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Behavioral Factors in Development of Tolerance to Ethanol's Effects[1]

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HOLLOWAY, F. A., D. C. BIRD, J. A. HOLLOWAY AND R. C. MICHAELIS. *Behavioral factors in development of tolerance to ethanol's effects.* PHARMACOL BIOCHEM BEHAV **29**(1) 105–113, 1988.—Dose-effect analyses were used to monitor the development of tolerance for ethanol's effects on FR30 operant performance in rats under different conditions of chronic ethanol exposure: (a) pre-session ethanol injections (PRE) vs. post-session ethanol injections (POST) in Experiment 1; and (b) an ethanol liquid diet (ED) vs. a control diet (CD) in Experiment 2. The PRE and ED groups developed tolerance at the conclusion of the chronic regimens, which declined by six months but not to baseline levels. These data suggest that tolerance results from learned compensatory adjustments (through intoxicated practice) to ethanol's disruptive effects. The POST, but not the CD, group developed a progressively increasing degree of tolerance after several ethanol challenge tests. These results suggest that some threshold level of passive ethanol exposure in the POST group interacted with their limited intoxicated practice. Finally, the tolerance developed under intoxicated practice conditions did not appear to reflect a generalized tolerance to rate-reducing properties of drugs, changes in ethanol kinetics, or age-related changes.

Ethanol Behavioral tolerance Physiological tolerance Intoxicated practice Rats
Stimulus control Liquid diet Operant performance Environment-dependent tolerance

TOLERANCE has been suspected of playing a role in the development of alcoholism because it allows one to consume increasing quantities of ethanol with fewer of the drug's debilitating effects on behavior. It has been suggested that conclusive evidence of tolerance development in experimental studies should include the demonstration of a shift-to-the-right in the dose-effect curve. That is, as a result of repeated ethanol exposure: (a) the effect of a given dose should be reduced; and (b) an increased dose should be required to reinstate the initial degree of impairment [4, 8, 23]. The latter requirement is important because conditions can exist (e.g., chronic toxic effects) under which the initial degree of drug impairment may not be reinstateable.

While tolerance to a variety of the behaviorally disruptive effects of ethanol has been reported [2, 6, 7, 11, 15, 16, 24–27, 32, 34, 35, 41–43], virtually all of the thoroughly documented tolerance effects are relatively short-lived, lasting several days to several weeks. In addition, with very few exceptions [25, 27, 34, 43], ethanol tolerance studies have examined only a single post-tolerance ethanol test dose. Studies using only one test dose can only satisfy the first criterion for tolerance mentioned above. Further, significant shifts in baseline response rates can change the shape of drug dose-effect curves [13]. Therefore, it is important to use complete dose-effect analyses, with contemporary saline baseline determinations, in order to fully characterize the changes which may result from chronic ethanol exposure. However, it should also be noted that the very act of measuring tolerance development and loss may affect the process being measured.

There is disagreement in the literature regarding the degree to which tolerance depends on physiological (i.e., changes in disposition, metabolism, or cellular sensitivity) versus behavioral (i.e., depending on intoxicated performance of the behavior in question) phenomena. Prior attempts to differentiate between "physiological" and "behavioral" tolerance have used an experimental design in which the behavioral group received intoxicated practice (pre-session injections) for several consecutive sessions while the physiological group received identical amounts of ethanol after the training session (post-session injections). The test session involved a pre-session ethanol injection for both groups. Such studies typically demonstrated tolerance development after a single ethanol exposure/test cycle in the behavioral group, but not the physiological group [6, 7, 24]. However, if this exposure/test cycle is repeated, the physiological group eventually developed the same degree of tolerance as seen in the behavioral group. This finding led to the suggestion that

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rapid tolerance development under conditions of intoxicated practice resulted from "behaviorally augmented" tolerance [24,26]. Some have suggested that the "physiological" tolerance induced by ethanol exposure *per se* is produced through the same mechanism as that responsible for "behaviorally augmented" tolerance [24]. An alternative explanation is that the post-session injections eventually result in tolerance purely as a consequence of the intoxicated practice received during the repeated ethanol test sessions [41,42].

At least two kinds of functional or behavioral tolerance have recently been described. One theoretical account invokes a Pavlovian conditioning model (see [18, 20, 39]), in which tolerance is attributed to the development of a conditioned compensatory response. Such conditioning results from the pairing of the environmental cues associated with the drug's administration with some "secondary" effect of the drug, which is antagonistic to the drug's "primary" unconditioned effects. This environmentally-dependent tolerance [29,30] would be expected to appear more strongly when the route of drug administration is the same in both the chronic exposure phase and the ethanol tests (e.g., tolerance produced by repeated injections) as opposed to when the route of administration differs between the chronic exposure phase and the ethanol tests (e.g., tolerance produced by liquid diet or inhalation).

