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Cumulative vs. Acute Dose–Response Procedures Produce Differential BAC and Behavioral Functions for Ethanol

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GAUVIN, D. V., R. J. BRISCOE, T. J. BAIRD, M. VALLET, K. L. CARL AND F. A. HOLLOWAY. *Cumulative vs. acute dose–response procedures produce differential BAC and behavioral functions for ethanol.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 397–403, 1997.—The discriminative stimulus attributes of ethanol (ETOH) were characterized in rats trained to discriminate between 1.25 g/kg ETOH and saline. The ETOH generalization functions were assessed using both acute and cumulative dosing procedures. The cumulative procedures differed in the individual incremented doses used to generate the functions. Acute dosing procedures produced discriminative functions that were significantly different from cumulative dose–response curves (DRCs). Similar cumulative DRC's were generated *within* each cumulative dosing procedure, whereas significant differences were produced *between* the two dosing incremented procedures. When blood alcohol concentrations (BACs) were quantified, a cumulative testing procedure produced significantly lower BACs than acute testing procedures at every dose above the initial or starting dose. Interestingly, response rate functions did not differ within or between cumulative and acute procedures. These data may suggest that differential ETOH dosing procedures may differentially influence the behavioral choice and BAC functions in rats, and cautions against the use of cumulative procedures to assess shifts in DRCs during chronic treatments without a concomitant assessment of BACs. © 1997 Elsevier Science Inc.

Drug discrimination Ethanol ETOH Dose–response curves Cumulative dosing

THE experimental control or arrangement of the relationship between drug administration and contingencies of reinforcement can result in drug discrimination. Once the discrimination has been established, a variety of techniques may be used to assess the effects of other doses of the drug. Dose–response curves (DRCs) or functions established in these ways are often referred to as generalization gradients and are used to describe fully the stimulus control established via drug-discrimination training procedures (1,10,13). In particular, two different dosing procedures have been established and used by a number of laboratories to generate DRCs in experimental animals. The most typical procedure used in DD studies is the acute dosing test, in which an experimental subject is tested in a single daily session after receiving a single (acute) injection of the drug of interest. Depending on the number of doses and drugs to be tested, training and test sessions are alternated throughout, what could be, a protracted period of time. In a growing number of DD studies investigating the development of tolerance and/or sensitization to the discriminative stimulus effects of drugs, a more rapid technique has been employed that allows for the determination of a complete DRC in a multicycle testing session conducted over the course of a single test day (9,10). The cumulative dosing procedure seems to be a critically important tool to the researcher simply because a complete DRC can be generated in a single test session during the course of chronic drug exposure. Thus, all experimental subjects have identical drug exposure histories and the rate of tolerance or sensitization development can be quantified and compared with control subjects at a single time point during the course of chronic drug exposure.

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A number of other laboratories have reported somewhat reliable DRCs using the cumulative dosing procedure (8,9,13–15). During our analysis of tolerance development to the discriminative stimulus effects of ethanol (ETOH) using similar cumulative dosing procedures to generate DRCs, we have found, and now report, an interaction between and within the cumulative and acute ETOH dose–response procedures and the resulting blood alcohol concentrations (BACs) in rats.

METHOD

Subjects

Twelve male Sprague–Dawley rats weighing 300–325 g were purchased from Sasco Laboratories, Inc. (Omaha, NE). Rats were housed individually in stainless steel suspended cages located in an American Association for Accreditation of Laboratory Animal Care-accredited colony room under the direct supervision of the Department of Animal Resources of the University of Oklahoma Health Sciences Center. The animal colony room was maintained on a 12-h light-dark cycle (lights on 0530) at 20–22°C, with a relative humidity of 60%. Each rat was initially given ad lib access to both food and water and were allowed a 1-week acclimation period to the new environment before the beginning of the experiments.

Care and use of animals were in accordance with the University of Oklahoma Health Sciences Center Institutional Animal Care and Use Committee and the National Institutes of Health Guidlines for the Care and Use of Animals in Research.

Apparatus

Experimental sessions were conducted in standard operant chambers (Lafayette Instruments, Lafayette, IN) equipped with two response levers, two stimulus lamps, a house lamp, and a pellet dispenser, housed within a sound-attenuating cubicle. Four operant chambers were located within a single isolated running room. Experimental contingencies and data collection were controlled by a Commodore 64C microcomputer system interfaced with the operant chambers (American Neuroscience Research Foundation, Yukon, OK).

