Transfer of incentive salience from a first-order alcohol cue to a novel second-order alcohol cue among individuals at risk for alcohol use disorder: electrophysiological evidence

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ABSTRACT

Background and aims In susceptible individuals, cues associated with drug use are theorized to take on incentive–motivational properties, including the ability to reinforce higher-order, drug-related associative learning. This study aimed to test this prediction among people varying in risk for alcohol use disorder. Design, setting and participants Repeated-measures experiment with a measured individual difference variable at a University psychology laboratory in Missouri, USA. One hundred and six young adults (96 contributed complete data) were pre-selected to represent the upper and lower quartiles of self-reported sensitivity to alcohol’s acute effects. Measurements Participants completed a second-order Pavlovian conditioning paradigm in which an initially neutral visual cue (second-order conditional stimulus; CS2) predicted onset of an olfactory cue (first-order conditional stimulus; CS1). Olfactory cues were isolated from alcoholic beverages, sweets and non-comestible substances, each presumed to have a natural history of first-order conditioning. Event-related potential responses to the CS2 across its conditioning and extinction, and to the CS1, provided neurophysiological indices of incentive salience (IS). Findings The IS of the alcohol CS1 was higher among participants low in alcohol sensitivity (LS), relative to their higher-sensitivity (HS) peers. The IS of the CS2 paired with the alcohol CS1 increased across the CS2 conditioning phase among LS but not HS participants. Also, LS (but not HS) individuals also experienced increases in alcohol craving following alcohol CS1 exposure, and this change was correlated with increases in the IS of the CS2 paired with the alcohol CS1. Conclusions Alcoholic beverage odor, a proximal cue for alcohol consumption, appears to reinforce conditioning of neurophysiological responses to a novel cue among low alcohol sensitivity (LS) individuals but not high alcohol sensitivity individuals, providing the first evidence that the LS phenotype may be associated with differences in the conditioned reinforcing properties of alcohol-related cues. These findings support the idea that the LS phenotype may increase alcohol use disorder risk via susceptibility to incentive salience sensitization.

Keywords Alcohol sensitivity, cue–reactivity, event-related potentials, incentive salience, sign-tracking, subjective response.

INTRODUCTION

Dopaminergic reward-learning circuits are critically involved in the development and maintenance of addiction [1,2], including alcohol use disorder (AUD) [3,4]. The Incentive Sensitization Theory of Addiction (ISTA) [5,6] posits that, in susceptible individuals [7], repeated drug use causes dopaminergic circuits to attribute increasing incentive–motivational value (incentive salience) to drug use-associated cues [8–11]. This incentive salience sensitization is posited to cause pathological, cue-induced motivation for drug use that translates into drug-seeking behavior. To date, ISTA has not been thoroughly tested in humans [12,13]. The current study tested the extent to which individual differences in neural correlates of incentive salience attribution to novel alcohol-related cues are

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evident in humans exhibiting a phenotype proposed to confer risk for AUD via incentive sensitization [14].

**ISTA: animal models**

According to ISTA, variability in cue–reactivity reflects individual differences in susceptibility to incentive salience sensitization [7,15]. In pre-clinical studies, this variability is expressed in the extent to which animals engage with conditioned reward-predictive cues as though the cues themselves were rewarding [7,15]. This ‘sign-tracking’ (ST) phenotype contrasts with a ‘goal-tracking’ (GT) phenotype, in which animals approach the location of reward delivery upon presentation of conditioned reward-predictive cues. The acquisition and expression of ST and GT phenotypes is mediated by dissociable neural systems [16–20] and probably represents different psychological processes [15,20]. The ST phenotype is argued to model vulnerability to incentive sensitization for at least two reasons. First, the ST phenotype is mediated by dopaminergic reward-learning circuits [18,21] and is highly resistant to cue extinction [22,23]. Second, rodents with the ST phenotype self-administer heavier doses of addictive drugs and exhibit greater drug cue-induced return of drug self-administration after extinction [12].

**ISTA: human models**

Translating pre-clinical models of addiction into human models of addiction risk poses major challenges [24,25], and yet consilience with pre-clinical paradigms may be critical to translational efforts [12–14]. For translating the ISTA, it is necessary to identify human phenotypes associated with ST-like responses [12]. One candidate phenotype is low sensitivity to alcohol (LS) [26]. LS is known to confer risk for AUD [27–29], and can be observed in both humans and rodents [30]. Additionally, LS is associated with exaggerated alcohol cue–reactivity in both laboratory [31–36] and natural environments [37,38]. Moreover, the genetic variants—particularly dopaminergic (DRD3, DRD4), cannabinoid (CB1) and μ1 opioid receptors (OPRM1)—associated with LS [39–42] are also associated with cue-induced alcohol craving [43,44] and alcohol cue–reactivity [45–47].