An alternative view of tolerance is exemplified by several researchers [10, 14, 37, 42] who suggest that the effective condition for the development of tolerance to a drug's effects on behavior depends on an interaction between the drug dose and the functional demands placed on the organism. This view also stresses the role of learning in tolerance development but emphasizes the acquisition of adaptive or compensatory responses through instrumental conditioning rather than through Pavlovian conditioning. In such models, little tolerance would be expected to develop in situations where the drug is administered after the behavioral task since the animal receives the drug while in its home cage where there is a low level of functional demand. Further, even when behavioral demands are placed on the animal following the post-session injections, the tolerance which develops appears to be specific to the behaviors for which there is intoxicated practice [13,28]. The role of such instrumental learning factors in the development of tolerance to amphetamine in operant tasks has been convincingly demonstrated [10, 13, 37]. However, comparable studies systematically investigating the role of instrumental learning factors in the development of ethanol tolerance are not yet available. The latter deficiency in the ethanol tolerance field is largely related to the inconsistent use of dose-effect analysis to detect changes in ethanol sensitivity.

In a recent study, significant shifts in dose-effect curves were found for ethanol's rate-decreasing effects on food-motivated schedule-controlled behavior in rats [3]. This tolerance effect persisted for up to six months, well past the usual report for metabolic or physiological tolerance. These data are consistent with a behavioral or "intoxicated practice" basis for such tolerance. However, in that study, the possibility that the persistence of the tolerance resulted from testing once or twice a month was not ruled out. The intent of Experiment 1 was to extend the latter study by applying the pre-/post-injection model (described earlier) to schedule-controlled performance in rats. Experiment 2 was designed to assess development and maintenance of ethanol tolerance when the animals were chronically exposed to an ethanol liquid diet or an isocaloric-control diet. Otherwise, the design features of Experiments 1 and 2 were virtually the same. The second experiment permitted assessment of whether any tolerance development is specific to the environmental context factors related to the chronic ethanol administration procedure. The liquid diet control group also provides a basis for assessing any tolerance development resulting from repeating dose-effect curve determinations *per se*. Each rat in both experiments was tested on a full range of ethanol doses prior to chronic ethanol exposure, after a period of chronic ethanol exposure, and six months after withdrawal from ethanol. Further, the course of ethanol sensitivity changes after termination of the chronic exposure period was assessed periodically with single dose ethanol challenges.

METHOD

Subjects

Eight adult male Sprague-Dawley rats weighing 300–350 g were used in each of the two experiments. Rats were obtained locally from the OUHSC Department of Comparative Medicine breeding colony. They were 90–110 days old at the beginning of each experiment. During each experiment, all rats were individually housed in standard wire-mesh, suspended cages with continuous access to water. The rats were food-deprived and maintained at 80% of their free-feeding weights. All supplementary feeding occurred at least 2–4 hours after the daily operant sessions. The home cages were located in a temperature and humidity controlled room with lighting on a 12 hour light/dark cycle. The behavioral sessions for each animal were run at the same time each day 2–6 hours after light onset.

Apparatus

Four rodent operant chambers (Model No. RTC-022, Lehigh Valley Electronics, Lehigh Valley, PA) were used in this study. Each chamber was housed within a sound and light attenuating chamber which included an exhaust fan and house light. The chambers were equipped with two response levers located 17.0 cm apart and 3.0 cm above the grid-rod floor. Approximately 18 g was required to deflect the lever sufficiently to result in a response. Food pellets (45 mg each, P. J. Noyes, Lancaster, NH), served as the reinforcer and were delivered into a food cup located equidistant between the response levers. The house lights functioned as the discriminative stimulus for food availability. All schedule programming and data collection was accomplished by solid-state and electromechanical devices located in an adjacent room.

Procedure

In each experiment, two groups of four animals were shaped by successive approximation to press the right lever for food pellets during 30-min sessions. Presses on the left lever (which had no programmed consequences) were also recorded but did not appear to vary in a dose-dependent manner. The fixed-ratio (FR) schedule of reinforcement was gradually increased over sessions until a terminal schedule of FR30 was attained. All subsequent training and test sessions lasted 30 min. As soon as responding was judged stable (less than ±10% variation on seven consecutive days), a 4-point dose-effect curve (DEC) for ethanol was obtained for each animal (0.375, 0.75, 1.125, and 1.5 g/kg). Each DEC also included a saline injection (baseline) session. All ethanol

doses were prepared as a 10% w/v solution in physiological saline and were administered intraperitoneally (IP). A minimum of three control sessions, again with less than ±10% between-session variation, were required prior to each test dose to allow time to recover baseline responding. The order of test doses at each DEC systematically varied across subjects within each group. Although this regimen does not exclude the possibility of short-term tolerance development, others have shown that rats given intoxicated practice every fourth day do not develop tolerance to ethanol's effects on treadmill performance [26]. Pilot data indicated that within 15 min following the IP injection of 1.5 g/kg ethanol, the blood level had reached its peak (190 mg/dl). Therefore, all ethanol test doses were administered 15 min prior to testing.

