Enhanced conditioned “liking” of novel visual cues paired with alcohol or non-alcohol beverage container images among individuals at higher risk for alcohol use disorder

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Abstract
Rationale/Objective This study used an evaluative conditioning (EC) procedure to assess the affective properties of a CS for ingested drug reward in humans. Specifically, the study tested whether the evaluative response (“liking”/“disliking”) to an arbitrary visual stimulus (“CS2,” e.g., a purple hexagon) could be changed through pairings with an alcohol or non-alcohol beverage cue (“CS1,” e.g., a full wine glass, a juice box), which is ostensibly a conditioned visual predictive stimulus for alcohol or non-alcohol liquid reward, respectively.

Methods Participants (N=369, 18–23 years, 66% female, 79% white, 21% reporting no alcohol use ever or in the past year) received 24 CS1 pairings with each CS2. CS2 and CS1 evaluations were assessed pre- and post-conditioning.

Results Alcohol and non-alcohol CS2 “liking” correlated with alcohol use. “Liking” of the alcohol but not non-alcohol CS1 also correlated with alcohol use. Alcohol CS1 “liking” also correlated with alcohol and non-alcohol CS2 ‘liking,” whereas non-alcohol CS1 ‘liking” correlated with non-alcohol but not alcohol CS2 “liking.”

Conclusions Taken together, findings support the idea that drug-related visual stimuli acquire appetitive (hedonic and/or incentive) properties as a function of individual differences in drug use, which entail individual differences in exposure to the conditioning effects of addictive substances like alcohol. Findings also suggest a link between drug use and the propensity to attribute affective/motivational significance to reward-predictive cues in general.

Keywords Addiction · Affective · Evaluative · Higher-order conditioning · Liking · Wanting

Introduction

Theorists have long appealed to associative learning accounts to explain how experimentation with drugs of abuse, like alcohol, can progress to regular and then problematic use, as well as why substance users so frequently relapse to problematic use despite verbalizing intentions to abstain or moderate use. Distinct learning-based accounts have been offered to explain these aspects of addictive behavior (for review, see (Niaura et al., 1988)). An especially influential and important model comes from Stewart et al. (1984). Based on studies conducted using people with substance use disorders (SUD) as well as with nonhuman animal models of drug cue reactivity, Stewart et al. (1984) proposed that a natural consequence of repeated drug use was the emergence of conditioned positive-affective or appetite-motivational reactivity to drug-predictive cues. Since then, observed drug cue reactivity in humans—often elicited using visual stimuli depicting drug use, self-administration devices, and paraphernalia—is routinely interpreted as evidence for affective/motivational properties putatively conditioned by drug use in the natural environment. The validity of such inferences, however, has remained largely untested (for review and discussion, see Robbins & Ehrman (1992)).

A stimulus that has been paired with a drug can be shown to possess some affective or motivational significance to an individual. With respect to drug stimuli, one commonly used method of testing for affective-motivational significance is the conditioned place preference/avoidance (CPP/CPA) paradigm (for review, see Bardo...
and Bevins 2000; Huston et al. 2013). In a drug CPP/CPA paradigm, animals are repeatedly exposed to two or more distinct contexts, one of which is repeatedly paired with the internal stimulus effects of the drug being tested. In a subsequent drug-free test, the amount of time an animal spends in the drug-paired vs. non-paired context is measured as an index of the animal’s evaluation of each context based on its experiences in each context. If the animal spends significantly more time in the drug-paired than non-paired context (i.e., displays drug CPP), it is inferred that the drug stimulus has appetitive (reinforcing) properties.

Although CPP/CPA and related paradigms are thought of as preclinical (nonhuman animal) models of human affective-evaluative learning, there is growing experimental evidence for the ability of post-ingestive drug psychopharmacology to induce conditioned cue preferences de novo in humans using CPP/CPA-like paradigms (e.g., Childs & de Wit, 2009, 2016; Mayo et al., 2013; Mayo & de Wit, 2016)). These de novo conditioned preferences arguably represent instances of affective-evaluative conditioning (EC). EC refers to a change in the evaluative response to a conditional stimulus (CS) due to its pairing with an affectively charged (valenced) unconditional stimulus (US, e.g., drug psychopharmacology). The evaluative response refers to expression of “liking”/“disliking” of the CS or what it represents (e.g., the US or responses to it). Humans can self-report their evaluative response to a stimulus (e.g., appeal or desirability ratings), eliminating the need to infer “liking” or “disliking” from movement toward or time spent near or away from the stimulus. Indeed, there exists an extensive body of work in the human experimental psychology literature devoted to EC effects on initially neutral or arbitrary stimuli repeatedly paired with affectively charged auditory and/or visual images (for review, see De Houwer et al., 2001; Hofmann et al., 2010)).

