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Presentation of an Ethanol-Paired Stimulus Complex Alters Response Patterns During Extinction

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SLAWECKI, C. J., H. H. SAMSON AND A. CHAPPELL. *Presentation of an ethanol-paired stimulus complex alters response patterns during extinction.* PHARMACOL BIOCHEM BEHAV **62**(1) 127–135, 1999.—It has been hypothesized that environmental stimuli previously paired with ethanol consumption play a role in excessive ethanol intake. This study examined the ability of orally self-administered ethanol to establish a tone-light stimulus complex as a conditioned reinforcer (CS^R). Male Long–Evans rats were trained to orally self-administer 10% ethanol (10E) using the sucrose-substitution procedure. During training, a tone–light stimulus complex was paired with ethanol presentation in a stimulus complex paired (SC-paired) group but not in a control group. Responding during extinction in the presence and absence of the stimulus complex was then examined. Following the initiation of ethanol self-administration, 10E maintained greater responding in the SC-paired group compared to the control group. When the stimulus complex was presented contingent on responding during extinction, the rate of extinction was slightly attenuated in the SC-paired group but not in the control group. The altered rate of extinction in the SC-paired group was characterized by: 1) a slight decrease in total session responding over successive days of extinction and 2) a transient attenuation of extinction burst response rate during the first extinction session. These data suggest the stimulus complex could function as a weak CS^R, but overall its ability to maintain lever pressing was minimal. © 1998 Elsevier Science Inc.

Ethanol self-administration Conditioned reinforcer Extinction Rats

THE ability of conditioned stimuli to elicit conditioned withdrawal and/or conditioned tolerance has been hypothesized to be an important link in the regulation of ethanol intake by environmental stimuli (20,31). However, a stimulus associated with ethanol consumption could also influence ethanol intake by functioning as a conditioned reinforcer (CS^R) or discriminative stimulus (CS^D). Although there are several studies indicating that a CS^D can influence ethanol intake in rats (6,8,9), few published studies have directly assessed the ability of ethanol to establish a CS^R (28,29). In one of these studies, Smith et al. (29) reported a buzzer paired with intragastric ethanol self-administration prolonged responding during extinction. This suggested the stimulus paired with ethanol self-administration functioned as a CS^R. Because several models of oral ethanol self-administration have now been developed (1,12, 22) that more adequately model human alcohol drinking, the demonstration that orally self-administered ethanol can establish a CS^R could have important implications for the role of conditioned reinforcement in human alcohol drinking.

Conditioned reinforcers have been suggested to influence appetitive behaviors (17,21,30). Samson and Hodge (24) have suggested that oral ethanol consumption is partially regulated by appetitive behaviors; therefore, it is reasonable to hypothesize that conditioned reinforcers might influence appetitive behaviors associated with ethanol self-administration. In particular, it is hypothesized that the onset and early maintenance of ethanol drinking would be susceptible to regulation by a CS^R because at this time there is no neuropharmacological activity associated with ethanol consumption. In partial support of this hypothesis, using the new response paradigm, Slawecki et al. (28) reported that a stimulus complex paired with orally self-administered ethanol did not function as a

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CS^R unless an intranucleus accumbens amphetamine infusion preceded the session. These data suggested the stimulus paired with oral ethanol self-administration could function as a weak CS^R whose reinforcing efficacy could be amplified by microinjection of amphetamine into the nucleus accumbens.

However, there were several issues that this study (28) did not address. First, the levels of responding maintained by the ethanol-paired stimulus complex were very low. These low levels of responding limited the ability to analyze response patterns maintained by the stimulus complex. In addition, the different operants employed in the new response study (i.e., lever press vs. lick response) made it difficult to compare response patterns maintained by ethanol and the stimulus complex. It was thought an extinction paradigm might resolve these issues. Using the extinction paradigm, the efficacy of a CS^R can be assessed by analyzing the rate of extinction in the presence vs. the absence of the stimulus complex. In addition, the relatively high levels of responding maintained early during extinction allows for a more in depth analysis of response patterns. The purpose of this study was to examine the ability of orally self-administered ethanol to establish a CS^R. It was hypothesized that a stimulus complex paired with oral ethanol self-administration would function as a CS^R and prolong responding during extinction in the presence of the stimulus complex.

METHOD

Animals

Male Long–Evans rats ($n = 60$) obtained from Harlan–Sprague–Dawley (Indianapolis, IN) were used in this study. At the start of the experiment, the stimulus complex paired (SC-paired, $n = 42$) group weighed an average of 217 ± 2 (SD) g (range: 184–239 g). The control (con, $n = 18$) group weighed an average of 219 ± 2 (SD) g (range: 204–229 g). All rats were housed individually in standard hanging cages with food and water available ad lib, except as noted below. The rats were maintained on a 12 L:12 D cycle (lights on at 0600 h). Animal care was in accordance with NIH guidelines.

Apparatus

Ethanol self-administration sessions were performed in modular operant chambers (Med-Associates; East Fairfield, VT). Operant chambers (30 × 30 × 24.5 cm) were equipped with two levers, two stimulus lights, an automatic dipper, a house light, and a sonalert (Mallory #SC268F). The house light was mounted 2 cm below the ceiling on the back wall of the chamber. The automatic dipper was located in the center of the front wall of the chamber. The active lever was mounted to the right of the automatic dipper. A stimulus light and sonalert were mounted immediately above this lever. The sonalert (2900 ± 500 Hz, 75–85 dB) was connected in series to a 3-K ohm resistor. Each chamber was housed in a sound-attenuating enclosure. A fan inside each enclosure masked external noise.

Drugs and Solutions

Ethanol solutions were prepared volume/volume (v/v) in water from 95% (v/v) ethanol. Sucrose solutions were prepared weight/volume (w/v) in water. The sodium pyrophosphate buffer used for the blood ethanol determinations was composed of: 7.4 mM sodium pyrophosphate ($Na_4P_2O_7$), 7.6 mM semicarbazide, 22 mM glycine, and 10 ml 2 N sodium hydroxide (NaOH)/300 ml buffer.