Experiment 1

The intent of this first experiment was to assess ethanol tolerance development and loss in rats receiving intoxicated practice on the operant task and in rats receiving equivalent amounts of ethanol but no intoxicated practice. Following completion of the first dose-effect curve, rats in Experiment 1 began to receive daily IP injections of ethanol. The behavioral or PRE group (N=4) received ethanol injections 15 min prior to the training sessions. The physiological or POST group (N=4) received ethanol 1.5 to 2.0 hours after the training session as a result of a report indicating progressive depression of response rates when the injections were administered immediately following the session [18]. During the period of chronic ethanol administration each member of the POST group was yoked, by weight, to a member of the PRE group. This procedure ensured identical ethanol exposure in the PRE and POST groups. An isocaloric IP injection control group was not employed in this study since the principal focus was on the PRE/POST conditions and since the isocaloric control issue was specifically addressed in Experiment 2. As the members of the PRE group demonstrated tolerance at a given dose, the daily maintenance dose was increased for both it and the yoked POST animal, until the maximally tolerable dose was reached. The maximally tolerable dose was defined as the dose that did not completely suppress responding but which reduced responding to less than 20% of the saline baseline performance on DEC-1 for 5 consecutive sessions. The escalating regimen of ethanol doses started at 1.125 g/kg, and in order, progressed to 1.5 g/kg, 2.25 g/kg, and 3.0 g/kg until the maximal dose was reached, at which time the second DEC (DEC-2) was obtained. The doses employed in this and the subsequent DECs were determined in part by the maximally tolerable dose achieved by each animal. If the test dose was less than the daily maintenance dose, the difference was administered following the test session. After completion of DEC-2, the daily ethanol injections were terminated. The mean number of sessions required for the PRE group to achieve maximal tolerance at the 1.125, 1.5, 2.25, and 3.0 g/kg doses, respectively, were 18.5, 13.75, 21.25, and 2.5 sessions. Two rats achieved maximal tolerance at the 2.25 g/kg dose and two at the 3.0 g/kg dose.

Operant responding continued on a daily basis between DEC-2 and DEC-3 determinations. The loss of tolerance was monitored with a 1.5 g/kg challenge dose administered once every second week for the next 10 weeks, and then once every fourth week for an additional 12 weeks. This was followed (four weeks after the challenge dose) by the third and final DEC (DEC-3) determination. Thus, the loss of tolerance was monitored for a total of 26 weeks before the final DEC determination.

Experiment 2

The intent of the second experiment (run after the completion of Experiment 1) was to minimize the effects of classical conditioning associated with the IP ethanol injection procedure by chronically exposing a group of rats (N=4) to ethanol in a standard liquid diet (BioServ, Inc.) containing 40–50% ethanol as calories. This group is designated as ED. A control group of rats (N=4, designated as CD) received an iso-caloric (maltrose-dextrin added) amount of the liquid diet each day. Each CD rat was matched with one of the ED rats to determine the amount of control diet it received. In order to maintain relatively stable blood ethanol levels throughout each day, each ED rat received its daily ethanol diet ration in three evenly distributed portions. Following the completion of DEC-1, rats in the two groups were given control diets for two days. The ED group then began a three-week period on the ethanol diet regimen (Week 1: 40% ethanol-derived calories; Week 2: 45% ethanol-derived calories; and Week 3: 50% ethanol-derived calories. One day after the start of the ED group's chronic regimen, the CD rats began to receive their iso-caloric amounts of control diet (again for three weeks). An independent group of four rats, receiving a similar ethanol diet regimen as the ED group in this experiment, had tail-vein blood samples taken two days before the end of the chronic ethanol period. Standard gas chromatography blood ethanol assays were performed and indicated a mean blood ethanol level of 85.0 mg% (SE=5.3).

At the conclusion of the chronic ethanol period, both groups were placed on ad lib control liquid diet for two weeks. During the latter period, the DEC-2 was determined. All animals continued to receive operant training sessions 2–3 times per week for the next five and one-half months. At intervals of four weeks following termination of the chronic ethanol diet period, challenge tests of 1.5 g/kg ethanol were administered IP 15 min before the operant session. Approximately 26 weeks following termination of the ethanol diet phase, a third DEC was determined (DEC-3).

For Experiment 2 groups, several other procedures were employed at the conclusion of DEC-3 tests. First, in order to compare ethanol absorption and elimination rates, blood ethanol levels after 1.5 g/kg IP injections of ethanol were determined for several post-injection time points. Next, in order to determine whether the tolerance observed reflected a generalized tolerance to the rate-reducing properties of drugs, caffeine dose-effect curves were obtained for performance on the FR30 operant task (3.2–56 mg/kg; 15 min pre-session). Caffeine has rate-reducing properties but obviously is not of the same drug class as ethanol [5]. Finally, in order to determine whether the chronic ethanol exposure regimen had lasting effects on behavior, acquisition performance on a one-way, active avoidance task was assessed. The methods for this last procedure have been described elsewhere [19]. Briefly, this procedure consists of training the rats to avoid shock by climbing onto a platform within 10 sec of its being introduced into the avoidance chamber. Respectively, these latter procedures permitted an assessment of possible differences in the ED and CD groups for: (a) peak blood ethanol levels and elimination rates; (b) drug sensitivity using a dissimilar drug; and (c) long-term chronic effects of ethanol exposure (see [40]).

