction in ethanol selection after involuntary intake of ethanol/chlordiazepoxide.* PHARMACOL BIOCHEM BEHAV **19**(2) 275–280, 1983.—C57BL/6J mice, after having been exposed to a free-choice condition between water and aqueous chlordiazepoxide (CDP, 25 mg/100 ml) or between water and ethanol/CDP, showed a significant trend for decreased preference for ethanol when tested 2 weeks later. Similarly, mice previously exposed to a no-choice intake of ethanol showed a significant decrease in ethanol preference when tested subsequently. A long-lasting (>20 weeks) reduction in ethanol selection developed after mice were previously exposed to ethanol/CDP in a no-choice condition. This was also accompanied by a decrease in the subsequent selection of ethanol/CDP, but not CDP. The exact mechanisms for the long-lasting decrease in ethanol selection was unknown, but it was not due to the development of fluid aversion. It is suggested that the combined central effects of ethanol/CDP might be partially responsible.

Chlordiazepoxide Ethanol Alcohol selection

BENZODIAZEPINES facilitate feeding behavior [4, 8, 10, 21] and influence drinking responses [12, 13, 15, 19] in different mammalian species. Such properties reflect the hyperphagic, hyperdipsic and antineophobic actions of these drugs [5, 7, 16, 22, 23]. We have recently reported [3] that the effects of chlordiazepoxide (CDP) on alcohol consumption in non-deprived mice vary with experimental designs. In a free-choice situation, the incorporation of CDP in ethanol solutions (2 to 20%, v/v) caused a significant decrease in the selection of ethanol. We hypothesized that the inhibition in alcohol consumption was due to the combined CNS effects of both drugs [2,3]. On the other hand, in a no-choice situation with intermittent (3 days for each 6-day cycle) incorporation of CDP in ethanol solutions, there was an increase in ethanol intake only on the first day of each cycle that CDP was present. This phenomenon was attributed primarily to a novelty effect which seemed to be present only in a no-choice situation [3].

This paper examines how the prior exposure to ethanol/CDP solutions affects the subsequent selection of alcohol in non-deprived mice.

METHOD

Animals

Male C57BL/6J mice (9 weeks old; Jackson Laboratories, Bar Harbor, ME) were acclimated for a week before use in a controlled-environment room (22°), with automatic light/dark (12/12 hr) cycle. They were housed singly in plastic cages throughout each experiment.

Procedure

Experiment 1. The first phase consisted of three groups of mice (N=33 to 48 each). They all received Teklad mouse diet (Teklad Mills, Winfield, IA) ad lib together with the following drinking conditions: Group 1 received a choice between water and an ethanol solution. The concentration of ethanol at the beginning of the experiment was 2% (v/v; from 95% ethanol) and this was increased (successively to 5, 8, 12.5, 15 and 20%) every 3 days until it reached 20%. Thus the first phase of the experiment lasted 18 days. Group 2 was given a choice between water and an aqueous CDP solution (25 mg/100 ml). The concentration of CDP did not change for the entire 18-day period. Group 3 had a choice between water and an ethanol solution containing CDP (25 mg/100 ml). The concentration of ethanol was gradually increased the same way as in group 1. Daily intake of each solution was recorded for each mouse. The positions of the drinking tubes were interchanged each day. Tubes containing CDP solutions were wrapped with aluminum foil as previously described [3]. At the end of the first phase, all groups received tap water and Teklad mouse diet ad lib for 2 weeks.

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In the second phase, each of the above 3 groups was divided into 3 subgroups (N=11 to 16 each). All of the mice received mouse diet ad lib and each subgroup was treated, respectively, with the following drinking conditions: (1) choice between water and an ethanol solution, with changes in ethanol concentrations identical with those for group 1 in phase 1; (2) choice between water and an aqueous CDP solution (25 mg/100 ml); (3) choice between water and an ethanol solution containing CDP (25 mg/100 ml) with changes in ethanol concentrations the same as those in (1).

Preference index for solutions other than water is defined as the ratio: Volume of solution consumed/total volume of fluid (water plus the other solution) consumed, and is expressed as a percentage.

Experiment 2. The number of mice involved was similar to that for the preceding experiment. The biphasic design was basically the same except that in phase 1, mice in groups 1–3 were given only the respective test solutions (namely, ethanol, aqueous CDP or ethanol/CDP); water was not offered as a second choice. The duration of the first phase was shortened to 12 days, up to 12.5% ethanol for groups 1 and 3. This was necessary because the mice tended to have lower ethanol intake at higher ethanol concentrations in the no-choice situation. Procedures for the second phase were the same as those described in Experiment 1.