Blood Sample Collection and Blood Ethanol Determination

Immediately following selected ethanol self-administration sessions, selected rats were restrained while a 100 μl blood sample from the tip of the tail was collected in a heparinized capillary tube. Evaluation of blood ethanol levels was accomplished by enzymatic analysis (3). Only selected rats had blood ethanol levels determined because blood was collected following completion of a second protocol (data not reported). Only 34 rats completed the second protocol.

Videotape Analysis

Observation during extinction sessions revealed dipper approach behavior in the SC-paired group. As a result, an ad hoc videotape analysis was performed during selected ethanol self-administration and extinction sessions. Only selected rats from the SC-paired ($n = 4$) and the con ($n = 3$) groups were videotaped to determine dipper entries following presentation of the stimulus complex because this behavior was not noted until late in the experiment. A dipper entry was defined as the placement of the head into the recessed dipper well. Total session dipper entries and dipper entries associated with stimulus complex presentations were recorded. The ratio of stimulus complex associated dipper entries to the total number of stimulus complex presentations was then calculated. Dipper entries associated with stimulus complex presentation occurred within 10 s of the stimulus complex presentation. Videotapes were rated by two individuals, and dipper entry estimations between observers concurred by >80%. *t*-Tests were used to examine differences in dipper entries between groups.

General Procedure

After the rats were received, two home-cage testing procedures were performed. First, a 3 day forced-ethanol test was performed, during which time 10% ethanol (10E) in water was the only fluid available to be consumed. This test was followed by a 14-day two-bottle home cage drinking test, during which time 10E and water were freely available. During home cage testing, the volume of each fluid consumed was recorded every 24 h. The bottles were then replenished and placed back onto the home cage. Placement of 10E with respect to water (left or right) alternated daily to prevent side preferences from being established.

Following home cage testing, operant training of a lever press began. The rats were fluid deprived 12 h prior to the first of three overnight (15-h) training sessions. During the first hour of the initial overnight session, lever pressing was shaped by reinforcing successive approximations. Each lever press (fixed ratio 1, FR1) was reinforced with a 3-s presentation of 0.1 ml of 20% sucrose. After the first overnight session, ad lib access to water in the home cage was reinstated and maintained for the duration of the experiment. After lever pressing was established (>500 responses/overnight session), daily 30-min sessions began.

Thirty-minute operant sessions were performed 5 days/week (Monday–Friday) at the same time each day. For the first 3 days, 10% sucrose (10S) was employed as the reinforcer. A sucrose-substitution procedure (22) was then used to initiate 10E as the reinforcer. Solution presentation during the sucrose-substitution procedure was as follows: 10% sucrose–2% ethanol for two sessions (10S2E), 10% sucrose–5% ethanol for two sessions (10S5E), 10% sucrose–10% ethanol for two sessions (10S10E), 5% sucrose–10% ethanol for two

sessions (5S10E), 2% sucrose–10% ethanol for five sessions (2S10E), 1% sucrose–10% ethanol (1S10E) for five sessions, and 10E for five sessions. When 10E maintained responding, over the course of 5–10 sessions the schedule of reinforcement was increased to an FR4. When responding was maintained by 10E on an FR4 with an average of <20% within-subject variability in total session responses, an ethanol concentration manipulation (ECM) procedure was performed. During the ECM procedure, the ethanol solution presented as the reinforcer was increased to 15% (15E), 20% (20E), and 30% (30E) for five sessions each. The ethanol concentration was then returned to 10E and remained at 10E for the duration of the experiment.

Pairing of a stimulus complex with reinforcer presentation in the SC-paired group began during the sucrose-substitution procedure. When ethanol was first added to the reinforcer (i.e., first session with 10S2E), a tone–light stimulus complex (tone on–light on) was paired with each reinforcer presentation during the session. All stimulus complex presentations were simultaneous and overlapping with reinforcer presentation. Pairing of the stimulus complex with reinforcer presentation was maintained for the duration of the experiment in the SC-paired group except during extinction. In the con group, there were no stimulus complex–reinforcer pairings during the initiation of ethanol self-administration. The con group was only exposed to the stimulus complex during extinction.

Extinction testing was performed following the ECM procedure. Each extinction test was run for five consecutive sessions (Monday–Friday) and at least five consecutive ethanol self-administration baseline sessions were performed between each extinction test. For the SC-paired group, the baseline ethanol self-administration condition was paired ethanol and stimulus complex presentation (ethanol + stimulus complex, ES condition). For the con group, the baseline ethanol self-administration condition was ethanol presentation without the stimulus complex (ethanol + no-stimulus complex, ENS condition). Two extinction conditions were employed. During the NENS extinction test, there were no programmed consequences associated with lever pressing (no-ethanol + no-stimulus complex, NENS condition). During the NES extinction test, only the stimulus complex was presented contingent upon completion of an FR4 (no-ethanol + stimulus complex, NES condition). During extinction, the dipper was not activated and solutions were not in the chamber. The order of the NES and NENS extinction tests was counterbalanced within each group. An additional manipulation for each group consisted of 5 consecutive days of ethanol self-administration according to the alternate group's baseline (SC-paired group = ENS condition; control = ES condition). Following completion of initial extinction testing, selected rats from the SC-paired and control groups were reexposed to selected extinction conditions for videotape analysis and/or blood ethanol levels were determined.

Data Analysis/Statistics

Daily ethanol intake in grams/kilograms/day (g/kg/day) was calculated from the volume of ethanol consumed during the forced ethanol test and two-bottle test. Ethanol preference ratios (10E ml/10E ml + H_2O ml) were calculated from the volume of each solution consumed during the two-bottle home cage test. Independent *t*-tests ($p < 0.05$) were used compare home cage drinking measures between groups during each home cage test (SigmaStat for Windows, Jandel Scientific).