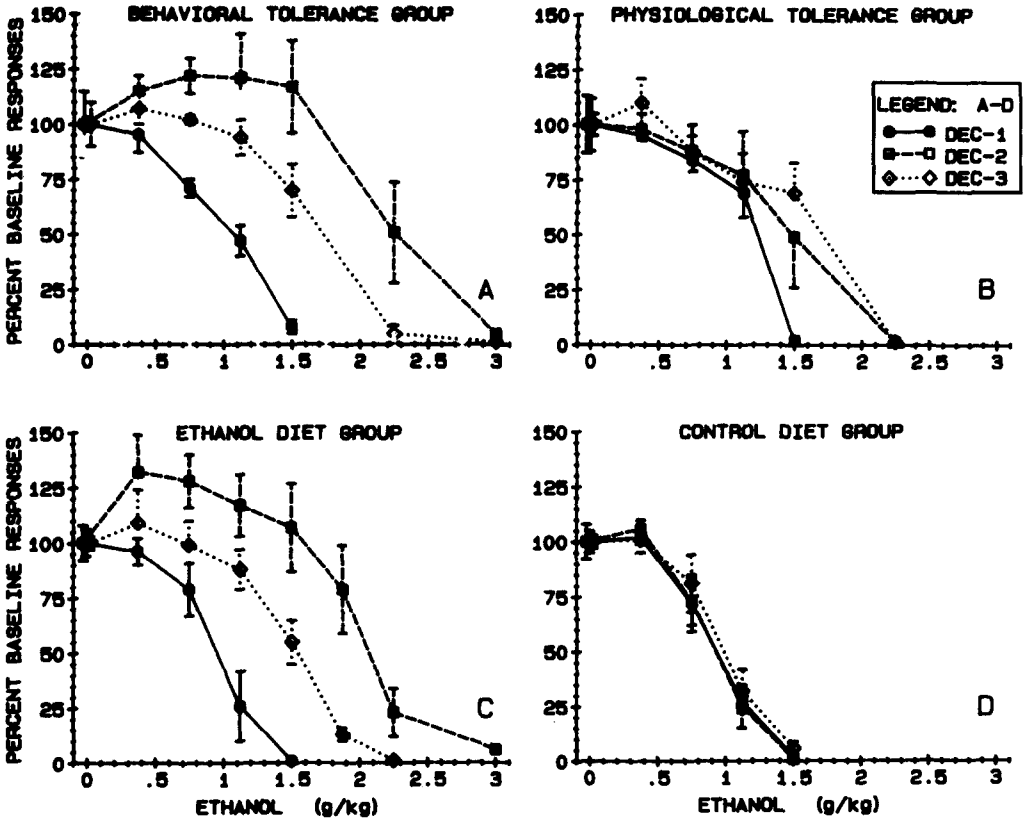

FIG. 1. Dose-Effect Curve (DEC) analysis of tolerance development. Means and standard errors for % contemporary baseline responses in the Behavioral (A=PRE) and Physiological (B=POST) Tolerance groups of Experiment 1 and the ethanol diet (C=ED) and control diet (D=CD) groups of Experiment 2. DEC-1 was determined before the chronic period, DEC-2, just after the chronic period, and DEC-3, six months after the chronic period. (N=4/group)

Data Analysis

The response rate data from individual animals were analyzed both as absolute numbers (responses/sec) and as the percent of saline baseline. The percent data were calculated by dividing the response rate at each dose by that of the saline session for that DEC. Since comparable results were obtained with both types of measure, only the percent of baseline data are presented in this report. Separate three-way analyses of variance (ANOVAs) were performed on the data from Experiments 1 and 2 to examine the between-subject main effects of group (PRE vs. POST or CD vs. ED) and the within-subject main effects of dose (0.375–1.50 g/kg) and DEC (1–3). Post-hoc comparisons were made with the Duncan's New Multiple Range Test. Since the doses higher than 1.5 g/kg were not included in DEC-1, separate two-way or three-way ANOVAs were employed to compare groups and DEC (2 and 3) at the higher dose(s). To compare the development and loss of tolerance among groups from both experiments, a three-way ANOVA was performed on the data from the 1.5 g/kg ethanal dose at each DEC and the monthly challenge doses, also 1.5 g/kg, following termination of the chronic ethanol regimen. This analysis examined the main effects of group (PRE, POST, ED and CD) and serial tests (1–8).

Finally, to quantify the extent of shift in ethanol sensitivity between DECs 1–3, the estimated ethanol dose which was effective in reducing response rates by 50% of saline baseline responding (ED50 scores) was calculated for each subject and for each DEC. This calculation was based on a linear regression of the log dose and the percent of saline baseline response rate (transformed to standard scores). A two-way ANOVA (groups by DEC) was used to examine main effects of groups (between) and DEC (within).

RESULTS

Experiment 1: % Baseline Responses

The data for % Baseline Responses for the PRE and POST groups at each DEC and dose level are represented in Fig. 1A and 1B. Analysis of these data indicated significant main effects for Dose, $F(3,18)=20.33$, $p<0.01$, DEC, $F(2,12)=17.44$, $p<0.01$, and the Group by DEC interaction, $F(2,12)=5.91$, $p<0.05$, plus a marginally significant Group by DEC by Dose interaction effect, $F(6,36)=2.29$, $p<0.10$. At DEC-2, the PRE group had significantly higher % baseline scores than the POST group at the 0.75 ($p<0.05$) and at the 1.125, 1.5, and 2.25 g/kg doses (p's<0.01).