Initial Drug Discrimination (DD) Training

Twelve rats were trained to discriminate between intraperitoneal injections of 1.25 g/kg ETOH and saline (SAL) in experimental sessions with several discrete trials using identical procedures to those previously described by Sannerud and Young (9) for a morphine discrimination and Sannerud et al. (8) for a chlordiazepoxide discrimination. Pressing the lever was established by successive approximations and maintained under a 15-min time-out (TO), 10-min time-in (TI) fixed-ratio 1 (FR 1) schedule of food presentation (See Fig. 1). During the TO, the stimulus and house lamps were off and responding had no programmed consequences. At the start of each TO, a subject received a saline, 1.25 g/kg ETOH, or sham injection. A sham injection was defined as handling of the rat and a slight touch to the skin of a hypodermic needle without penetration of the skin. After the 15 min elapsed, the TI was initiated, at which time the house and stimulus lamps were illuminated and the discrimination contingencies were in effect. The TI ended after 10 min and another injection was given; the 25 min between injections remained constant.

The appropriate lever for reinforcer delivery was determined by which of the discriminative stimuli was administered. Saline trials were preceded by saline injections, and completion of the response on the left lever produced food.

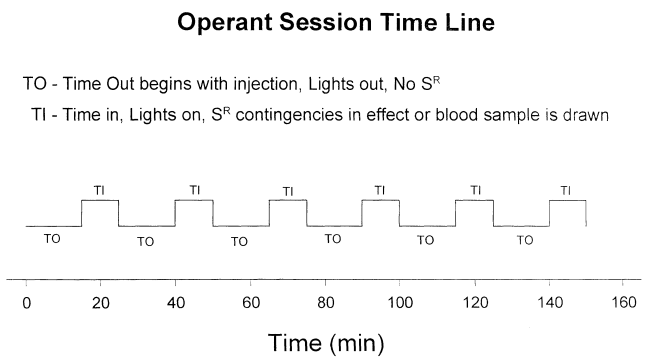

FIG. 1. Time-line for operant sessions, ETOH injections, and BAC sampling.

The first ETOH trial was preceded by a 1.25 g/kg ETOH injection; the second ETOH trial was preceded by a sham injection. During ETOH trials, completion of the response on the right lever produced food. The number of consecutive responses on the appropriate lever required for a reinforcer delivery was gradually increased to 10 (FR 10). Responses on the inappropriate lever reset the FR requirements on the injection appropriate lever.

Each daily training session varied in length and in the number of ETOH and saline trials; 0–6 saline trials preceded 0 or 2 ETOH trials. A two-month sequence of trials was arranged for each rat so that: 1) each rat received a different daily sequence; 2) an equal number of ETOH and saline trials were conducted within each week; and 3) no more than two consecutive ETOH trials were run on any given day. Previous work by Sannerud et al. (8) and Sannerud and Young (9) have previously established that this protocol prevented the session length or individual trial number from gaining stimulus control and minimized differences among successive generalization gradients (see below). Maintaining these stringent training criteria has established that the only reliable stimulus available to an individual subject to solve the discrimination task is the interoceptive cues produced by the injection of saline or ETOH. Training sessions were conducted 5–7 days per week and continued until the individual rats emitted fewer than 20 responses before the first reinforcer delivery, distributed at least 90% of the total session responses on the correct lever, and had earned at least two reinforcers within each trial.

Initial Testing

Once stimulus control was established, cumulative and acute DRCs for ETOH were generated in every rat. A series of acute dose test sessions consisted of a single TO and TI period. A single injection of saline or a selected dose of ETOH was administered at the beginning of the TO period and the individual rat was removed at the completion of the TI period (25 min in duration). During a cumulative test session, saline or a dose of ETOH was administered at the start of each TO period. In order to assess the replicibility of the DRCs for both discriminative stimulus control and rate-altering effects by ETOH, the following independent factors were varied in cumulative dose tests: the initial ETOH dose, the size of the cumulative dose test increments, and the number of consecutive trials. Two different dosing procedures were used. In Procedure #1, single injections of 0.0 (SAL), 0.25, 0.5, 0.5, 0.5, 0.5 g/kg ETOH were administered at the start of each TO, which resulted in cu-

mulative doses of 0.0, 0.25, 0.75, 1.25, 1.75, 2.25 g/kg ETOH during each successive TI. In Procedure #2, single injections of 0.0 (SAL), 0.625, 0.625, 0.625, 0.625 g/kg ETOH were administered at the start of each TO, which resulted in cumulative doses of 0.0, 0.625, 1.25, 1.875, and 2.5 g/kg ETOH during each successive TI. During test sessions, 10 consecutive responses on either lever produced food.