Reinforcer presentations were used to calculate ethanol intake (g/kg) in both groups and the number of stimulus complex–reinforcer pairings in the SC-paired group. To calculate momentary response rates, a response episode was defined as at least four consecutive responses (i.e., completion of an FR4) with interresponse times (IRTs) no greater than 1 min between each response. IRTs greater than 1 min signaled the end of a response episode. The total time spent responding (total session time) was defined as the cumulative time required to emit all session responses with IRTs >1 min and postreinforcement pauses not included in the cumulative time measurement. Similar, cumulative time measurements were calculated for the first response episode and the first half of total session responses. Momentary response rates (total session rate, half session rate, and first episode rate) were then calculated by dividing the appropriate number of responses (total responses, half session responses, first episode responses) by the appropriate time required to emit those responses (total session time and half session time, first episode time). These measures have previously been described (27).

Unless otherwise noted data are presented as the mean ± standard error of the mean (SEM). Two-way repeated-measures (RM) analysis of variance (ANOVA) were used to analyze: 1) total session responses and ethanol intake during the sucrose-substitution and ECM procedures, and 2) total session responses and response rates during extinction testing using SPSS for Windows Statistical Software Package (SPSS Inc.). Two-way RM ANOVA employed Type III Sum of Squares to account for the unequal group size. A Friedman's test (nonparametric repeated-measures ANOVA) as described by Zar (33) was used to compare the rank sums of responding in the SC-paired group between the NES and NENS conditions. Pearson product moment correlations were used to determine the relationship between ethanol intake and blood ethanol levels to ensure the ethanol solutions presented were being consumed. All *t*-tests and Pearson product moment correlations were performed using SigmaStat for Windows (Jandel Scientific). Visual analysis of cumulative response records was accomplished with SoftCR (Med-Associates; East Fairfield, VT).

RESULTS

Home Cage Testing

Average ethanol intake and ethanol preference ratios during the forced ethanol and two-bottle preference tests (Table 1) were similar to those previously reported by this laboratory (11,23). During the forced ethanol test, ethanol intake in the SC-paired group was significantly less ($p < 0.05$) than ethanol intake in the con group. Individual *t*-tests reported no significant difference between groups in consumption measures recorded during the two-bottle test.

Ethanol Self-Administration Training

At completion of ethanol self-administration training, there were no significant differences ($p = 0.11$) in body weight between the SC-paired (545 ± 50 g, SD) and the con (569 ± 61 g, SD) groups. There were significant decreases in responding, $F(6, 348) = 22.14$, $p < 0.001$, in both groups as the sucrose was removed from the solution during the sucrose-substitution procedure (Fig. 1: left, bars), but there was no significant difference, $F(1, 58) = 1.92$, $p = 0.171$, between groups (Fig. 1: left, open vs. closed bars). There were significant changes in ethanol intake, $F(6, 348) = 46.25$, $p < 0.001$, during

TABLE 1

HOME-CAGE DRINKING MEASURES DURING THE FORCED ETHANOL TEST AND TWO-BOTTLE HOME CAGE TEST IN THE SC-PAIRED AND CON GROUPS

	SC-Paired (n = 42)	Con Group (n = 18)
Forced ethanol intake (g/kg/day)	6.77 ± 0.16*	8.20 ± 0.30
Two-bottle ethanol preference ratio (%)	18.9 ± 2.0	19.8 ± 3.0
Two-bottle ethanol intake (g/kg/day)	1.54 ± 0.17	1.53 ± 0.23
Two-bottle ethanol volume (ml/day)	5.6 ± 0.5	5.5 ± 0.9
Two-bottle water volume (ml/day)	25.8 ± 0.9	23.2 ± 1.3
Total fluid volume (ml/day)	30.7 ± 0.8	28.57 ± 1.0

Data are presented as the mean ± SEM. Significant difference ($p < 0.05$): *difference between groups.

the sucrose-substitution procedure. Ethanol intake rose to a peak when 10S10E was employed as the reinforcer and decreased as the sucrose was removed. Overall ethanol intake in the SC-paired group during the sucrose-substitution procedure was significantly greater, $F(1, 58) = 4.37$, $p = 0.041$, than ethanol intake in the con group (Fig. 1: left, open vs. closed circles). Changes in responding and ethanol intake observed during sucrose-substitution are similar to those previously observed by this laboratory (11,22).

Statistically significant, $F(4, 232) = 16.78$, $p < 0.001$, changes in responding were observed during the ECM procedure. Total responding decreased compared to 10E in both groups when 20E and 30E were employed as reinforcers and increased back to initial 10E levels when 10E was reinstituted as the reinforcer (Fig. 1: 10E-2 vs. 10E-1). Increasing the ethanol concentration significantly increased ethanol intake, $F(4, 232) = 49.47$, $p < 0.001$, in both groups when 15E, 20E, and 30E were employed as reinforcers. These changes in responding and ethanol intake are similar to those previously observed in this laboratory (11,22). Ethanol-maintained responding, $F(1, 58) = 5.39$, $p = 0.024$, and ethanol intake, $F(1, 58) = 7.62$, $p = 0.008$, in the SC-paired group was significantly greater during the ECM procedure compared to the con group (Fig. 1: right, bars and circles). Differences in ethanol maintained responding between groups can be attributed to trends toward greater responding across all ethanol solutions by the SC-paired group, but ethanol intake in the SC-paired group was significantly greater than ethanol intake in the con group only when 30E and 10E (10E-2) were presented as the reinforcers.