The PRE group also displayed significant overall differences across DECs ($p<0.01$), these differences being significant at the 0.75, 1.125, and 1.5 g/kg doses (p's<0.01). At each of the latter doses, the PRE group displayed higher scores on DEC-2 and DEC-3 than on DEC-1. At the 1.5 and 2.25 g/kg doses, the PRE group displayed lower scores on

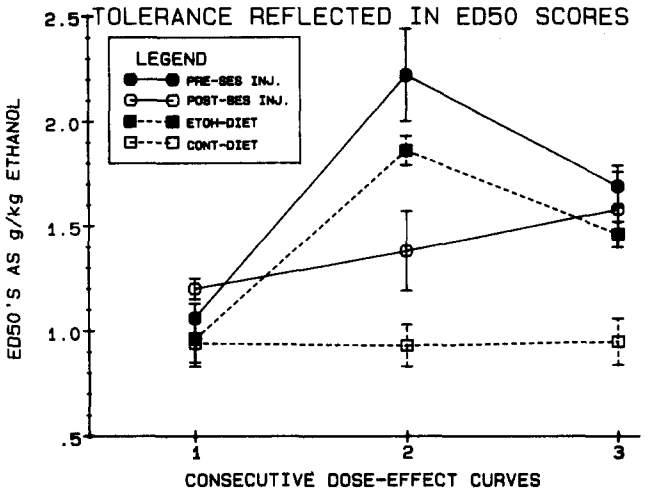

FIG. 2. Tolerance development as indexed by ED50 scores (the ethanol dose at which responding is reduced to 50% of contemporary baseline levels). Means and standard errors for groups from Experiments 1 and 2 at each DEC.

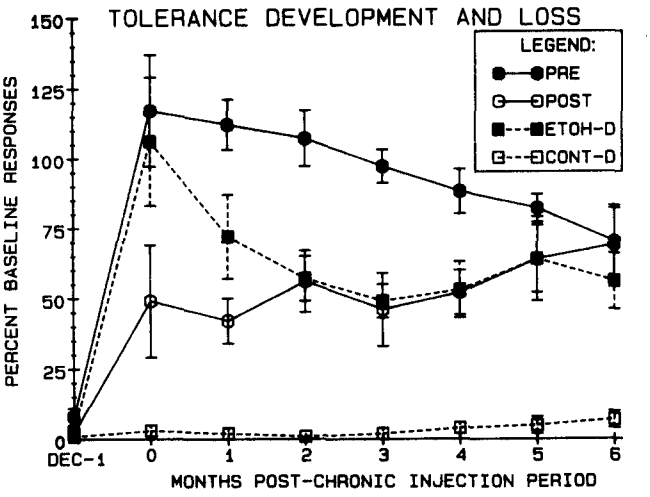

FIG. 3. Tolerance development and loss reflected by % baseline responses (means and standard errors) to 1.5 g/kg ethanol challenge tests for group from Experiments 1 and 2. The DEC-1 test is indicated on the figure and Tests 0 and 6 were from DEC-2 and DEC-3, respectively. Tests 1–6 were given one month apart beginning one month after the chronic period. (N=4/group)

DEC-2 than on DEC-3. The overall differences across DECs for the POST group approached significance ($p<0.10$), however significant DEC differences were evident at the 1.5 g/kg dose ($p<0.01$). At the latter dose, the POST group displayed higher scores on DEC-2 and DEC-3 than on DEC-1 (p's<0.01).

This pattern of results reflects several trends: (a) greater tolerance development in the PRE than in the POST groups at the end of the chronic injection period (DEC-2); (b) a persistent but declining level of tolerance in the PRE group six months after termination of the chronic injection regimen (DEC-3); and (c) a lesser level of tolerance development in the POST group at the end of the chronic injection period, which persisted, and possibly increased slightly (1.5 g/kg dose), during the six months after the chronic period.

Experiment 2: % Baseline Responses

The data for % Baseline Responses in the Control (CD) and Ethanol Liquid Diet (ED) groups is presented in Figs. 1C and 1D. Analysis of these data indicated significant main effects for Dose, $F(3,18)=38.33$, $p<0.01$, DEC, $F(2,12)=6.47$, $p<0.05$, Group, $F(1,6)=6.47$, $p<0.05$, Group by DEC interaction, $F(2,12)=6.07$, $p<0.05$, and the Group by DEC by Dose interaction, $F(6,36)=2.59$, $p<0.05$. At DEC-2, the ED group displayed higher % baseline scores than the CD group at the 0.75, 1.125, 1.5, and 1.875 g/kg doses (p's<0.01). At DEC-3 levels, the ED group scores were only higher than the CD group scores at the 1.125 and 1.5 g/kg doses (p's<0.01).

The ED group, but not the CD group, displayed significant differences among the three DECs ($p<0.01$). This latter difference was significant at the 1.125 and 1.5 g/kg dose levels (p's<0.01). At each of the latter doses, the ED group displayed higher scores on DEC-2 and DEC-3 than on DEC-1 (p's<0.01). Further, the ED group scores were lower on DEC-3 than on DEC-2 at the 1.5 and 1.875 g/kg dose levels (p's<0.01).