A valuable approach to assessing the affective and motivational properties of a CS is to test its ability to support new learning. This test would examine if the CS can serve as a conditioned reinforcer (or punisher) for new actions. Alternatively, it would test if the CS can support classical conditioning of novel CS. For instance, the sight of an alcohol beverage container (e.g., a beer can) is ostensibly a visual CS for alcohol. One approach to examining its affective and motivational properties would be to test if it can support classical conditioning of a new visual CS, like a neutral object (e.g., green triangle). If the beverage container has acquired affective and/or motivational properties from its association with alcohol, then it may be able to transfer these properties to an initially neutral object (e.g., green triangle) via beverage container-neutral object pairing, such that the initially neutral object comes to possess the same affective and/or motivational properties as the alcohol beverage container.

Given that clinical researchers in the area of addiction have long hoped to reshape the evaluative response of individuals to a drug and/or its cues, it is not surprising that there are many reports of EC experiments in which drug related-images are the CSs (e.g., Houben et al., 2010a, b; Houben et al., 2010a, b; Magurean et al., 2016; Noel et al., 2019; Tello et al., 2020)). In contrast, reports of EC experiments in which drug-related images are the USs are rare (e.g., Deweese et al., 2016; Littel & Franken, 2012)). Few published experiments exist that pair an arbitrary stimulus with an image that depicts a drug. Evidence that drug images (viz., visual cues for drug) promote conditioning to an arbitrary stimulus would support the idea that drug images have conditioned reinforcing (appetitive) properties (cf. Freeman et al. 2012, 2013 as notable exceptions). The absence of such experiments from the scientific record is surprising given that their results would speak to the validity of the idea that observed drug cue reactivity reflects conditioned affective and motivational properties and to the proposal of Stewart et al. (1984) that drug use begets drug cue-conditioned appetitive-motivational states that promote drug use.

The current study and hypotheses

Few studies have used EC as an index of the affective properties of a CS for ingested reward US in humans (e.g., Christoffersen et al., 2017; Fleming et al., 2021; Viemose et al., 2013)). The current study tested whether the evaluative response to a simple visual stimulus, a colored shape (“CS”), could be changed by pairings with the sight of an alcohol or non-alcohol beverage container (“CS”), each of which is ostensibly a visual CS for alcohol or non-alcohol liquid reward, respectively. Based on the conditioned incentive account of drug cue reactivity proposed by Stewart et al. (1984), the primary hypothesis was that the alcohol beverage container (drug CS) would support conditioning of a positive evaluative response to an arbitrary colored shape (CS), as a function of the heaviness of alcohol use history. The non-alcohol beverage container (non-drug CS) also was expected to support conditioning of a positive evaluative response to an arbitrary colored shape (CS), but independently of alcohol use history.

Method

Participants

Participants were undergraduates who earned credit toward a research experience requirement in Introductory Psychology courses at a large, public university in the Midwestern USA. A total of 448 participants were recruited between
June 2020 and May 2021. A total of 28 were excluded from the analyses due to the following: (a) age outside the target range (target age: 18–23 years; n = 1 self-reported age as 38 years); (b) failure to follow instructions (e.g., took a break during the conditioning task [n = 4]); (c) Internet connectivity loss (n = 1); or (d) reported lack of sleep the night before study days 1 (n = 7) or 2 (n = 15). Of the remaining 420 individuals, 369 (88%) completed both study 1 and study 2 procedures. Supp See Table 1 for participants’ demographic characteristics. Neither inclusion status nor day 2 completion status was associated with sociodemographic characteristics.

Alcohol use was assessed using a question set recommended by the National Institute on Alcohol Abuse and Alcoholism (2003) (for item language and response scaling, see Online Supplemental Information). Specifically, participants were asked to indicate typical drinks per drinking day and drinking days per week, heaviest drinking episode and frequency of such episode, and frequency of binge drinking (5+/4+ drinks in 2 h for men/women), all during the past year. Participants also reported the maximum drinks consumed in one episode in their lifetime, age at first drunk, and age at start of regular drinking. Participants also completed the Brief Young Adult Alcohol Consequences Questionnaire (BYAACQ) Total score. Scale max. 24. By scores ≥10, individuals are likely to be experiencing several psychosocial consequences of alcohol use, and by scores ≥15, multiple AUD symptoms and increasing distress or impairment are likely (Kahler et al., 2005). Data from the N = 293 individuals who reported alcohol use in the past year. Pearson correlation coefficients are shown in rightmost column. ***p < .001