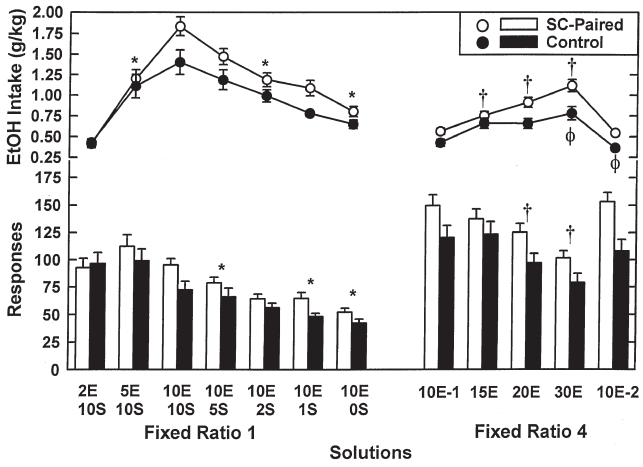

FIG. 1. Responses (bars) and ethanol intake (circles) during the sucrose-substitution (left) and ethanol concentration manipulation (right) procedures. Open symbols represent the SC-paired group (n = 42) and filled symbols represent the con group (n = 18). Data represent the mean ± SEM of all sessions for each solution. The order of the solutions on the x-axis represent the order of presentation during training: 10% sucrose–2% ethanol = 10S2E; 10% sucrose–5% ethanol = 10S5E; 10% sucrose–10% ethanol = 10S10E; 5% sucrose–10% ethanol = 5S10E; 2% sucrose–10% ethanol = 2S10E; 1% sucrose–10% ethanol = 1S10E; 10% ethanol = 10E; 15% ethanol = 15E; 20% ethanol = 20E; 30% ethanol = 30E. Significant differences ($p < 0.05$): *Significant difference (both groups) from prior solution; †significant difference (both groups) from first 10E exposure during ECM ϕ = significant difference between groups.

Extinction Tests

During extinction there were no statistically significant differences across baseline sessions in total session responding, $F(14, 770) = 1.49$, $p = 0.109$, total session momentary response rate, $F(14, 770) = 1.40$, $p = 0.148$, half-session momentary response rate, $F(14, 770) = 1.65$, $p = 0.06$, and first-episode momentary response rate, $F(14, 770) = 1.111$, $p = 0.345$. In addition, during the alternate self-administration conditions (SC-paired: ENS, con: ES) there were no differences in total session responding, $F(4, 220) = 0.95$, $p = 0.0.435$, total session momentary response rate, $F(4, 220) = 0.57$, $p = 0.683$, half-session momentary response rate, $F(4, 220) = 0.53$, $p = 0.716$, and first-episode momentary response rate, $F(4, 220) = 0.55$, $p = 0.699$, across successive sessions. Therefore, baseline and alternate ethanol self-administration data are presented as the group mean ± SEM calculated from within-subject averages of baseline ethanol self-administration sessions (n = 15) and alternate self-administration sessions (n = 5). There were also no statistically significant differences in total session responding, $F(1, 55) = 1.28$, $p = 0.263$, total-session momentary response rate, $F(1, 55) = 0.14$, $p = 0.712$, half-session momentary response rate, $F(1, 55) = 2.75$, $p = 0.103$, or first response episode response rate, $F(1, 55) = 0.05$, $p = 0.828$, based on the order of extinction conditions (i.e., NES first or NENS first). Therefore, extinction tests are not presented separately based on the order of extinction.

At the end of the extinction phase of the experiment there were no statistically significant differences ($p = 0.19$) in body weight between the SC-paired (570 ± 58 g, SD) and con groups (592 ± 61 g, SD). For the SC-paired group, an average of 82 ± 10 (range: 68–100) sessions were run prior to extinction testing with an average of 42 ± 12 reinforcers per session (range: 24–64) presented. As a result, >3000 stimulus complex–ethanol pairings were experienced by rats of the SC-paired group prior to extinction. During the extinction tests, three rats in the SC-paired group were sacrificed due to health issues (i.e., broken teeth and skin infection). These subjects are not included in the extinction results.

Total Session Responses

Baseline responding in the SC-paired group was significantly greater, t-test, $p = 0.010$, than responding in the con group (Fig. 2: top, SC-paired:ES vs. bottom, con:ENS). In the SC-paired group, omission of the stimulus complex during ethanol self-administration did not significantly alter respond-

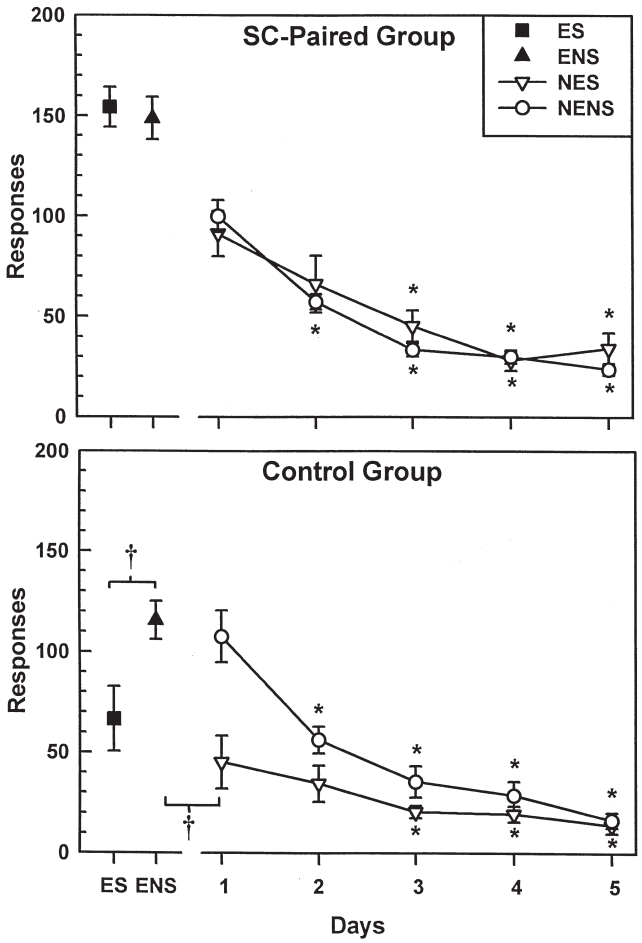

FIG. 2. Total session responding in the SC-paired (top, $n = 39$) and control (bottom, $n = 18$) groups. Filled symbols represent total session responses during ethanol self-administration (ES = filled squares, ENS = filled triangle). Open symbols represent responding during extinction testing (NES = open triangles, NENS = open circles). Data are presented as mean ± SEM. ES = ethanol + stimulus complex. ENS = ethanol + no stimulus complex. NES = no ethanol + stimulus complex. NENS = no ethanol + no stimulus complex. Significant differences ($p < 0.05$): †significant difference between bracketed symbols; *significant difference from day 1 of the same extinction condition.

ing (paired t-test, $p = 0.329$) compared to baseline (Fig. 2: top, ES vs. ENS). Presentation of the stimulus complex during ethanol self-administration in the con group significantly decreased responding (paired t-test, $p = 0.003$) compared to baseline (Fig. 2: bottom, ES vs. ENS).