Experiment 3. In phase 1, one group of mice (N=12) received mouse diet ad lib and an ethanol solution containing CDP (25 mg/100 ml) as the sole source of fluid. The time course for increasing ethanol concentrations was the same as in Experiment 2 (group 3). In phase 2, the mice were treated for ethanol preference as described above, and the same test was repeated at 8 and 13 weeks after the end of phase 1. The purpose was to ascertain whether the aversion to ethanol, developed after completion of phase 1, dissipated with time.

Statistics

A multivariate analysis of variance (MANOVA; version 9.1 of SPSS program) was used for statistical evaluations of the data. Levels of significance ($p<0.05$ considered significant) were tested for the main factor which was the comparison of the preference indices (P.I.) from previously-treated mice with those from naive mice over the 18-day (or 14-day when there were missing data) test period. In essence, the shapes of the curves in the sub-groups were compared. Depending on the experimental designs, some results were analyzed as a within-subject design while others were analyzed as a between-subject design. Thus data for A1, B2 and C3 of Fig. 1 and data shown in Fig. 3 were examples of the within-subject design since in each case, the sets of data were compiled from the same mice. The rest of the data were analyzed as between-subject comparisons.

RESULTS

Experiment 1. Results obtained in the first phase were used as data for "previously untreated" animals concerning choice conditions, 1, 2 or 3 as depicted in Fig. 1. These data were not significantly different from those (not shown) obtained from mice which received mouse pellets and water ad lib for the first phase and offered choice conditions 1, 2 or 3. The rest of Fig. 1 depicts results from the second phase. Part A (1 and 3) of Fig. 1 illustrates that mice with prior exposure to a choice of water and ethanol did not differ significantly from naive mice in their subsequent preference for ethanol, $F(1,10)=0.74$, $p>0.42$, or ethanol/CDP, $F(1,31)=0.12$, $p>0.7$; however, they differed significantly, $F(1,42)=9.77$, $p<0.005$, from naive mice in their subsequent preference for aqueous CDP, the tendency being for a slightly higher preference. Mice previously treated with a choice of water and aqueous CDP had a significantly lower preference index profile, $F(1,25)=4.95$, $p<0.05$, compared to previously-untreated animals (B1). These mice also showed a trend for significantly lower preference for aqueous CDP (B2), $F(1,10)=7.04$, $p<0.05$, and ethanol/CDP (B3), $F(1,31)=9.45$, $p<0.005$. Mice which previously had a choice of water and ethanol/CDP showed a significantly lower preference for ethanol (C1), data for 20% not used; $F(1,31)=14.5$, $p<0.005$, a slightly higher preference for aqueous CDP (C2), $F(1,42)=8.55$, $p<0.01$, but no difference in preference for ethanol/CDP, $F(1,10)=0.94$, $p>0.3$.

There was no difference in the volume of total fluid consumed by each group in both phases of the experiment. The daily volume ranged from 4.8–7.3 ml.

Experiment 2. In Fig. 2, data for "previously untreated" animals were those from the first experiment. These were compared with results obtained from the second phase of this experiment. As depicted in Fig. 2, mice which were previously exposed to ethanol only showed significant changes in the preference index profile for ethanol (D1), $F(1,24)=5.08$, $p<0.005$, CDP (D2), $F(1,38)=5.76$, $p<0.05$, and ethanol/CDP (D3), $F(1,27)=5.39$, $p<0.05$. In contrast, the prior treatment of mice with CDP only did not affect their subsequent preference for ethanol (E1), $F(1,31)=0.35$, $p>0.5$, CDP (E2), $F(1,42)=2.96$, $p>0.09$, and ethanol/CDP (E3), $F(1,30)=0.01$, $p>0.9$. However, there was a dramatic decrease in preference for ethanol, $F(1,27)=70.38$, $p<0.001$, in mice which were previously exposed to ethanol/CDP (F1). There was a significant decrease in preference for ethanol/CDP (F3), $F(1,31)=18.74$, $p<0.001$, for animals which had a similar prior treatment, but there was no significant change in preference for aqueous CDP (F2), $F(1,42)=2.34$, $p>0.1$. The daily mean volume (range 4.8–7.5 ml) of total fluid intake for each group in phase 2 of this experiment was comparable to those observed for mice in Experiment 1.