Prior to cumulative DRC tests, each rat was tested under the following conditions: 1) six consecutive saline trials; 2) five consecutive saline trials followed by a single ETOH trial; 3) four consecutive saline trials followed by two consecutive ETOH trials (ETOH injection + sham); 4) three consecutive saline trials followed by a single ETOH trial; 5) two consecutive saline trials followed by two consecutive ETOH trials; 6) a single saline followed by a single ETOH trial; 7) a single saline trial; and finally, 8) a single ETOH trial. Cumulative ETOH dose–response test sessions were conducted at the end of each month. Initial acute dose–response test sessions were alternated with pairs of successful SAL and ETOH training sessions during the months of continued training. If an individual rat failed to reach criterion during either SAL or ETOH training sessions, further testing was postponed until two sessions of criterion performance occurred. Once the acute DRCs were completed, rats were run in daily training sessions during the month and cumulative dose–response tests were conducted at the end of each month. A single acute dose–response function was generated over the course of two months and four cumulative dose–response functions (two under each cumulative procedure) were generated across the four-month period.

Once these behavioral dose–response tests were completed, the time- and dose-dependent changes in blood alcohol concentrations (BACs) were quantified using identical temporal schedules of injections used in the behavioral testing sessions. At time points equivalent to the start of each TO period, rats were injected with ETOH. A 20 μl tail blood sample was drawn from each rat at the beginning of each TI period. During cumulative BAC tests, each rat's tail was secured by the experimenter at the onset of each TI, the tail was gently "milked" to provide a 20 μl blood drop at the tip of the tail, at which time the blood sample was drawn up into a calibrated barosilicate micropipette (Fisher Scientific Products). Once the sample was collected (see BAC procedure, below) the rat was left undisturbed until the beginning of the next TO period (depending upon the testing procedure used). BACs were quantified for both acute and cumulative testing procedures. During cumulative behavioral dose–response tests rats have the opportunity to consume food pellets (ranging from 10–17 pellets per TI period). It could be suggested that the consumption of the 45 mg food pellets during the 10-min TI periods (total food consumed ranging from 450 to 765 mg food) could modify the pharmacokinetics of supplemental IP ETOH injections. Therefore, BACs were quantified in both "food restricted" (23.5 h food deprived) and "fed" subjects. In the "fed" condition, each rat received the identical number of reinforcer pellets earned during each TI period of the behavioral DEC immediately after the ETOH blood draw. This time period corresponded to the behavioral TI periods and would provide identical stomach contents to those interacting with the behavioral DECs.

Blood Alcohol Concentration Analysis

BAC's were quantified using a gas chromatographic headspace sampling technique. A 20-μl blood sample was drawn from the tip of the tail at various time points after an acute or cumulative dose of ETOH was administered. The blood sample was immediately deposited in a 20-ml serum bottle with 1-ml of 0.02% v/v solution of 1-propanol in distilled water (internal standard). The blood aliquot was immediately sealed and incubated for 20 min in a 50°C metabolic shaking water bath (Precision Scientific Co, Chicago, IL) before blood alcohol measurements. A 1.0-ml air sample was drawn from each sample bottle using a gas-tight syringe and immediately injected into the entry port of a Hewlett-Packard Gas Chromatograph (5890A) equipped with a hydrogen-fueled flame ionization detector. The chromatograph was also equipped with a 6′ × 1/8′ stainless steel column with 5% Carbowax, 20 M, 60/80 mesh Carbopack B packing. This system utilized a nitrogen carrier gas with a pressurized air purge. The oven temperature was set at 135°C, and injection and detector temperatures of 200 and 300°C, respectively. A Hewlett-Packard integrator (HP3396A) calculated the area under the curve of all detectable peaks. For each of 20 samples run through the chromatograph, a complete set of ETOH standards of known concentrations were made by serial dilution (equivalent to 400, 200, 100, 50, 25, 0 mg/dl) and quantified on the chromatograph. An additional set of diagnostic standards purchased from Sigma Chemical Co. (St. Louis, MO; 99.99% accuracy) were run to validate the accuracy of our standards (300, 100, and 50 mg/dl). The area of each ETOH-associated peak was normalized using two different procedures: 1) expressing the area of each individual peak as a percentage of the total area of all peaks in the chromatogram; and/or 2) expressing the area of the ethyl alcohol peak as a percentage of the internal standard peak. Both procedures yielded equivalent (statistically nonsignificant) results. Blood ETOH concentrations were assessed using a 7-point linear regression analysis (least-squares procedure) from the ethanol standards. Five separate BAC functions were generated in subgroups of 8–9 rats over the 10-month course of the study. The order of tests was varied randomly between and within subjects for the acute dose, and both cumulative dose "restricted" food conditions to minimize order effects. The cumulative BACs were retested in the "fed" condition at the completion of the study.