In the SC-paired group responding significantly decreased, $F(5, 185) = 59.78$, $p < 0.001$, during the NES extinction test. Responding on days 3–5 was significantly less than responding on day 1, and responding on day 1 was significantly less than during the ethanol self-administration baseline (Fig. 2: top, open triangles). During the NENS extinction test responding significantly decreased, $F(5, 185) = 100.05$, $p < 0.001$, in the SC-paired group. Responding on days 2–5 was significantly less than responding on day 1, and responding on day 1 was less than during ethanol self-administration baseline (Fig. 2: top, open circles). These data indicate responding decreased slightly more rapidly during the NENS extinction tests compared to the NES extinction tests in the SC-paired group once extinction began.

In the con group, responding significantly, $F(5, 80) = 41.72$, $p < 0.001$, decreased during the NES extinction test. Responding on days 3–5 was less than on day 1, and responding on day 1 was less than during ethanol self-administration baseline (Fig. 2: bottom, open triangles). Responding also significantly decreased in the con group during the NENS extinction test, $F(5, 80) = 37.06$, $p < 0.001$. During the NENS extinction test responding on days 2–5 was significantly decreased compared to day 1 (Fig. 2: bottom, open circles). A comparison of the rate of extinction during the NENS extinction test in both groups revealed a significant group by day interaction, $F(5, 275) = 3.46$, $p = 0.005$, because a significant decrease in responding on day 1 compared to baseline was observed in the SC-paired group but not in the control group (Fig. 2). Responding on days 2–5 during the NENS extinction test compared to day 1 was not different between groups.

Representative response patterns during ethanol self-administration and extinction in the SC-paired group are depicted in Fig. 3. Response patterns maintained by 10E (ES and ENS) were characterized by high response rates and discrete response episodes as previously observed by our laboratory (25). Further, responding during the NES extinction test tended to be greater compared to the NENS extinction test in the SC-paired group (i.e., greater on days 1, 3, and 5). This tendency was not apparent when examining mean levels of responding in the SC-paired group (Fig. 2, top). However, it is more apparent when the distribution of responding during the NES and NENS conditions is depicted with box plots (Fig. 4). A Friedman RM ANOVA on Ranks reported a significant difference ($\chi^2 = 186.5$, $p < 0.0001$) in responding between the NES and NENS conditions in the SC-paired group. This test indicates the rank sum is greater on days 1–4 (days 2 and 4, not presented) of the NES condition compared to the NENS condition (Fig. 4). That is, the range of responses above than the median was larger in the NES condition (approximately 75–400) compared to the NENS condition (approximately. 80–350) on day 1 of each condition.

Response Rates

During the NES extinction test [first-episode rate, $F(4, 220) = 0.2$, $p = 0.94$; half-session rate, $F(4, 220) = 0.34$, $p = 0.853$; total-session rate, $F(4, 220) = 1.57$, $p = 0.183$], and NENS extinction test there were no statistically significant differences in response rates [first-episode rate, $F(4, 220) = 0.90$, $p = 0.468$; half-session rate, $F(4, 220) = 1.03$, $p = 0.392$; total-session rate, $F(4, 220) = 1.97$, $p = 0.100$] across the five consecutive sessions within each condition in either group. However, there were trends indicating differential response rates on day 1 of the NES and NENS extinction tests. Extinction response rate data for the NES and NENS extinction tests are presented as the average ± SEM for each group on day 1 (Fig. 5).

Statistically significant differences in response rate were observed within each group between extinction conditions [first-episode rate, $F(3, 165) = 4.76$, $p = 0.003$; half-session rate, $F(3, 165) = 10.80$, $p < 0.001$; total-session rate, $F(3, 165) = 20.52$, $p < 0.001$]. There was also an interaction [group × extinction test] for the first response episode rate between groups, $F(3, 165) = 3.15$, $p = 0.027$. In the SC-paired group, total-session, half-session, and first-response episode response

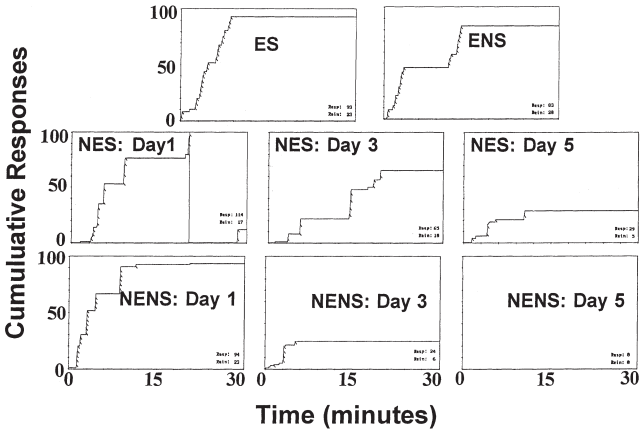

FIG. 3. Representative patterns of responding from a single subject in the SC-paired group. Ethanol was presented contingent on lever pressing during the ES and ENS conditions. NES day 1–NES day 5 represent response patterns on days 1, 3, and 5 of the NES extinction test. NENS day 1–NENS day 5 represent response patterns on day 1, 3, and 5 of the NENS extinction test. Upward pen deflections represent responses, and diagonal crosshatches represent completion of the fixed ratio 4 schedule of reinforcement. Total session length was 30 min. ES = ethanol + stimulus complex. ENS = ethanol + no stimulus complex. NES = no ethanol + stimulus complex. NENS = so ethanol + so stimulus complex.