Table 1 Sample characteristics

| Characteristic | Med (IQR) | n (%) |
|----------------|-----------|-------|
| Age, yr        | 18 (1)    |       |
| Female         | 242 (66)  |       |
| Ethnicity      |           |       |
| Hispanic       | 14 (4)    |       |
| Race           |           |       |
| American Indian/Alaskan Native | 1 (< 1)  |       |
| Native Hawaiian/Pacific Islander | 0 (0)    |       |
| Asian          | 13 (3)    |       |
| Black          | 37 (10)   |       |
| White          | 292 (79)  |       |
| Multiple selected | 25 (7)   |       |
| Other          | 1 (< 1)   |       |
| Handedness     |           |       |
| Right dominant | 331 (90)  |       |
| Total N        | 369       |       |

Table 2 Alcohol use measures

| Measure                      | Med (IQR) | Min–max | Correlation with AlcQF composite scores |
|------------------------------|-----------|---------|----------------------------------------|
| Age first drunk (yr)         | 17 (2)    | 14–21   | −0.27***                                |
| Age reg. drink (yr)          | 18 (1)    | 14–22   | −0.18**                                 |
| DDPW past year               | 0.62 (0.85)| 0.03–5.50| 0.87***                                |
| DPDD past year               | 3.50 (2.00)| 1.00–21.50| 0.44***                                |
| MaxQ past year               | 6.00 (8.50)| 1.00–29.50| 0.48***                                |
| MaxF past year               | 0.03 (0.11)| 0.03–3.50| 0.32***                                |
| BPW past year                | 0.25 (0.48)| 0.03–5.50| 0.76***                                |
| MaxQ lifetime                | 9.50 (8.50)| 1.00–29.50| 0.49***                                |
| Alcohol problems             | 4 (6)     | 0–23    | 0.57***                                |

First drunk, first time got drunk (speech slurred or unsteady on your feet). Reg. drink, regular drinking (at least once a month for 6 months of more). DDPW, drinking days per week. DPDD, drinks per drinking day. AlcQF, alcohol quantity-frequency product (DDPW×DPDD). MaxQ, maximum quantity (no. of drinks) consumed in a 24-h window. MaxF, frequency of MaxQ per week. BPW, binges per week. Alcohol problems, past month Brief Young Adult Alcohol Consequences Questionnaire (BYAACQ) Total score. Scale max. 24. By scores ≈10, individuals are likely to be experiencing several psychosocial consequences of alcohol use, and by scores ≥15, multiple AUD symptoms and increasing distress or impairment are likely (Kahler et al., 2005). Data from the N = 293 individuals who reported alcohol use in the past year. Pearson correlation coefficients are shown in rightmost column. ***p < .001

1 Of the 369 individuals who completed both study day 1 and study 2 procedures, 49 (13%) reported alcohol use the night before study day 1, and 44 (12%) reported alcohol use the night before study day 2. Alcohol use the night before study day 1 could affect their learning on study day 1, and alcohol use the night before study day 2 could affect memory expression on study day 2. However, given that these individuals were on heavier end of the alcohol use spectrum (see Online Supplemental Information), exclusion of their data would limit the range of individual differences in alcohol use represented in our analyses. Thus, their data were included in all analyses. Importantly, however, exclusion of their data from analyses did not change the pattern of results, although it did increase the significance of some model-estimated mean comparisons.
Materials

Visual stimuli

Stimuli for the CS₁ (made in Inkscape; www.inkscape.org) consisted of simple geometric shapes filled with a solid color: a blue hexagon, a green triangle, a purple circle, a red pentagon, and a yellow square. A smaller solid, white-filled version of the same shape was placed inside each stimulus. The green triangle, purple circle, and yellow square were used in the conditioning task. The blue hexagon and red pentagon were not used and served as nonconditioned control (NC) stimuli. Stimuli for the CS₂ consisted of alcohol and non-alcohol beverage containers photographed in a real-world context: a wooden table with wooden booth-style seating typical of pubs and restaurants. These were taken from a standardized picture set for alcohol cue reactivity studies, from which most, but not all, overt branding elements have been removed and which has been validated for alcohol craving induction in American adults (Lovett et al., 2015; Veilleux et al., 2018).

Conditioning task

A simple target detection decoy task was used to ensure that participants would be attentive and responsive to the occurrence of one stimulus category (the “targets”: fixation crosses) embedded in a train of ostensibly irrelevant stimuli (the “non-targets”: colored shapes, alcohol beverages, non-alcohol beverages). Target and nontarget stimuli were overlaid on an image of a wooden table and booth, and this background image was displayed throughout all inter-trial intervals (3 s fixed). Each participant was incidentally exposed to three stimulus relations in the stream of nontarget stimuli. One colored shape (henceforth, the alcohol CS₂ + paired with alcohol CS₁) predicted alcohol beverage container images (2 beers, 2 liquors, and 2 wine pictures). A second colored shape (henceforth, the non-alcohol CS₂ +) predicted non-alcohol beverage images (an orange juice, chocolate milk, water, soda pop, lemonade, and iced tea picture). A third colored shape (henceforth, CS₂ −) predicted the wood table/booth background image.