Drugs

Ethyl alcohol USP (190 proof; U.S. Industrial Chemicals Company, Houston, TX) was diluted to 10% w/v in normal (0.9%) sterile saline. Injection volumes for the training dose of ETOH, expressed in milliliters, were 12.5 times greater than the individual rats' body weight expressed in grams [For example, a rat weighing 300 g (0.300 kg) would receive a 3.75-ml injection of 10% w/v ETOH in order to administer 1.25 g/kg ETOH]. On training days, the volumes of saline injections were varied from 1 ml/kg to 5 ml/kg to insure that the rats could not reliably use the volume of the injections to solve the discrimination task.

Data Analysis

Discrimination data were analyzed as the percentage of total session responses emitted on the ETOH-appropriate lever. The individual ED_{50}s were calculated by linear regression using a least-squares procedure and averaged for each dose–response procedure; the group mean ED_{50}s were compared using a simple Student's *t*-test. Overall response rates were expressed in responses per second, and provided a second measure of behavioral effects of ETOH that appears to be independent of the distribution of response choice on the two levers (2). Blood alcohol levels are expressed in mg/dl, and

were fitted to the best fitting linear function (least-squares procedure). All data were analyzed using a mixed-factor (subject × treatment, repeated measures) ANOVA with a posteriori tests of dose and/or time comparisons with Duncan's multiple range test. All data analyses were conducted by the CSS:Statistica personal computer software system (Complete Statistical System, Tulsa, OK). Acute and cumulative dose–response functions were compared an ANOVA and by fitting the data to the best fitting straight line, calculating the slopes of each function, and testing the lines for parallelism (11,12) using an IBM-based Pharmacological Calculation System (Pharm/PCS, Ver. 4; MicroComputer Specialists, Philadelphia, PA). Statistical significance was set at the 0.05 level.

RESULTS

ETOH stimulus control was established in all 12 rats with a mean number of sessions to criterion performance of 58 days (range 41–67 days). The initial multicycle tests of stimulus control by the two training stimuli provided clear evidence that each rat demonstrated differential response patterns that correlated perfectly with differential cycle-dependent injection patterns (data shown in Table 1). In all of these tests, each rat emitted less than 13 responses prior to the first reinforcer (FRF), greater than 90% on the injection-appropriate lever, and earned more than 10 reinforcers in each test cycle.

Figure 2 compares the ETOH DRCs generated in a subgroup of eight randomly selected rats using the single or acute injection procedure (closed circles) and a cumulative dose–response function (open symbols). The group mean percentage (±SE) of total test cycle (session) responses emitted on the ETOH-appropriate lever is expressed as a function of test dose. The best-fitting straight-line functions were fitted to the acute (solid) and the two cumulative (dashed and dotted) functions. Under the acute testing procedure, rats emitted 100% ETOH-appropriate responding after injections of the 1.25 g/kg training dose of ETOH, however, the cumulative testing procedure did not engender equivalent response patterns until a cumulative dose of 2.25 g/kg ETOH had been administered. The two-way ANOVA of acute vs. cumulative DRCs demonstrated a significant Main Test × Group Interaction ($F(8,48) = 3.43$, $p = 0.003$: left panel; $F(8,48) = 6.71$, $p = 0.00007$). Statistical analyses of the cumulative DRCs demonstrated that there were significant main (dose) effects for both discriminative (DRC #1: $F(5,30) = 9.43$, $p < 0.0001$; DRC #2: $F(4,24) = 16.4$, $p < 10^{-6}$ DRC #3: $F(4,24) = 9.3$, $p < 10^{-4}$ DRC #4: $F(4,24) = 20.6$, $p < 10^{-6}$) and rate-of-responding functions (see Fig. 4) (DRC #1: $F(5,30) = 11.7$, $p < 0.10^{-5}$ DRC #2: $F(4,24) = 6.5$, $p < 0.001$; DRC #3: $F(4,24) = 8.0$, $p < 0.0003$); DRC #4: $F(4,24) = 3.8$, $p < 0.01$). Cumulative behavioral dose–response functions were similar when generated *within* each cumulative procedure (DRC #1 vs. DRC #3, $F(1,6) = 0.23$: n.s.; DRC #2 vs. DRC #4, $F(1,6) = 0.13$: n.s.). However, significant differences were produced *between* the