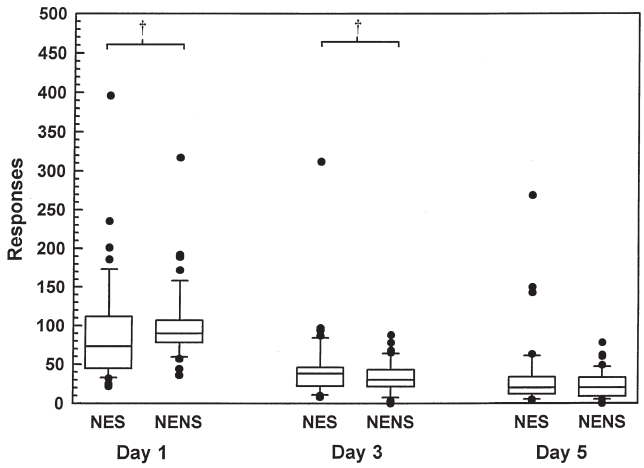

FIG. 4. Box plot depicting the distribution of responses in the SC-paired group ($n = 39$) during days 1, 3, and 5 of the NES and NENS extinction tests. Each box covers the 25th to the 75th percentiles. Error bars indicate the 10th and 90th percentiles. Filled circles depict responses outside of the 10th and 90th percentiles. NES = no ethanol + stimulus complex. NENS = no ethanol + no stimulus complex. Significant differences ($p < 0.05$): = significant difference between brackets.

rates during the NENS condition were greater than baseline ethanol self-administration response rates (Fig. 5: open bars, ES vs. NENS). During the NES condition half-session response rate and total-session response rate were significantly greater than baseline ethanol self-administration response rates, but the first episode response rate was not (Fig. 5: open bars, ES vs. NES). These data indicate that contingent presentation of the stimulus complex during the NES extinction test attenuated the "extinction burst" responding observed in the first response episode during the NENS extinction test. Total-session, half-session, and first-response episode response rates during the NENS extinction test were greater than baseline ethanol self-administration response rates in the con group (Fig. 5: closed bars, ENS vs. NENS). The first-episode response rate also tended to be greater than baseline ethanol self-administration in the con group during the NES and NENS extinction test (Fig. 5: closed bars, ENS vs. NES). There were no significant differences in ethanol self-administration response rates between the SC-paired and con groups (Fig. 5: open ES vs. Closed ENS).

Cumulative records depicting the changes observed in response rates on the first day of the NES and NENS extinction tests in the SC-paired group are shown in Fig. 6. Bracketed regions (|———|) represent the areas of interest. Visual examination of response patterns reveals the initial response rate in the NES condition (Fig. 6: middle) is similar to the response rate during baseline (Fig. 6: top). In contrast, during the first day of the NENS extinction test (Fig. 6: bottom), response rates are elevated compared to both baseline and the NES extinction. The cumulative response record depicting response patterns during the NES extinction test (Fig. 6: middle) also demonstrates the shift to higher response rates observed during NES extinction (i.e., similar to the NENS condition) as the session progressed, thus showing the transient nature of this difference in response rate.

Dipper Entries

Videotape analysis of rats in the SC-paired and con groups when the stimulus complex was presented contingent on responding during extinction (NES extinction test) suggested different patterns of dipper entry between the groups, but these differences were not statistically significant ($p = 0.091$), most likely due to the low number of subjects examined. Rats in the SC-paired group tended to make a dipper entry when the stimulus complex was presented, but rats of the con group did not (Table 2: stimulus complex associated). In the SC-paired rats a dipper entry was associated with 92 ± 6% of the stimulus complex presentations during the NES condition. In the con group, dipper entry was only associated with 28 ± 15% of stimulus complex presentations during the NES condition. Differences in dipper entry cannot be attributed to differences in the tendency of these rats to make dipper entries because there was no difference in total session dipper entries during ethanol self-administration or during the NES extinction test.

Blood Ethanol Levels

Combined ethanol intake of both groups (SC-paired, $n = 21$; con , $n = 13$) averaged 0.31 ± 0.04 g/kg on the day blood samples were collected. This average intake is significantly ($t = 3.46$, $p = 0.0015$) less than the average ethanol intake of 0.48 ± 0.04 g/kg observed following completion of self-administration training, and resulted from significant increases body weight ($t = -3.08$, $p = 0.004$) and decreases in responding ($t = 3.24$, $p = 0.0028$) observed over the duration of the experiment. *t*-Tests reported no significant differences in ethanol intake, ($t = 1.22$, $p = 0.23$), or blood ethanol levels ($t = 1.92$, $p = 0.065$) between the SC-paired and con groups. Ethanol intake averaged 0.35 ± 0.06 g/kg in the SC-paired group. In the con group, ethanol intake averaged 0.25 ± 0.05 g/kg. Combined blood ethanol levels from both groups averaged 16.0 ± 2.5