Each beverage image used was presumed to be a CS₁ for the depicted beverage and, more broadly, for the beverage category. Each unique beverage image was presented 4 times. A total of 24 shape-image pairings was given per shape, and the order of pairings was randomized. Unique shape-image mappings were counterbalanced across participants. Shape and image presentations each lasted 4 s. Time between shape and image onset was varied across participants via random assignment to delay, simultaneous, or trace conditioning procedures. For more details, see Online Supplemental Information.

Appeal ratings task (evaluative response measurements)

Participants rated the colored shapes on three occasions and rated the beverage images on two occasions (see Fig. 1). One image was presented at a time, and the participant had to answer six questions about it, one question at a time, before the next image was presented. A 101-point visual analog scale anchored at 0 (not at all) and 100 (extremely) was used to measure image ratings. For more details, see Online Supplemental Information. The order of images (within stimulus sets) and the order of questions (within stimulus presentations) were randomized. Beverage images were presented unaltered (on the wooden table/booth background). Colored shapes were presented against a solid white background (i.e., in the absence of the wooden table/booth background).

Preliminary correlational analyses of the rating data are presented in Online Supplemental Information. In summary, for each stimulus set, (i) there were significant, large, positive correlations among ratings within measurement occasions (see Tables S24 and S67), suggesting that ratings indexed the same underlying construct, and (ii) there was fair-to-good test–retest reliability within and between days (see Tables S5 and S8), indicating that change in ratings reliably indexed between-person variation.

Analytic approach

The hypothesis concerned the extent to which a specific between-person difference, alcohol use as captured by the modified AlcQF index, moderates change in the evaluative response to an arbitrary CS₂ after pairings with an alcohol or non-alcohol CS₁. For completeness, we also examined whether that key between-person difference, alcohol use, moderated the evaluative response to the CS₁ (i.e., the images of alcohol and non-alcohol beverage containers). Evaluative ratings were analyzed using linear mixed models (LMMs). Following best practices (Page-Gould, 2019; Volpert-Esmond et al., 2021), we first determined the best random effects structure by comparing the fit of the unconditional (i.e., within-person factors only)
model varying in random effect structures. An unstructured correlation matrix was specified. Restricted maximum likelihood estimation (RMLE) was used. Upon finding the best random effects structure (for more details, see Online Supplemental Information), we switched to maximum likelihood estimation (MLE) and proceeded with hypothesis testing by adding interactions with individual differences in alcohol use history, i.e., the modified AlcQF index. Satterthwaite’s method (1941) was used for fixed effects parameter estimations (due to >3000 observations) to compare model-estimated population means at levels of the modified AlcQF index chosen to span its range (i.e., −1, 0, 4, 16, 32). The threshold for statistical significance was p < 0.050.

Procedure

Due to the global COVID-19 pandemic, all procedures were conducted online using desktop or laptop computers. All study tasks and materials were implemented on Gorilla (www.gorilla.sc), an online experiment builder and study hosting platform (Anwyl-Irvine et al., 2019). Upon accessing the study on either day 1 or day 2, participants were reminded of the following: (i) to move to quiet, private place where they would not be disturbed or distracted; (ii) to pay attention; (iii) to turn off or put away phone, email, or music; and (iv) to not eat or drink anything during the study session. Timelines for study days 1 and 2 are shown in Fig. 1. Study procedures took place at approximately the same time across the two consecutive days (see Table S9). For more details, see Online Supplemental Information.

Table 3 Summary of ANOVA F-tests in model of beverage container image appeal

|          | F     | df    | p     | η²   |
|----------|-------|-------|-------|------|
| Sex      | 10.29 | 1.368 | <.001 | 0.115|
| Alcohol use | 11.35 | 1.368 | <.001 | 0.126|
| Type     | 17.81 | 1.29  | <.001 | 0.198|
| Alcohol use×type | 50.30 | 1.368 | <.001 | 0.560|

Type was a within-subjects factor (2 levels: alcohol CS₁, non-alcohol CS₁). Sex was a between-subjects factor (2 levels: female, male). Alcohol use was a between-subjects covariate (−1 = never used, 0 = used but not in the past year, >0 = use frequency in the past year typically used quantity). Data (88,439 observations) represent N = 369 participants, n = 20 unique images, and n = 6 unique rating questions. For the random intercept for participants, SD was 15.26. For the random intercept for images, SD was 7.68. For the random intercept for rating questions, SD was 0.87. For the random slope of type within participant random intercepts, SD was 10.60. Random slopes of type within participant random intercepts were correlated −0.05 with the latter. For the random slope of type within rating questions, SD was 1.31. Random slopes of type within rating questions random intercepts were correlated −0.66 with the latter. Residual SD was 21.77.