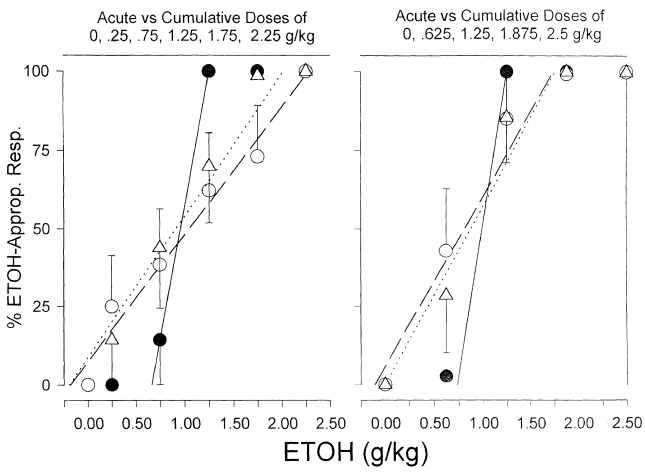

FIG. 2. The mean (±SE) percentage of total session responses emitted on the ethanol-appropriate lever during two different cumulative dose testing procedures are compared with the dose–response curves generated using acute testing conditions. Acute dose–response functions are depicted in closed circles; the best-fitting straight line fitted to the acute dose response functions are also depicted as solid lines. The left panel compares the acute DRC with cumulative DRC #1 (open circles and dashed lines) and #3 (open triangles and dotted lines) using cumulative ethanol doses of 0.25, 0.75, 1.25, 1.75, and 2.25 g/kg. The right panel compares the acute dose–response function (solid circles) and its best-fitting straight line function (solid line) to DRC #2 (open circles and dashed line) and DRC#4 (open triangles, dotted line), which used cumulative doses of 0.625, 1.25, 1.875, and 2.5 g/kg. The DRC number reflects the month in which the tests were conducted relative to reaching training criteria for stimulus control.

TABLE 1

RESULTS OF TEST TRIALS CONDUCTED TO INSURE THAT RATS WERE UNAFFECTED BY TEST SESSION DURATION AND THAT RESPONSE CHOICE WAS NOT UNDER THE CONTROL OF ANY INDIVIDUAL CYCLE

	Group mean (± SE) % ETOH-AApprop. Resp.					
Testing conditions	ø 1	ø 2	ø 3	ø 4	ø 5	ø 6
6 saline ø s	2 ± 2	0 ± 0	0 ± 0	0 ± 0	1 ± 1	0 ± 0
5 saline ø s, 1 ETOH ø	0 ± 0	4.6 ± 0	0 ± 0	3 ± 1	0 ± 0	100 ± 0
4 saline ø s, 1 ETOH ø, 1 Sham ø	0 ± 0	0 ± 0	4 ± 4	0 ± 0	100 ± 0	100 ± 0
3 saline ø s, 1 ETOH ø	5 ± 2.5	0 ± 0	0 ± 0	99 ± 0.6	—	—
2 saline ø s, 1 ETOH ø, 1 Sham ø	0 ± 0	4.2 ± 2	99 ± 0.1	100 ± 0	—	—
1 saline ø, 1 ETOH ø	0 ± 0	100 ± 0	—	—	—	—
1 saline ø	2 ± 2	—	—	—	—	—
1 ETOH ø	100 ± 0	—	—	—	—	—

ø - cycles; ETOH - ethanol.