Experiment 3. Figure 3 illustrates the long-lasting nature of the reduction in ethanol preference developed in mice after an initial treatment with ethanol/CDP under a no-choice condition. The results (for 2 weeks) are qualitatively similar to those presented in Fig. 2 (F1), although slightly higher preference indices were observed for the present experiment. There was no significant recovery, $F(2,10)=0.91$, $p>0.4$, of the inhibition in ethanol selection (Fig. 3) even after 13 weeks from the end of the initial exposure to ethanol/CDP.

DISCUSSION

The C57BL/6J mice are well known for their high preference for a 10% ethanol solution [14,17]. Our results indicate that prior exposure of these mice to the free choice condition of water and ethanol did not affect the subsequent (2 weeks later) selection of ethanol or ethanol/CDP, but there was a slight but significant increase in the selection of aqueous CDP (Fig. 1). We have previously shown that the preference for ethanol/CDP was significantly less than that for ethanol alone [3]. The present investigation indicates that mice previously exposed to the free choice condition of water and ethanol/CDP showed a significantly lower preference index profile for ethanol, a slight increase in preference for aqueous CDP, but no change in preference for ethanol/CDP (Fig. 1, C1–3). The prior exposure to the free choice condition

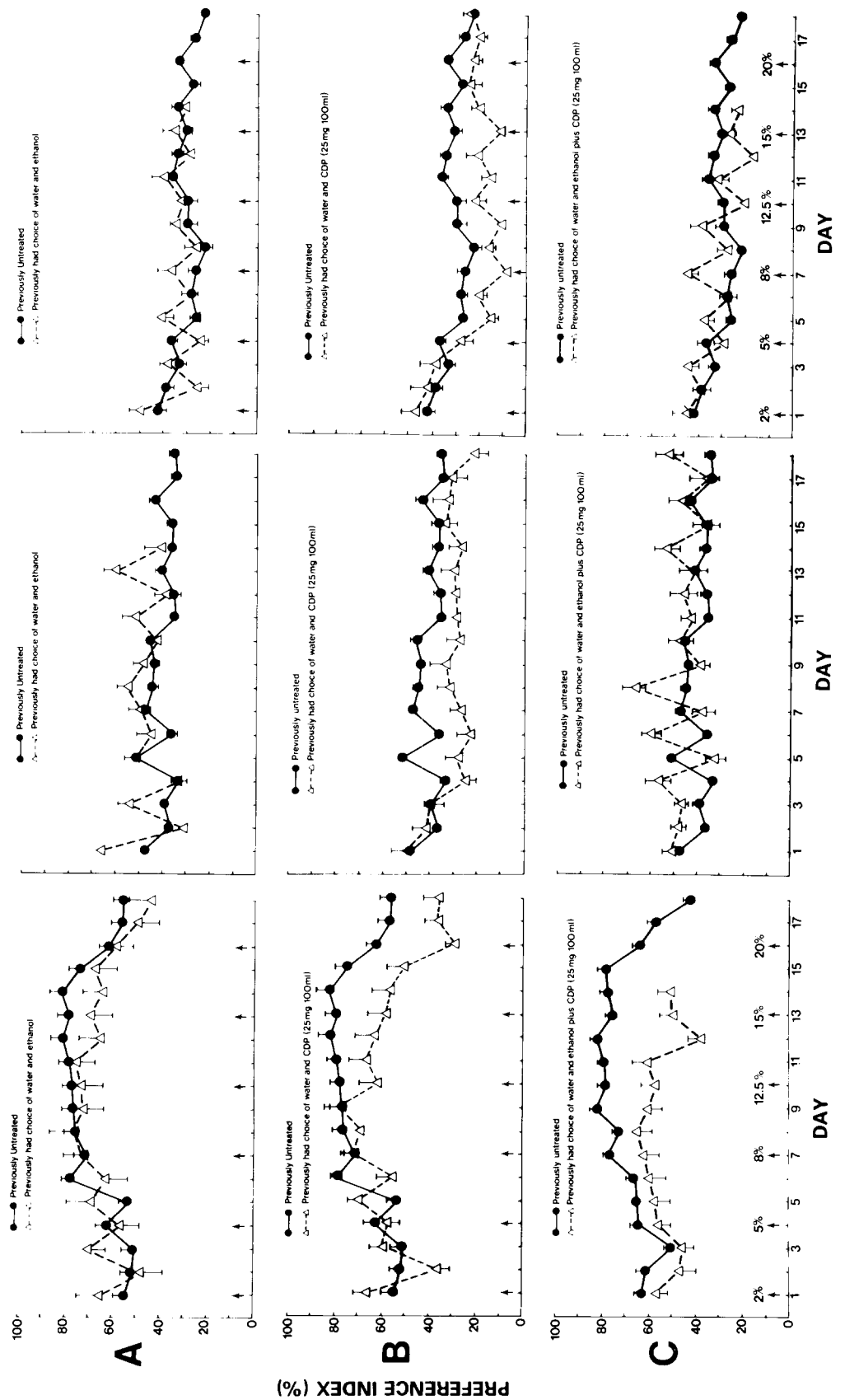

FIG. 1. Preference indices for solutions (other than water) under free-choice conditions 1, 2 and 3 for naive mice and mice which had previously undergone the following conditions (horizontal rows): A, choice of water and ethanol; B, choice of water and aqueous CDP (25 mg/100 ml); C, choice of water and ethanol containing CDP (25 mg/100 ml). Preference index is the ratio: volume of solution (other than water) consumed/total volume of fluid intake (solution plus water), and is expressed as a percentage. Vertical arrows denote the days on which ethanol concentrations were changed. Each point represents the mean of 11–16 observations, with S.E. (+ or −) denoted as shown.