Again, this pattern of results reflected tolerance development in the ethanol diet group but not the control diet group. While the level of tolerance declined by six months post-withdrawal, a significant level of tolerance still remained.

The next two segments of the Results section compare tolerance development and loss across groups in Experiments 1 and 2, examining first the ED50 metric and then the 1.5 g/kg ethanol challenge tests.

Experiments 1 and 2: ED50 Analysis

The mean ED50 scores for all groups and DECs in Experiments 1 and 2 are presented in Fig. 2. Analyses of these data indicated significant main effects by Groups, $F(3,12)=9.77$, $p<0.01$, DEC, $F(2,24)=28.32$, $p<0.01$, and the Group by DEC interaction, $F(6,24)=7.34$, $p<0.01$. Significant group differences were found at DEC-2 and DEC-3 (p's<0.01), but not at DEC-1. At the DEC-2 tests, the CD group had significantly lower ED50 scores than the ED ($p<0.01$), PRE ($p<0.01$), and POST ($p<0.05$) groups, and the POST group had significantly lower ED50 scores than the PRE ($p<0.01$) and ED ($p<0.05$) groups. At the DEC-3 tests, the CD group had significantly lower ED50 values than all other groups (p's<0.01), and the PRE, POST, and ED groups were not different from one another.

The ED and PRE groups displayed significant differences in their ED50 scores across DECs ($p<0.01$). The CD group showed no changes across DECs and DEC differences in the POST group only approached significance ($p<0.10$). Both the ED and PRE groups displayed significant increases in their ED50 scores from DEC-1 to DEC-2 and DEC-3 (p's<0.01). Both of these latter groups also displayed significant decreases in their ED50 scores from DEC-2 to DEC-3 (ED: $p<0.05$; PRE: $p<0.01$). The only significant shift in ED50 scores in the POST group was an increase from DEC-1 to DEC-3 ($p<0.05$).

These data confirm the % Baseline analyses presented earlier. For the behavioral tolerance groups: (a) regardless of whether intoxicated practice is attained from pre-session IP

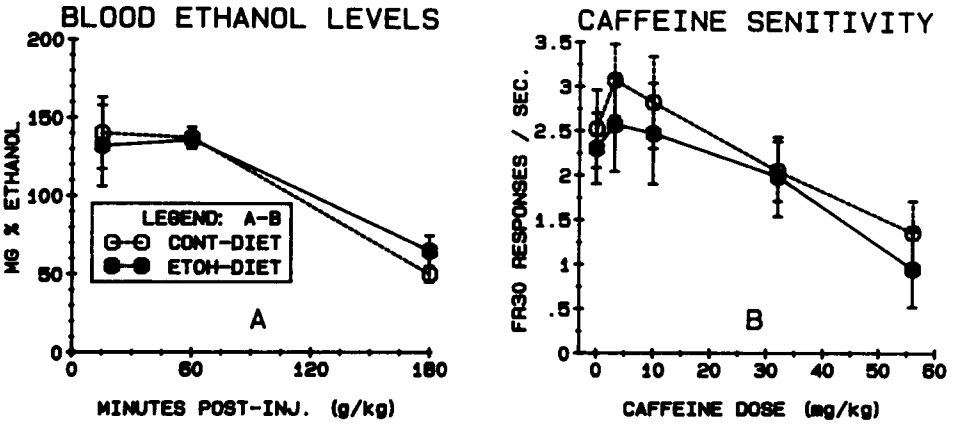

FIG. 4. Other tests for Experiment 2 groups (ED and CD) after DEC-3 determinations. (A) Blood ethanol levels and (B) caffeine dose-effect curve for operant performance (means and standard errors).

injections (PRE group) or from drinking the ethanol diet (ED group), significant shifts-to-the-right in the ethanol DEC were produced; (b) a significant portion of the tolerance acquired through intoxicated practice was lost after six months following the chronic ethanol regimens; but (c) the level of ethanol sensitivity displayed at this six-month post-withdrawal test was still significantly less than that for the original DEC. The physiological tolerance group displayed a different pattern, with a small amount of tolerance evident at the termination of the chronic ethanol regimen (significant POST vs. CD difference at DEC-2 but no significant difference between DEC-1 vs. DEC-2 in the POST group), and a continued development of tolerance evident at DEC-3 (significant difference between DEC-1 vs. DEC-3 in the POST group).

Experiments 1 and 2: Ethanol Challenge Tests

The % Baseline scores on eight 1.5 g/kg ethanol tests (the three DECs and the five monthly tests between DEC-2 and DEC-3) for all groups in Experiments 1 and 2 are presented in Fig. 3. Analysis of these data indicate significant main effects for Groups, $F(3,12)=43.68$, $p<0.01$, Ethanol Tests, $F(7,84)=25.79$, $p<0.01$, and the Groups by Test interaction, $F(21,84)=5.75$, $p<0.01$. Significant group differences were apparent at all tests except the first one (all p's<0.01) and significant differences across tests were evident in all groups except the CD group (all p's<0.01). The scores for the ED, POST, and PRE groups remained significantly higher than those for the CD group on all tests after Test 1 (p's<0.01).