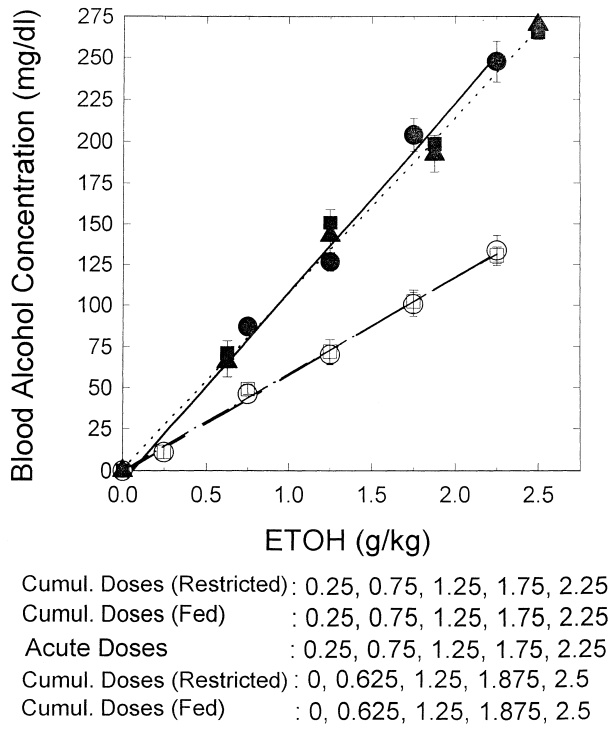

FIG. 3. Comparison between tail blood alcohol concentrations quantified from 20-μl samples serially drawn at time-points corresponding to operant time-in periods during cumulative dose–response functions or acutely drawn during acute dose–response functions. Each point represents the group mean (±SE) of 8 or 9 rats, randomly selected from the trained subject pool of 12. Two cumulative dose increments and a range of acute ETOH doses were tested over the course of the study. Details of analysis are described in Method.

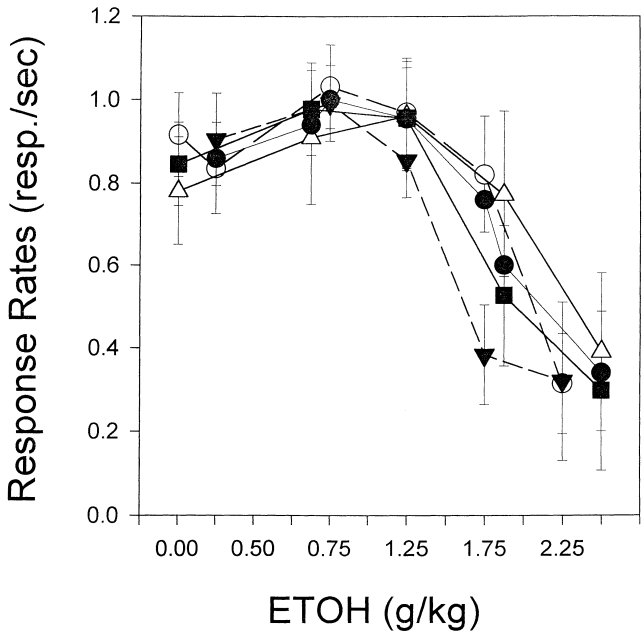

FIG. 4. Dose- and procedural-dependent changes in rates of lever press responding during cumulative and acute dose–response generalization test sessions. Rates of responding are expressed in responses/s and are plotted as a function of ETOH test dose. The rates-of-responding correspond to the response-choice data displayed in Figs. 2 and 3, above. For each dose–response function there was a significant Dose main effect; however, upon comparison, there were no significant Test main effects nor Dose × Test interaction effects.

two dosing procedures: ED_{50}s: DRC #1 vs. #3: 1.06 ± 0.2 vs. 1.06 ± 0.23; DRC #2 vs. #4: 0.74 ± 0.15 vs. 0.73 ± 0.17. Using Simple Effects tests to compare each acute DRC with the cumulative DRCs generated with the same incremented dosing procedure (Fig. 2 left panel vs. right panel), significant Time × Dose interactions resulted between the acute and cumulative DRC's (acute vs. DRC#1: $F(5,30) = 5.46$, $p = 0.001$; acute vs. DRC#3: $F(5,30) = 5.6$, $p < 0.001$; acute vs. DRC#2: $F(4,24) = 5.5$, $p = 0.002$; acute vs. DRC#4: $F(4,24) = 6.2$, $p < 0.001$). Because the slopes of the acute DRCs spanned a limited dose range of only 2 or 3 doses, individual dose and time comparisons were conducted. In DRCs 1 and 3, the cumulative doses of 0.75 and 1.25 g/kg ETOH were significantly different from acute injection tests conducted with either 0.75 (ps <.01) and 1.25 g/kg (ps <.01). Similarly, in the DRCs #2 and #4, the cumulative doses of 0.625 ($p < 0.01$) and 1.25 ($p = 0.049$) were significantly different from the acute tests.