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4 To confirm that any observed effects of the modified AlcQF index, a pseudo-continuous predictor variable, were not artefactual consequences of including assigned scores of −1 and 0 to represent individuals reporting never/not yet using alcohol and individuals reporting alcohol use in the lifetime but not in the past year, respectively; all analyses were rerun excluding these individuals (n = 76 [21% of the final analytic sample]). Results were unchanged. Thus, effects of the modified AlcQF index reported in the main text are robust to the exclusion of individuals reporting zero exposure to alcohol pharmacology in the past year or in the lifetime.
Results

Appeal of beverage images

As shown in Table 3, there was a significant interaction between alcohol use history and image type on rated appeal. As shown in Fig. 2, among individuals reporting no alcohol use (ever or in the past year) and light alcohol use in the past year, non-alcohol CS1 items were “liked” more than alcohol CS1 items, whereas the opposite was true among individuals reporting heavier alcohol use. Simple slopes analysis indicated that there was a significant simple slope for the effect of alcohol use on the rated appeal of alcohol CS1 items, $b \pm SE = 1.07 \pm 0.16$, $z = 6.63$, $p < 0.001$, but a nonsignificant simple slope on the rated appeal of non-alcohol CS1 items, $b \pm SE = -0.19 \pm 0.15$, $z = -1.22$, $p = 0.223$. The difference between slopes was significant, $b_D \pm SE_D = 1.26 \pm 0.18$, $z = 7.09$, $p < 0.001$. Thus, the preference reversal observed among individuals reporting heavier alcohol involvement was driven by alcohol use-related increases in alcohol CS1 “liking” more so than by alcohol use-related decreases in non-alcohol CS1 “liking”. That is, increases in alcohol involvement were related to increases in alcohol CS1 “liking” rather than decreases in non-alcohol CS1 “liking.”

Appeal of colored shapes

Table 4 shows model-estimated mean baseline appeal rating for each shape cue type (alcohol CS2+, non-alcohol CS2+, CS2−, NC). As shown in Table 5, alcohol use history, type, and time significantly interacted in predicting appeal. As shown in Fig. 3, there were robust evaluative conditioning (EC) effects, defined as those in which alcohol or non-alcohol CS2+ appeal was significantly changed from its pre-conditioning baseline, and that change differed significantly from change in CS2− appeal over the same time frame. Figure 3 also shows that there were robust familiarity effects, defined as significant increases in NC appeal relative to its baseline. In keeping with our primary hypothesis, among individuals reporting either no alcohol use (ever, or in the past year), there was a robust negative alcohol EC effect, whereas there was a robust positive alcohol EC effect among individuals reporting heavy past-year alcohol use (Fig. 3). In fact, the alcohol EC effect (i.e., variable (AlcQF Past Year) at 4. Medium Past Year indicates $M \pm SE$ were estimated while holding the alcohol use moderator variable (AlcQF Past Year) at 16. Heavy Past Year indicates $M \pm SE$ were estimated while holding the alcohol use moderator variable (AlcQF Past Year) at 32. Alcohol CS1=images of alcohol beverage containers. Non-alcohol CS1=images of nonalcohol beverage containers. Asterisks (*) = $p < .05$ for Cue Type comparison.

5 Ancillary analyses presented in Online Supplemental Information indicated that, ignoring alcohol use history, post-conditioning increases in non-alcohol CS2+ appeal relative to pre-conditioning baseline measured immediately after conditioning were retained to the next day (see Fig. S3). Increases in NC appeal also were retained to the next day.
post-conditioning-preconditioning alcohol CS₂+ evaluation) became more intensely positive with increasing alcohol involvement, \(b \pm SE = 28.84 \pm 7.61, \ z = 3.79, p < 0.001\). There were robust positive non-alcohol EC effects as well as familiarity effects among individuals spanning almost the entire spectrum of alcohol involvement (Fig. 3). However, contrary to our predictions, the magnitude of non-alcohol EC effects (i.e., post-conditioning-preconditioning non-alcohol CS₂+ evaluation) also increased with increasing alcohol involvement: \(b \pm SE = 19.43 \pm 2.55, p = 0.011\). In contrast, the magnitude of familiarity effects (i.e., post-conditioning-preconditioning NC evaluation) was unrelated to alcohol involvement: \(b \pm SE = 8.80 \pm 6.77, z = 1.30, p = 0.193\). Finally, Table 6 shows that changes in the evaluative response to the alcohol and non-alcohol CS₂+, CS₂−, and NC-colored shapes were significantly positively correlated with the evaluative response to alcohol CS₁ items, whereas only changes in the evaluative response to the non-alcohol CS₂+ and NC colored shapes were significantly positively correlated with the evaluative response to non-alcohol CS₁ items.