Although the PRE and ED groups did not differ at Tests 1 and 2 (the first two DECs), by one month after (Test 3) and continuing until four months after (Test 6) termination of the chronic regimen, the ED group's scores dropped to levels significantly lower than the PRE group (p's<0.01). Further, the ED group differed significantly from the POST group at Tests 2 and 3 (p's<0.01 and 0.05, respectively) but not on the remaining tests. Finally, the PRE group had significantly higher scores than the POST group (after DEC-1) at all but the last two tests (p's<0.01). These latter two findings were due both to declining tolerance in the PRE and ED groups and to increasing tolerance in the POST group. The PRE group displayed significant tolerance loss relative to Test 2 on all tests after Test 4 (all p's<0.05), while the ED group displayed significant tolerance loss on all tests after Test 2 ($p<0.01$). In contrast, the POST group demonstrated a progressive increase in tolerance on all tests after Test 2 (p's<0.05).

Experiment 2: Other Procedures

In order to gain some additional understanding of the nature of the residual ethanol sensitivity differences between the ED and CD groups in Experiment 2, several other procedures were performed in the first month following completion of DEC-3. In order, these procedures were: (a) determination of ethanol blood levels at several time points following a 1.5 g/kg ethanol IP dose; (b) caffeine DECs on the FR30 operant task; and (c) acquisition performance in a one-way active avoidance task (criterion of four consecutive avoidances).

Figure 4A shows the blood ethanol levels for the ED and CD groups. Significant time-dependent decreases across time are evident ($p<0.01$), but there are no significant group differences at any time point. Figure 4B illustrates the caffeine DECs for the two groups. Again, significant dose-related decreases in response rate are evident in both groups ($p<0.01$), but no significant differences between groups are evident at any dose. Finally, examination of avoidance acquisition performance indicates that the ED group took significantly more trials, $t(6)=3.97$, $p<0.01$, to meet the acquisition criterion (mean=37 trials, SE=5.7) than the CD group (mean=17.3 trials, SE=1.3).

DISCUSSION

The data from Experiments 1 and 2 indicate several interesting features regarding the development and loss of ethanol tolerance as reflected by FR30 operant performance. These points are summarized as follows. First, the opportunity for intoxicated practice clearly enhanced the degree of tolerance generated. Tolerance, as measured by shifts in the DEC after chronic ethanol exposure, is greatest in the two groups which experienced prolonged intoxicated practice (i.e., the PRE and ED groups). The physiological tolerance group (i.e., POST) displayed tolerance at a level significantly lower than the PRE or ED groups but significantly higher than the CD group, which showed no tolerance development at all. Secondly, both the "behavioral" tolerance groups (PRE and ED) displayed some tolerance loss when tested six months

after the end of the chronic ethanol period, but the level of tolerance was still significantly greater than that for the CD group. Analysis of ethanol tests during this six-month interval revealed that the PRE group's decrease in ethanol tolerance was gradual, while the decline in the ED group was quite abrupt. The POST group never displayed tolerance loss but, in fact, showed a slight, but progressive, increase in tolerance during this six-month interval. Third, blood ethanol determinations suggested that the differences in ethanol sensitivity between the ED and CD groups are not attributable to differences in absorption or elimination of ethanol. Fourth, age-related changes cannot account for the persistence of decreased ethanol sensitivity since no such changes were detected in the CD group. Fifth, there did not appear to be a general decline in drug sensitivity in the ED group (no differences between the ED and CD groups in their sensitivity to caffeine was detected). Finally, the ED group displayed a deficit in acquiring a learned active avoidance behavior compared to the CD group. The latter finding is similar to that reported earlier for rodents exposed to a chronic ethanol diet [19].

One particularly noteworthy aspect of these data is the persistence of decreases in ethanol sensitivity in the PRE, POST, and ED groups. Few reports indicate that ethanol tolerance persisted as long as described here [5, 16, 35], and fewer still document such tolerance effects with complete dose-effect analyses [5]. It is quite possible that the infrequent and widely spaced ethanol challenges given during the six-month post-withdrawal period may have acted to maintain tolerance in these groups. However, this schedule of tests clearly cannot induce tolerance by itself. No trace of tolerance development was evident in the CD group which received no chronic ethanol, but did receive a similar pattern of DEC determinations and ethanol challenges (17 sessions in all). This is compatible with another report indicating that tolerance was not produced for treadmill performance with ethanol exposure every four days [26]. However, in animals which had reached a significant degree of tolerance earlier (PRE and ED groups), this schedule of intoxicated practice opportunities may have been sufficient to maintain a significant portion of this tolerance for at least six months after chronic ethanol exposure. In addition, in animals which had extensive exposure to ethanol, but without intoxicated practice or significant tolerance development (POST group), this schedule of intoxicated practice opportunities was sufficient to induce a progressive increase in tolerance even after chronic ethanol exposure was discontinued. Further work is underway to assess the role of these continued intoxicated practice sessions in the maintenance or the further development of tolerance. A comparable long-term tolerance effect in humans would appear to constitute an obvious risk factor in "recovering" alcoholics and, consequently, could have important implications for treatment strategies.