Figure 3 shows the blood alcohol curves. Acute injections of 0.25, 0.75, 1.25, 1.75, and 2.25 g/kg ETOH were administered individually in a modified single cycle (see above). The filled circles and the best-fitting straight line function (solid black line) for these acute injection tests are compared with two different incremented cumulative testing procedures. A series of repeated injections of 0.25, 0.5, 0.5, 0.5, and 0.5 g/kg ETOH, which corresponded to cumulative doses of 0.25, 0.75, 1.25, 1.75 and 2.25 g/kg ETOH, are depicted in open circles (food restricted condition) and open squares (fed condition) and fitted to the best-fitting (dashed) lines. The BACs corresponding to the second incremented cumulative dosing procedures were generated after injections of 0.0 (saline), 0.625, 1.5, 1.875, and 2.5 g/kg ETOH. These results are also shown in Fig. 3 for both food-restricted (closed diamonds) and fed conditions (closed squares) and fitted with the best-fitting (dotted) line. These latter BACs did not differ from each other or the best-fitting line of the acute injection BAC function (All Fs less than 0.5). Prior to statistical confirmation, these latter BACs were visually different from the acute BAC. Therefore, a sixth BAC was generated using a different incremented cumulative procedure that was initiated with a slightly higher dose of ETOH. A series of repeated injections of 0.0 (SAL), 0.5, and 0.75 g/kg ETOH provided cumulative doses of 0.0, 0.5, and 1.25 g/kg ETOH, respectively, (data not shown). Significantly different BAC functions resulted from acute (filled circles) and the first cumulative dosing procedure (open circles; $F(1,6) = 38.6$, $p < 10^{-6}$). However, The small amount of food earned during each TI period of the behavioral DECs failed to affect the BACs significantly ($F(1,6) = 0.1$, $p = 0.998$). Raising the dose of the first ETOH injection from 0.25 to 0.5 g/kg in the second cumulative procedure produced quantitatively similar BAC functions to those produced by the acute injection procedure (data not shown).

As detailed, above, the dose–response functions for the group average rates-of-responding are shown in Fig. 4. Similar dose-related changes in the rates-of-responding were engendered irrespective of the dosing procedure used (acute vs. cumulative or cumulative procedures 1 and 2; all comparative Fs < 1.0, ps were n.s.).

DISCUSSION

We have demonstrated shifts in ETOH discriminative stimulus generalization functions differentially generated by acute injection and cumulative dosing procedures. Tests conducted with the training dose of ETOH (1.25 g/kg) using an acute testing procedure always engendered a group mean percentage of ETOH-appropriate responding that met our training criteria (>90%), whereas both of the cumulative procedures used required a higher cumulative dose of ETOH in order to engender equivalent criterion performance. The initial comparisons between acute and cumulative dosing procedures produced similar ED_{50}s. However, although the acute test of the 1.25 g/kg training stimulus produced 100% ETOH-appropriate responding, the cumulative dose of the 1.25 g/kg dose engendered approximately 70%. This differential response topography engendered by the training dose of ETOH was associated with differential group mean BACs of approximately 125 and 75 mg/dl, for acute and cumulative procedures, respectively.

The differential stimulus generalization functions were contrasted with extremely reliable rates-of-responding functions. We have previously suggested that ETOH's discriminative stimulus effects were critically dependent on blood alcohol levels only, and not on any of ETOH's metabolic byproducts (acetaldehyde, methanol, acetone, etc.); response rates, on the other hand, are sensitive to the algebraic summation of the rate-disruptive effects of ETOH and all of its behaviorally active metabolites (2). The data from the present study support our previous suggestions.

Although we have demonstrated similar DRCs generated in two-month intervals using the same incremented cumulative procedure (Months 1 and 3: 0.0, 0.25, 0.75, 1.25, 2.25 g/kg ETOH), these DRCs differed significantly from the DRCs generated in the two intervening months using a different incremented cumulative procedure (Months 2 and 4: 0.0, 0.625, 1.25, 1.875, 2.5 g/kg ETOH). The month to month reproducibility of these functions suggests that changes in discriminative sensitivity (i.e., tolerance or sensitization) resulting from the repeated injections of the training dose of ETOH, and various other patterns of ETOH dose injections that occurred throughout the duration of the experiment, cannot account for the differential behavioral choice data.