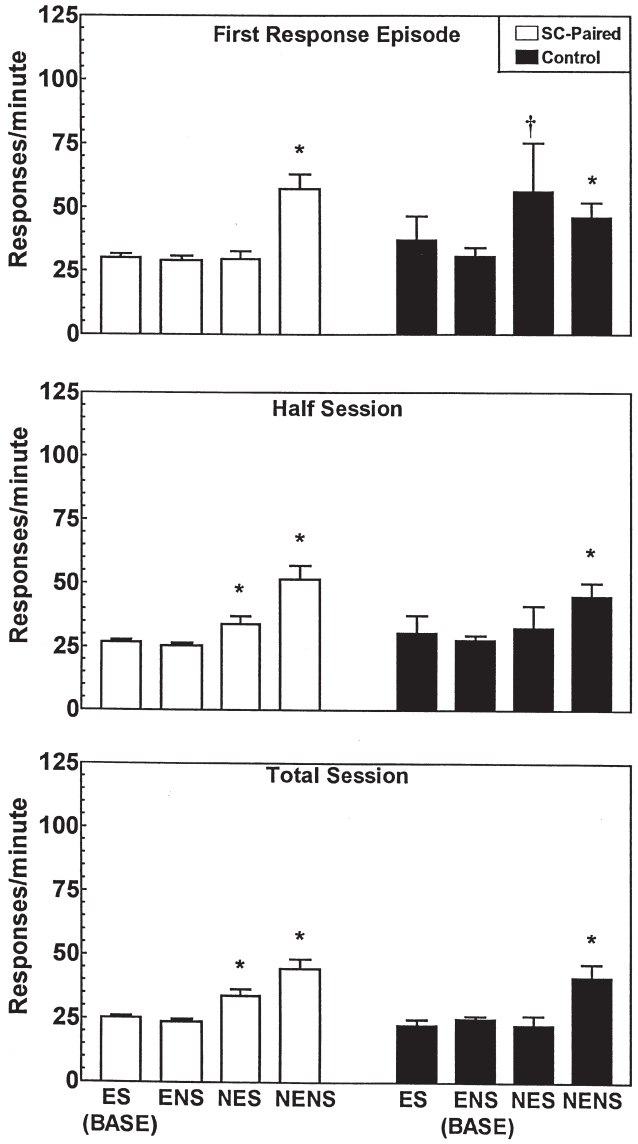

FIG. 5. First episode momentary response rate (top), half-session momentary response rate (middle), and total session episode momentary response rate (bottom) during ethanol self-administration and extinction in the SC-paired group (open bars, $n = 39$) and con group (filled bars, $n = 18$). Response rates for extinction tests (NES and NENS) represent only day 1. Data are presented as mean ± SEM. ES = ethanol + stimulus complex. ENS = ethanol + no stimulus complex. NES = no ethanol + stimulus complex. NENS = no ethanol + no stimulus complex. Significant differences ($p < 0.05$): *significant differences from baseline response rate (SC-paired = ES, con = ENS) within groups, †significant differences in response rates between groups during the same extinction condition.

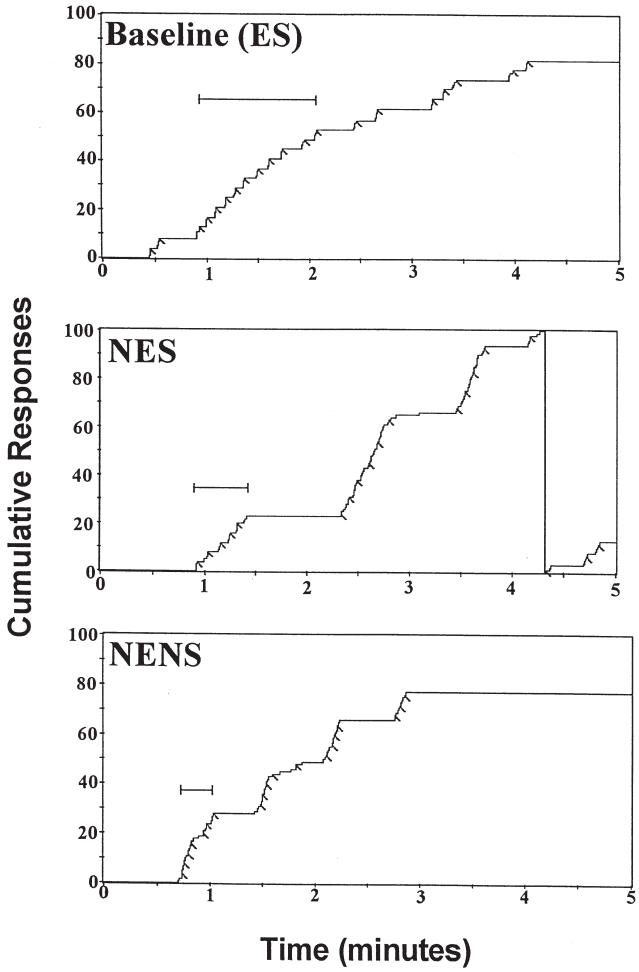

FIG. 6. Representative patterns of responding during the first 5 min of three operant sessions from a single subject in the SC-paired group. Baseline (ES) depicts response patterns when ethanol was presented contingent on lever pressing. NES depicts response patterns on day 1 of the NES extinction test. NENS depicts response patterns on day 1 of the NENS extinction test. Upward pen deflections represent responses, and diagonal crosshatches represent completion of the fixed ratio 4 schedule of reinforcement. Total session length was 30 min. ES = ethanol + stimulus complex. NES = no ethanol + stimulus complex. NENS = no ethanol + no stimulus complex. Bracketed region (|———|) in each cumulative record depicts the areas of interest.

mg/dl. In the SC-paired group, blood ethanol levels averaged 19.5 ± 3.4 mg/dl (range = 0–49.6 mg/dl). In the con group, blood ethanol levels averaged 10.0 ± 2.7 mg/dl (range = 0.0–27.3 mg/dl). There was a significant positive correlation ($r^2 = 0.78$, $p < 0.0001$) between ethanol intake and blood ethanol levels when both groups were combined ($n = 33$), suggesting both groups were consuming the ethanol.