Ancillary analyses presented in Online Supplemental Information indicated that the post-conditioning decrease in alcohol CS₂+ appeal relative to pre-conditioning, measured immediately after conditioning, among individuals with limited histories of alcohol use was significantly larger than when measured the next day (see Fig. S4). In contrast, the post-conditioning increase in alcohol CS₂+ appeal relative to preconditioning, measured immediately after conditioning, among those reporting heavier use histories was not significantly different when tested the next day (see Fig. S4). Thus, retention of conditioned responses to the alcohol CS₂+ from study days 1 to 2 increased with increasing alcohol involvement.

**Table 4** Baseline appeal of colored shapes by type by conditioning procedure

| Cue type          | M (SE)     |
|-------------------|-----------|
| Alcohol CS₂+      | 37.20 (3.82) |
| Non-alcohol CS₂+  | 34.90 (3.81) |
| CS₂−              | 35.30 (3.83) |
| NC                | 38.90 (4.50) |

M (SE) shown are estimates from linear mixed model adjusting for biological sex. Alcohol CS₂+ = colored shape that was paired with images of alcohol beverage containers during the conditioning task. Non-alcohol CS₂+ = colored shape that was paired with images of non-alcohol beverage containers during the conditioning task. CS₂− = colored shape that was paired with the image of wooden booth/table (i.e., the background in images of alcohol and non-alcohol beverage containers) during the conditioning task. This image was also present during all inter-trial intervals during the conditioning task. NC refers to the colored shapes that were not presented during the conditioning task.

**Table 5** Summary of ANOVA F-tests in model of colored shape appeal

|                      | F     | df  | p     | η² |
|----------------------|-------|-----|-------|----|
| Sex                  | 5.26  | 1.368 | .022  | 0.032 |
| Alcohol use          | 1.35  | 1.369 | .246  | 0.017 |
| Time                 | 1.93  | 1.378 | .165  | 0.003 |
| Type                 | 0.19  | 3.12 | .904  | 0.010 |
| Alcohol use × time   | 6.80  | 1.378 | .009  | 0.052 |
| Alcohol use × type   | 0.33  | 1.371 | .800  | 0.012 |
| Type × time          | 29.55 | 3.3129 | <.001 | 0.718 |
| Alcohol use × time × type | 4.64  | 3.3129 | .003  | 0.155 |

Type was an effect-coded within-subjects factor (4 levels: alcohol CS₂+, non-alcohol CS₂+, CS₂−, NC). Time was an effect-coded within-subjects factor (2 levels: preconditioning, post-conditioning). Sex was a between-subjects factor (2 levels: female, male). Alcohol use was a between-subjects covariate (−1 = never used, 0 = used but not in the past year, >0 = use frequency in the past year typically used quantity). Data (33,180 observations) represent N=369 participants, n=5 unique colored shapes, and n=6 unique rating questions. For the random intercept for participants, SD was 17.38. For the random intercept for shapes, SD was 5.97. For the random intercept for rating questions, SD was 2.65. For the random slope of time (effect-coded dummy variable: −1 = pretest, 1 = posttest) within participant random intercepts, SD was 4.28. Random slopes of time within participant random intercepts were correlated 0.15 with the latter. For the random slope of type1 (effect-coded dummy variable: −1 = non-alcohol CS₂+, 1 = alcohol CS₂+) within participant random intercepts, SD was 16.02. Random slopes of time within participant random intercepts were correlated 0.05 with the latter and −0.07 with the random slopes of time. For the random slope of type 2 (effect-coded dummy variable: −1 = non-alcohol CS₂+, 1 = CS₂−) within participant random intercepts, SD was 11.91. Random slopes of type 3 within participant random intercepts were correlated 0.02 with the latter, 0.03 with the random slopes of time, and −0.34 with the random slopes of type 1. For the random slope of type 3 (effect-coded dummy variable: −1 = non-alcohol CS₂+, 1 = NC) within participant random intercepts, SD was 14.25. Random slopes of type 4 within participant random intercepts were correlated 0.05 with the latter and −0.07 with the random slopes of time.

**Discussion**

**On the affective-motivational properties of alcohol beverage cues**

We observed that (i) “liking” of the presumably naturally learned alcohol CS₁ scaled with alcohol use, which is consistent with prior reports (Lang & Yegiyan, 2014; López-Caneda & Carbia, 2018; Pronk et al., 2015; Stauffer et al., 2017), (ii) “liking” of the de novo conditioned alcohol CS₂+ scaled with alcohol use, and (iii) alcohol CS₂+ “liking” scaled with alcohol CS₁ “liking.” Together, these observations provide strong support for the ideas of Stewart et al. (1984), regarding the ability of repeated drug use to imbue drug use-predictive cues with appetitive/positive affective
properties. These observations are also consistent with the sign-tracking model of alcohol abuse (Tomie, 1996; Tomie & Sharma, 2013).