Differential blood alcohol concentrations were produced by the acute and cumulative testing procedures, but only if the cumulative dosing procedure was initiated by a low dose of ETOH (0.25 g/kg). When a slightly higher initial dose of 0.5 g/kg ETOH was used in a cumulative procedure, acute and cumulative BACs were quantitatively similar (data not shown). The small amount of food in the stomach resulting from the ingestion of food during the TI periods of the behavioral DRCs did not alter the BAC functions resulting from IP injections.

Using a home-cage ETOH self-administration procedure, we have demonstrated previously that a 15-min pretreatment with a low dose of 0.25 g/kg ETOH behaviorally primed the rats to consume a significantly greater volume of ETOH [125% of control; (4)]. This 15-min pretreatment is identical to the interval used in the present study and may suggest that some differential absorption or distributional mechanism may be involved in these differential behavioral responses.

The BACs resulting from the acute injections of 0.25 g/kg in either the single cycle test condition and the initial cycle of the cumulative procedure were quantitatively similar (acute: 10.92 ± 3.7 mg/dl; cumulative: 10.94 ± 3.7 mg/dl). The 0.75 g/kg ETOH dose, when administered acutely, resulted in a group mean BAC of 87.0 ± 1.9 mg/dl, whereas the third cycle of the cumulative dosing procedure associated with a cumulative dose of 0.75 g/kg ETOH dose (0.0 + 0.25 + 0.5 g/kg) resulted in a group mean BAC of 46.1 ± 2.9 mg/dl.

The procedural-induced changes in ETOH's pharmacokinetics may explain why the cumulative and acute testing procedures do not provide equivalent reproducibility of DRCs reported by other laboratories. The rat BAC function typically does not demonstrate a temporal plateau when IP injections are administered. With its short half-life, ETOH's BAC is most usually characterized by a rapid ascending limb (absorption & distribution phase), a single peak, and a consistent (dispositional) descending limb. Other laboratories, reporting reproducible DRCs, have utilized drugs with relatively longer half-lives, such as morphine (9) and chlordiazepoxide (8), which are characterized by a plateau in the serum drug concentration functions. Within a cumulative dosing procedure, this plateau and extended half-life may allow for the simple additivity of the stable blood drug concentration of the initial dose injection and the second cumulative injection, and so on. Recently, Lytle, Emmett-Oglesby, and Stephens (5) reported similar differential response topography in the discriminative functions of midazolam. Similar to the kinetics of ethanol, the benzodiazepine, midazolam, has a rapid onset of action with a short half-life. In cumulative tests with the training drug, full substitution of midazolam for itself did not occur until doses higher than the training dose (1.0 mg/kg) were used. The authors suggested that, within the cumulative procedure, the metabolism of some of the initial low doses of midazolam caused the 1.0 mg/kg dose to be slightly less efficacious then when the training drug was administered in a single injection during training. Caution is advised even with this explanation because extremely robust and reproducible DRCs for the discriminative stimulus effects of midazolam have been previously reported for both a qualitative two-choice [midazolam-saline; (7)] and a quantitative three-choice [0.32 mg/kg-saline-3.2 mg/kg; (6)] operant procedure in rats. The training and test procedures used in the present study are strikingly similar to those used by Sannerud et al., (6–9). The procedural similarities between our study and those of Sannerud may suggest that more subtle, as yet unidentified, variables may influence the shape and distribution of the generalization functions.

Similar acute vs. cumulative behavioral and breath-alcohol dose–response functions have been reported with ETOH administration in the rat by Hiltunen and Järbe (4). These authors compared a single injection of the training dose of 1.0 g/kg ETOH to cumulative doses of 0.25, 0.25, and 0.5 g/kg ETOH in female Sprague–Dawley rats responding under an FR-10 schedule of liquid reward. The disparity between the present report and those of Hiltunen and Järbe may be due to the differential stock (ALAB AB, Sollentuna, Sweden vs. Sasco Inc. Omaha, NE), gender (female vs. male), ETOH samples that were utilized (breath vs. tail-blood), or reinforcer delivered (liquid vs. pellet).

Current studies are examining the dispositional, clearance, or time-course effects produced by the acute and cumulative testing procedures in rats. These studies will help to illuminate the role of metabolic processes in these effects.

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