**Current study**

The current study sought to demonstrate the acquisition of incentive salience for novel alcohol-related cues among individuals varying in alcohol sensitivity using a Pavlovian conditioning paradigm in which neutral visual cues (colored squares) were paired with isolated olfactory cues for appetitive stimuli [alcoholic beverage odors, sweet odors and non-comestible appetitive control (NCAppC) odors]. Each visual cue (the second-order conditional stimulus, CS2) predicted onset of an olfactory cue (the first-order conditional stimulus, CS1), such that three novel associations (alcohol CS2-CS1, sweet CS2-CS1 and NCAppC CS2-CS1) could be conditioned within each participant. This paradigm tests whether the conditioned reinforcing value of alcohol odors—theoretically conditioned as predictors of alcohol-related reward in daily life—varies as a function of individual differences in alcohol sensitivity.

Incentive salience attribution to the visual CS2 and olfactory CS1 was measured with event-related brain potentials (ERPs). Decades of research indicate that the amplitude of the P3 ERP elicited by visual stimuli and the late positive complex (LPC) elicited by olfactory stimuli reflect the eliciting stimuli’s incentive–motivational significance (see [48,49]). P3 and LPC amplitudes are sensitive to varying monetary value (e.g. [50,51]), varying predictive value with respect to affective events (e.g. [52,53]) and varying physiological states such as hunger (e.g. [54]). P3 and LPC amplitudes also are sensitive to pharmacological manipulations of dopaminergic neurotransmission (e.g. [55–58]) and correlate with event-related activation of brain regions innervated by the mesocorticolimbic dopamine system (e.g. medial pre-frontal cortex and ventral striatum) (e.g. [59,60]). Thus, these ERP responses provide a relatively direct measure of stimulus incentive value in humans that does not rely on conscious awareness of affective–motivational experiences or locomotor behavior.

We predicted that LS participants would show a pattern of experiential and neural responses consistent with a ST phenotype. Specifically, relative to HS counterparts, LS participants were expected to show greater increases in self-reported alcohol (but not sweet) craving as a result of alcohol CS1 exposure (H1). In addition, because alcohol odor (but not sweet odor) probably has more pre-existing incentive salience for LS individuals, the alcohol CS1 should support more effective conditioning of the alcohol CS2 among LS than HS individuals. Hence, the alcohol CS2 (but not sweet CS2) was expected to elicit larger P3 amplitude among LS versus HS participants during CS2 acquisition (H2). More specifically, the P3 elicited by the alcohol CS2 was expected to increase across pairings among LS but not HS individuals (H3). The P3 elicited by the newly conditioned alcohol CS2 was expected to remain larger among LS versus HS individuals during CS2 extinction (CS1 omission) (H4), reflecting short-term maintenance of incentive salience for the alcohol CS2. Also, given evidence for associations between neurophysiological and craving responses to drug-related cues [61,62], we predicted that alcohol CS1-induced craving would correlate with P3 response to the alcohol CS2 (H5).

Following previous demonstrations of larger neurophysiological response to alcohol but not to other appetitive visual CS1 among LS versus HS individuals [31,32,36], we
expected larger LPC amplitude for alcohol but not for other olfactory CS1 among LS compared to HS individuals (H6). Because alcohol CS2–CS1 pairings involved presentation of alcohol CS1 without subsequent alcohol ingestion [unconditional stimulus (US)], alcohol CS2 acquisition involved de-facto alcohol CS1 extinction (US omission). Thus, LS versus HS LPC amplitude differences were expected to be largest at the beginning of CS2 acquisition (H7). Finally, given that ST is resistant to cue extinction [22,23], less extinction of CS1 response—i.e. less decrease in LPC amplitude across non-reinforced CS1 presentations—was expected among LS versus HS participants (H8).

MATERIALS AND METHODS

The University of Missouri Institutional Review Board reviewed and approved all procedures used in this experiment. Variable selection and analyses were planned prior to data collection as part of the grant application (F31 AA022551) that funded this study. However, the analyses were not formally pre-registered, and therefore results should be considered exploratory.

PARTICIPANTS

One hundred and six undergraduates participated in a laboratory session in exchange for course credit or $14/hour. Data from eight participants were unusable due to equipment errors (n = 7) or excessive electroencephalogram (EEG) artifact (n = 1); two additional participants showed impaired olfaction during screening (see Supporting information). Thus, data from 96 participants (51% LS; 90% white; 48% male; mean age = 19.65 years) contributed to the analyses. Recruitment and screening procedures are detailed in the Supporting information.