The observation of conditioned “disliking” of the alcohol CS$_2$+$ from among individuals reporting little or no alcohol use warrants consideration. Given that individuals reporting little alcohol use also “liked” the alcohol CS$_1$ less than they “liked” the non-alcohol CS$_1$, the experience of viewing the alcohol CS$_1$ may have been aversive when contrasted against the experience of viewing the non-alcohol CS$_1$. This potential explanation for conditioned “disliking” of the alcohol CS$_2$+$ is a “contrast” effect. For these individuals, the plausibility of such a “contrast” effect increases if we suppose that individuals reporting little or no alcohol use also are more likely to endorse explicit beliefs or expectancies involving aversive (punishing) outcomes of alcohol use than explicit beliefs or expectancies involving appetitive (rewarding) outcomes of alcohol use—given that the former are associated with abstention from alcohol use, whereas the latter are associated with heavier alcohol use (Leigh & Stacy, 2004; Sher et al., 1996; Wiers et al., 2002; Wood et al., 1996). Since explicit beliefs or expectancies about alcohol use outcome expectancies were not assessed in the current study, this possibility remains to be tested in future research.

**On the affective-motivational properties of non-alcohol beverage cues**

All individuals were assumed to have used, regularly across their lifespans, one or more of the nondrug liquid rewards depicted among the non-alcohol CS$_1$ items, providing a non-drug reward comparison condition against which to evaluate the specificity of the link between drug use and related evaluative conditioning effects. Specifically, we expected null relationships between alcohol use, non-alcohol CS$_1$ “liking,” and non-alcohol CS$_2$+ “liking.” Although we did not observe a link between alcohol use and non-alcohol CS$_1$ “liking,” we did observe that non-alcohol CS$_2$+ “liking” scaled with alcohol CS$_2$+ /NC Appeal as a Function of Alcohol Use History. Note. LMM-estimated MD$\pm$ SED are shown. Never/Not Yet indicates MD$\pm$ SED were estimated while holding the alcohol use moderator variable (AlcQF Past Year) at -1. None Past Year indicates MD$\pm$ SED were estimated while holding the alcohol use moderator variable (AlcQF Past Year) at 0. Light Past Year indicates MD$\pm$ SED were estimated while holding the alcohol use moderator variable (AlcQF Past Year) at 4. Medium Past Year indicates MD$\pm$ SED were estimated while holding the alcohol use moderator variable (AlcQF Past Year) at 16. Heavy Past Year indicates MD$\pm$ SED were estimated while holding the alcohol use moderator variable (AlcQF Past Year) at 32. Alcohol CS$_2$+ = colored shape that was paired with images of alcohol beverage containers during the conditioning task. Non-alc. CS$_2$+ = colored shape that was paired with images of non-alcohol beverage containers during the conditioning task. CS2 - = colored shape that was paired with the image of wooden booth/table (i.e., the background in images of alcohol and non-alcohol beverage containers) during the conditioning task. This image also was present during all inter-trial intervals during the conditioning task. NC refers to the colored shapes that were not presented during the conditioning task. Conditioning took place on day 1. Post-conditioning measures were obtained on both day 1 and 2, but day was not considered in this model (see main text). Ampersand ($\&$) = $p<.050$ for the pre-post comparison, indicating significant changes from baseline appeal. Asterisks (*) = $p<.050$ for comparison of the pre-post comparison against the corresponding pre-post comparison for CS$_2$-, indicating that change in appeal from pre-conditioning test on day 1 to the post-conditioning test on day 1 was significantly different from change in the appeal of the CS$_2$- over the corresponding timeframe.

![Image](https://example.com/image.png)
use. Additionally, non-alcohol CS$_2$+ “liking” scaled with alcohol and non-alcohol CS$_1$ “liking.” One potential explanation for these unexpected findings is that individuals reporting heavier compared to lighter alcohol use in the sample may not have distinguished between alcohol CS$_1$ and non-alcohol CS$_1$ during the conditioning task. This could be due to the use of intermixed alcohol and non-alcohol CS$_2$-CS$_1$ trials rather than discrete blocks of alcohol and non-alcohol CS$_2$-CS$_1$ pairings. Alternatively, it could be due to individuals reporting heavier compared to lighter alcohol use being more likely to “find” alcohol-relatedness in the non-alcohol beverages (e.g., seeing them as mixers for certain alcohol beverages). A third potential (non-mutually exclusive) explanation is that the use of sweetened (sugary) beverages among the non-alcohol beverages may have tapped into shared appetitive mechanisms for alcohol and sweets (Fletcher & Kenny, 2018; Fortuna, 2010). A fourth potential (non-mutually exclusive) explanation comes from the idea that the propensity to attribute “incentive salience” to reward-predictive cues, a “bottom-up” motivational property theorized to underlie the conditioned reinforcing value of reward-predictive cues, is a trait-like risk factor for substance use disorders (Flagel et al., 2009; Robinson et al., 2014; Saunders & Robinson, 2013) including alcohol use disorders (Cofresi et al., 2019).