DISCUSSION

When presented contingent on responding during extinction, a stimulus complex paired with ethanol presentation slightly attenuated the rate of extinction. This effect in the SC-paired group during the NES extinction test was characterized by 1) slightly more responding during extinction, 2) a larger absolute range of responding above the median, and 3) a transient attenuation of extinction burst responding. The maintenance of responding by a reinforcer-paired stimulus has been suggested to indicate the stimulus functions as a CS^R (4,7,13,14,29). Using the extinction paradigm, Hill (14) and Bugelski (4) have reported that the sound made by a food hopper previously associated with food reinforcement main-

TABLE 2

TOTAL SESSION DIPPER ENTRIES AND DIPPER ENTRIES ASSOCIATED WITH STIMULUS COMPLEX PRESENTATIONS IN THE SC-PAIRED GROUP (n = 4) AND CON GROUP (n = 3) DURING ETHANOL SELF-ADMINISTRATION (SC-PAIRED = ES, CON = ENS) AND DURING EXTINCTION (NES EXTINCTION TEST)

	SC-Paired (n = 4)		Control (n = 3)	
Dipper Entries	Baseline (ES)	NES	Baseline (ENS)	NES
Total session	61.2 ± 7.6	52.0 ± 6.2	51.3 ± 8.0	44.3 ± 12.3
Stimulus complex associated	14.5 ± 3.1	11.3 ± 4.0	NA	1.3 ± 0.9

Data are presented as the means ± SEM. ES = Ethanol + Stimulus complex. ENS = Ethanol + No Stimulus complex. NES = No Ethanol + Stimulus complex.

tained and reinitiated lever pressing during extinction. Similar effects have been observed for self-administered drugs of abuse, such as ethanol (29) and morphine (7). Therefore, the tendency towards prolonged responding during the NES extinction test in the present study suggests the stimulus complex might function as a CS^R. However, it is important to note that the effects observed, although statistically significant, were small and transient, suggesting the contribution of the stimulus complex to the maintenance of responding was low. It might be suggested the slight maintenance of responding observed could be due to the previous association of the stimulus complex with sucrose–ethanol solutions. Although this cannot be ruled out with the present data, we feel this is unlikely given the more recent and prolonged association of the stimulus complex with ethanol solutions compared to sucrose–ethanol solutions.

The stimulus complex suppressed responding in the con group when presented during ethanol self-administration or extinction. This can most likely be attributed to external inhibition of responding by the novel stimulus complex (26). Ideally, the stimulus complex would not have altered responding in the control group; however, this decrease is not a confounding factor. Traditionally, randomly paired control groups are employed to show that the maintenance of responding by reinforcer-paired stimuli cannot be attributed to the stimulus complex functioning as a primary reinforcer (2). As such, the decrease in responding in the control group during NES extinction suggests the stimulus complex is not a primary reinforcer, and the maintenance of responding in the SC-paired group can be attributed to the association of the stimulus complex with ethanol reinforcement. It should also be noted that the traditional random control was not employed in the present study due to the pattern of responding during ethanol self-administration sessions. It was our concern that the high concentration of ethanol-maintained responding early in the operant session might result in unintended pairings of the stimulus complex with ethanol presentation in a random pairing situation. The use of a naive control group allowed us to avoid this problem.

As previously observed in the new response paradigm (28), a stimulus complex paired with orally self-administered ethanol did not function as an efficacious CS^R. The reasons for the stimulus complex not functioning as an efficacious CS^R might be partially explained by a number of procedural factors such as the pairing procedure (16), the number of pairings (10), and the level of food restriction (19). However, these data are generally consistent previous studies that have employed ethanol to examine conditioned reinforcement (13,29), and although there are indications of conditioned reinforcement to varying degrees, the effect is usually transient. Smith et al. (29) reported the maintenance of responding by a stimulus paired with intragastrically self-administered ethanol, but responding was only maintained for 2–4 h. Recently, Heyser et al. (13) reported the maintenance of responding during extinction and reinstatement of responding by a stimulus complex paired with orally self-administered ethanol, but the effect was small and transient. These studies (13,28,29) suggest that environmental stimuli paired with ethanol reinforcement do not function as highly effective conditioned reinforcers, which maintain operant behavior. However, the increased responding in the SC-paired group might suggest discriminative stimuli play an important role in the acquisition of ethanol self-administration.

When the stimulus complex was presented to the SC-paired group a dipper entry followed, but similar behavior was not observed in the con group. Although only observed in a small sample of subjects, this response is similar to the discriminated approach behavior observed during training in the new response paradigm (5). This suggests the stimulus complex functioned as a discriminative stimulus (CS^D). Although not established as a traditional CS^D, the stimulus complex was established as a cue, indicating that upon dipper approach ethanol would be available. Therefore, the tendency for response patterns during the NES extinction test to be similar to those observed during ethanol self-administration could be the result of generalization (10,32) in the presence of a CS^D. That is, the differences between extinction and ethanol self-administration conditions were minimized when the stimulus complex continued to be presented.

Discriminative stimuli associated with ethanol consumption (6,9,18) have been reported to increase ethanol intake. The increased responding and ethanol intake in the SC-paired group suggests pairing of the stimulus complex with ethanol also enhanced ethanol-maintained responding in this study. This increased responding is not attributed to an innate preference for ethanol in the SC-paired group because this group consumed less ethanol during the forced-ethanol test, and ethanol intake during the two-bottle preference test was not different from the control group. However, it should be noted that ethanol intake and ethanol-maintained responding in the SC-paired group was not greater than intake and responding typically observed in this laboratory (11,15), and responding was not altered when the stimulus complex was omitted during ethanol self-administration. Potentially, prolonged self-administration training in the presence of a stimulus complex that indicated reinforcer delivery might have increased stimulus control over responding. That is, the stimulus complex may have enhanced the relationship between lever pressing and ethanol delivery, as suggested by Falk (8). Therefore, the rats remained engaged in lever pressing and ethanol consumption.

This study examined the ability of orally self-administered ethanol to establish a tone–light stimulus complex as a CS^R. The data suggest that the stimulus complex could function as a CS^R, but its overall contribution to the maintenance of lever pressing was minimal. This weak efficacy of the stimulus complex as a CS^R might suggest other stimuli more closely associated with ethanol intake might be better suited to maintain responding prior to the onset of ethanol's neuropharmacolog-

ical activity. The association of ethanol's taste with ethanol's neuropharmacological activity during initiation suggests these taste stimuli might function as conditioned reinforcers. There were also indications the stimulus complex was established as a CS^D, suggesting the discriminative stimulus properties of the stimulus complex might have influenced responding during extinction. Overall, these data suggest that in the present paradigm the ability of a stimulus complex paired with orally self-administered ethanol to function as a conditioned reinforcer is minimal.

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