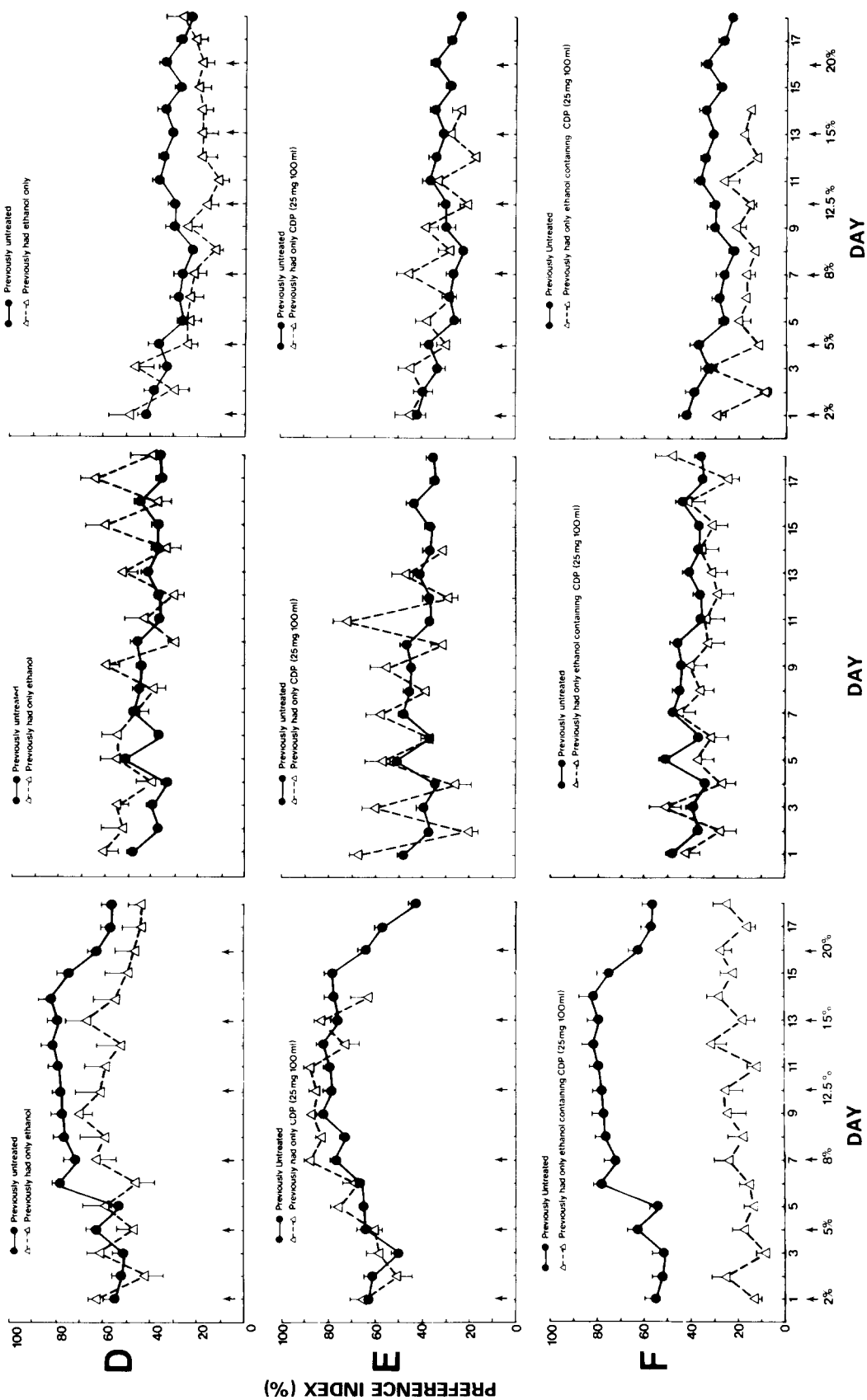

FIG. 2. Preference indices for solutions (other than water) under free-choice conditions 1, 2 and 3 for naive mice and mice which had previously undergone the following no-choice conditions (horizontal rows): D, ethanol solutions of different concentrations; E, aqueous solution of CDP (25 mg/100 ml); F, ethanol solutions containing CDP (25 mg/100 ml). Vertical arrows have the same denotations as those in Fig. 1. Each point represents the mean of 11–16 observations, with S.E. (+ or −) denoted as shown.

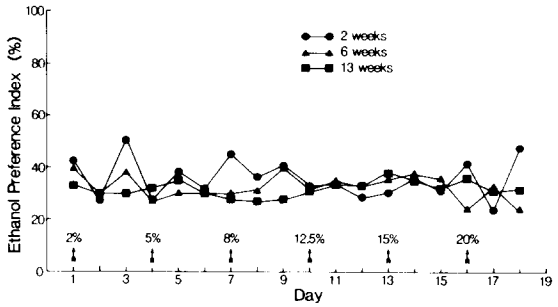

FIG. 3. Ethanol preference index for mice which had been treated previously with ethanol/CDP under a no-choice condition, according to the protocol for Experiment 2 (group 3). The mice were subsequently tested for ethanol preference at 2, 6 and 13 weeks (as shown) after the end of the first part of the experiment. They received water and food pellets ad lib in between the testing periods. Vertical arrows have the same denotations as those in Fig. 1. Each point represents the mean of 12 observations.

between water and aqueous CDP also induced a subsequent decreased preference for ethanol, aqueous CDP and ethanol/CDP (B1–3). However, mice previously subjected to the no-choice intake of aqueous CDP did not subsequently show any change in ethanol preference. The decrease in ethanol preference in B1 and C1 could not have been due to any carry-over effect of CDP or its metabolites, since the mice were retested two weeks after their initial exposure to CDP or ethanol/CDP. CDP is metabolized very rapidly in mice [2]. We do not know why an analogous decrease in preference for ethanol/CDP was not observed in C3. This might be related to the fact that the preference for ethanol/CDP was already low even for naive mice.