As noted earlier, some have suggested that behavioral tolerance may develop through Pavlovian conditioning when cues associated with ethanol administration come to elicit a conditioned compensatory response opposite in direction to ethanol's acute effects [29,30]. Such stimulus-control of operant behavior also can occur [40]. An environmentally-dependent model does not appear appropriate here, however, since in Experiment 2 the ED group developed a considerable degree of tolerance, despite the fact that the cues associated with the chronic diet and those associated with the IP test injections are clearly different. It is interesting that the ED group displayed a more rapid decline in tolerance after withdrawal from ethanol than did the PRE group. It will be recalled that the liquid diet groups remained on the control diet regimen during the DEC-2 determinations but were subsequently placed on ad lib lab chow. It is possible that the absence of the liquid diet during all subsequent ethanol tests contributed to the more rapid decline in tolerance seen in the ED group. However, the present data are not conclusive regarding this point. The slower loss of tolerance in the PRE versus the ED group may be due to higher blood ethanol levels in the PRE group during chronic injections (a 1.5 g/kg dose yields levels ranging from 140–190 mg%) relative to the blood levels produced by the ethanol diet at the time of testing (i.e., an average of 85 mg%). Further work is underway to examine these possibilities.

It has been proposed that the extent of tolerance development may reflect an interaction between the ethanol dose and the specific behavioral demands placed upon the animal while intoxicated [39,42]. In Experiment 1, it would appear that passive exposure to ethanol in the POST group interacted with the limited amount of intoxicated practice experienced by that group during challenge sessions to produce the level of tolerance noted at the end of the experiment. The CD group, which had a comparable number of intoxicated practice sessions during DEC determinations and the ethanol challenge session, developed no tolerance at all. The POST group had a degree of ethanol exposure equivalent to the PRE group. However, only a modest amount of tolerance was evident in the POST group at DEC-2, after the chronic injection period. The POST group in the present study, however, eventually did display a significant shift in ED50 scores (relative to DEC-1), but only at the end of the study (DEC-3) after numerous ethanol challenge tests. These latter challenge doses would appear to provide additional intoxicated practice experience with minimal additional ethanol exposure. These results are consistent with the suggestion [41,42] that such intoxicated practice in post-injection groups during ethanol test or challenge sessions may have prompted learned behavioral adjustments in the POST as well as the PRE groups.

From the above discussion, the question remains concerning the nature of the post-session ethanol intoxication experience. One recent study [38] suggests that such post-session ethanol injections also may produce a "learning" influence on the target behavior, even though that behavior occurred without intoxicated practice. In the case of the POST group in this present study, the animal may have learned some unspecified adaptive behavior while intoxicated in its home cage (e.g., adjusting to ethanol's ataxic effects). Further, it is documented that behaviors not under the explicit control of external stimuli (as in the post-injection condition) are more sensitive to control by internal stimuli [31]. Thus, the ethanol cue itself, according to this argument, may be sufficient to re-elicit any learned adaptive behavior on subsequent tests with ethanol. Clearly, more research is required to assess this hypothesis.

Finally, the exact nature of the putative compensatory responses which are supposed to be acquired through intoxicated practice also is unknown at this time. From the present results it is unknown whether such compensatory processes are under stimulus control by context factors related both to the cue properties of ethanol and to the specific stimuli associated with its administration. Similar context-specific tolerance effects have been reported for the effects of chlordiazepoxide in a Geller-Seifter conflict task [14] and recently for the effects of midazolam on spontaneous locomotor ac-

tivity and shock-induced suppression of locomotor activity [9]. Additionally, it has proven difficult to directly measure conditioned compensatory responses. Investigators seeking to detect a compensatory hyperactive response in experiments employing activity-depressing drugs sometimes have been successful [21,33] and sometimes not [17]. King and colleagues [21] have suggested at least three possible sources of difficulty in assessing the compensatory response effect. First, the drug cue itself, alone or in combination with other context cues, may be an important component of the stimulus which elicits the compensatory response. The results of Experiment 2 of the present investigation indicate that the control diet plus the ethanol state produced by IP injections was sufficient to produce ethanol tolerance effects observed at DEC-2 in the ED group. Second, some conditionable physiological mechanism (e.g., peptide release) might counteract drug effects but produce no noticeable effect on behavior in the absence of the drug. Such a mechanism may prove difficult to demonstrate aside from showing that manipulation of one or more endogenous agents acts to enhance or prevent tolerance development. Finally, the conditioned tolerance effect may not depend on compensatory processes at all, but rather on some other associative mechanism, such as habituation (see [36]). One potential solution to the problems in assessing possible compensatory responses may lie in contrasting such conditioned effects against another behavior. Dickenson [12] cites this strategy as useful in detecting conditioned inhibitory effects. In the present context, tolerance developed to ethanol's depressant effects on FR30 operant performance might be tested in a task sensitive to the hypothetical "rate-increasing compensatory behavior" (e.g., a DRL task). Such a study is currently underway in this laboratory.

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