**Role of familiarity effects in detection of evaluative conditioning (EC) effects**

Familiarity effects, i.e., increases in NC cue appeal over time (Zajonc, 1968), were consistently observed. Importantly, changes in NC cue appeal were often similar in size to changes in alcohol CS$_2$+ and non-alcohol CS$_2$+ appeal over time, so comparisons would have yielded no significant difference. In contrast, changes in alcohol CS$_2$+ and non-alcohol CS$_2$+ appeal over time often differed significantly from corresponding changes in CS$_2$− appeal over time. The latter is evidence in favor of the idea that changes in alcohol CS$_2$+ and non-alcohol CS$_2$+ appeal reflected associative learning (viz., *conditioned* changes in evaluative response) rather than non-associative learning (i.e., familiarity or habituation). Inclusion of, and comparison against, the CS$_2$− condition was thus critical for detecting EC effects here.

**Limitations**

Several limitations apply to this study. The first set of limitations is common to online behavioral studies: (i) we had no way to verify compliance with our instructions (e.g., to maximize the size of the browser window, to turn off or put away smartphones); (ii) beyond restricting the study to run only on desktop or laptop computers, we were unable to standardize hardware or software (although this is more a problem for reaction time than self-report measures); and (iii) testing location (and, hence, physical environment) was not standardized across participants. Despite these limitations, we were able to observe *discriminative* second-order conditioning of an evaluative response with only 24 reinforced trials.

A potentially important limitation of the current study is that fewer than a third of participants naturally developed any conscious awareness of (viz., were able to verbalize) the programmed CS$_2$−CS$_1$ contingencies. Contingency awareness is widely regarded as an important determinant of EC effects in humans (Bar-Anan et al., 2010; Brewer, 1974; Dawson et al., 2007; Gast et al., 2012). Additional analyses and discussion of the relationships among alcohol use, evaluative conditioning, and contingency awareness are presented in Online Supplemental Information.

An additional limitation is that the current study was unable to determine whether the magnitude (and/or valence) of conditioned evaluative responses to the CS$_2$+ depends on the hedonic or incentive value assigned to the CS$_1$ or the hedonic or incentive value assigned the US. Disentangling those possibilities requires a de novo conditioning experiment with CS$_1$-US and CS$_2$-CS$_1$ acquisition stages.

A final, albeit important, limitation worth noting is that the sample was comprised of emerging adults (age

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7 Nonassociative learning here refers to changes in the response of the central nervous system to a stimulus due to its repeated exposure to that stimulus. Associative learning here refers to changes in the response of the central nervous system to one stimulus due to learning of a contingent relationship to another stimulus.
18–23 years) enrolled in a predominantly white, Midwestern university. The sociodemographic makeup of the sample is thus limited in its representativeness of the broader population of emerging adults in the USA. The sample also was not stratified for alcohol use levels, so the alcohol use distribution was sampled randomly, leading to uneven sampling across the range of possible use levels. Additionally, the levels of alcohol use history (as captured by the modified AlcQP index) at which simple effects of CS₂-CS₁ pairings were estimated for follow-up analyses were chosen to span the range of observed use history levels. Thus, effects observed at different levels of alcohol use here remain to be replicated in a sample with more consistent sampling across the range of alcohol use behavior.

Conclusion

In keeping with predictions of Stewart et al. (1984), the current study demonstrates that novel, arbitrary visual cues can come to be evaluated more positively (i.e., “liked” more) after repeated pairing with existing, “naturalistic” visual cues for alcohol (associations acquired through pairings encountered in daily life), and that this effect scaled with the heaviness of alcohol use. Additionally, the current study found that arbitrary visual cues can come to be evaluated less positively (i.e., “liked” less) after pairings with “naturalistic” alcohol cues among nondrinkers. Both findings are surprising given the extensive non-reinforced exposure to visual alcohol cues (i.e., exposure without subsequent alcohol ingestion) experienced by teetotalers and lushes alike since alcohol beverage advertising aims to pair brands with positively valenced suggested outcomes of alcohol use (Jackson & Bartholow, 2020).

Supplementary Information

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Declarations

Ethics approval. All procedures were approved by the University of Missouri Institutional Review Board.

Conflict of interest. The authors declare no competing interests.

Reprints. Reprint requests should be directed to Dr. Roberto Cofresi, cofresi@missouri.edu.

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