The major finding of this investigation is the long-lasting reduction in selection of ethanol solution in mice previously exposed to a no-choice intake of ethanol/CDP. This is a reproducible phenomenon. In fact in a repeat experiment, the same reduction persisted more than 20 weeks (data not shown). The pretreatment did not alter the subsequent selection of aqueous CDP, but it significantly decreased the subsequent selection of ethanol/CDP. The decrease in ethanol preference could not have been due to a general fluid aversion because the daily total fluid volume (water and ethanol solution) consumed by these animals was not significantly different from that ingested by naive mice. Since there was either no significant change or a slight reduction in the selection of ethanol or ethanol/CDP resulted from the initial no-choice experience of either CDP alone (Fig. 2; E1 and E3) or ethanol by itself (Fig. 2; D1 and D3), respectively, it must have been the initial combined effects of CDP/ethanol which led to the subsequent dramatic reduction in the selection of ethanol or ethanol/CDP. Our earlier work [2] has shown that mice were rendered more sensitive to the CNS effects of ethanol after they had been given an acute dose of CDP/ethanol. We can only speculate that the development of the long-lasting decrease in ethanol preference might originate from such an effect, although the mechanisms involved are unknown. Rodgers and McClearn reported [8] that forced alcohol intake in C57BL mice tended to increase alcohol preference. On the contrary, we observed a significant trend for a lower preference for ethanol (D1).

The consumption of alcohol together with the benzodiazepines in humans is not uncommon [9]. These two drugs have also been reported to be involved together in overdose cases [11], although it is not known whether alcohol consumption is curtailed in patients after such an experience. The existence of benzodiazepine dependence in alcoholics [1,20] also suggests that in general no aversion to ethanol develops after alcohol-benzodiazepine intake. Therefore, the present finding may not have its counterpart in the human situation. It remains to be determined whether abstinence rate is higher or lower in alcoholics treated with the benzodiazepines compared to those who receive other drug treatment.

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