MEASURES

Self-report measures

Typical alcohol use and related measures are summarized in Table 1. Full description of these measures is provided in the Supporting information.

Alcohol sensitivity

Self-reported sensitivity to 15 effects associated with alcohol consumption (e.g. feeling talkative; feeling dizzy) was measured using the alcohol sensitivity questionnaire (ASQ) [63,64]. The ASQ’s construct validity has been demonstrated in research showing that scores predict subjective responses to alcohol in the laboratory [64]. Internal consistency in the current sample was excellent (α = 0.98). A full description is given in the Supporting information.

Craving

Alcohol craving was measured with the eight-item alcohol urge questionnaire (AUQ) [65], in which respondents rate their current desire for alcohol (e.g. ‘Nothing would be better than a drink right now’) using seven-point scales. To permit comparison of alcohol craving with craving for another consumable, participants also completed a modified AUQ assessing desire for sweets (achieved by replacing ‘alcohol’ or ‘drink’ in each item with ‘sweets’ or ‘candy’). Reliability in the current sample was very good for the alcohol AUQ (time 1, α = 0.87; time 2, α = 0.90) and the modified sweets AUQ (time 1, α = 0.90; time 2 α = 0.91). Change in craving was quantified by regressing time 2 AUQ scores on time 1 AUQ scores (i.e. residual scores) separately for alcohol and sweets.

Table 1 Alcohol use and problems as a function of alcohol sensitivity group

<table>
<thead>
<tr>
<th></th>
<th>LS (n = 49)</th>
<th>HS (n = 47)</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>χ²</td>
</tr>
<tr>
<td>AUD FH+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past-month drinks/week</td>
<td>12.46 (10.67)</td>
<td>9.00 (11.50)</td>
<td>5.93 (7.66)</td>
</tr>
<tr>
<td>Past-month binges</td>
<td>1.15 (0.87)</td>
<td>0.62 (0.87)</td>
<td>0.37 (0.23)</td>
</tr>
<tr>
<td>Past-month maximum drinks/hour</td>
<td>2.50 (1.19)</td>
<td>2.17 (1.65)</td>
<td>1.97 (1.19)</td>
</tr>
<tr>
<td>Negative consequences</td>
<td>7.47 (6.40)</td>
<td>5.90 (8.8)</td>
<td>4.54 (5.09)</td>
</tr>
<tr>
<td>AUD-related negative consequences</td>
<td>4.17 (4.34)</td>
<td>2.00 (6.00)</td>
<td>2.42 (3.11)</td>
</tr>
</tbody>
</table>

FH+ = positive family history. Past-month drinks/week was calculated as the number of drinking occasions per week over the past month multiplied by the typical number of drinks consumed per occasion. Past-month binges = number of binge drinking episodes (4+ drinks for women; 5+ drinks for men) during the past month. Past-month maximum drinks/hour = maximum number of drinks consumed/hour during the heaviest drinking episode in the past month. Negative consequences and alcohol use disorder (AUD)-related negative consequences were assessed as in [86]. LS = participants (n = 49) with low sensitivity to alcohol as defined by alcohol sensitivity questionnaire (ASQ) scores. HS = participants (n = 47) with high sensitivity to alcohol, as defined by ASQ scores. SD = standard deviation; IQR = interquartile range.
Laboratory measures

Olfactory stimuli

Three classes of odorants were delivered using a custom-built olfactometer (details in the Supporting information). Alcohol odors were tailored to each participant’s two most frequently consumed alcoholic beverages, and were produced by passing an airstream through the liquids. Sweet odors, consisting of peppermint and chocolate, and NCAppC odors, consisting of leather and cedar, were produced by passing an airstream over 1” × 1/4” strips of cotton paper containing droplets of odorant produced by a local perfumer. The sweet and NCAppC odors were chosen based on pleasantness ratings provided by a pilot sample drawn from the same population as the study participants.

Second-order appetitive conditioning and extinction task

This task (derived from [66]) paired each type of odorant (CS1: alcohol, sweet and NCAppC) with a previously unassociated visual cue (CS2: red, green and blue 2” squares) while EEG was recorded. The task was divided into three blocks, two for acquisition of CS2-CS1 associations and one for extinction of these associations. Acquisition trials began with a black fixation cross (1000 ms), followed 3 sec later by the presentation of a CS2 (1000 ms), which was followed 3 sec later by presentation of a CS1 (2000 ms). CS1 presentation was accompanied by the word “Sniff” appearing on the monitor, which was followed 2 sec later by a tone signaling participants to cease inhalation (see Fig. 1). Trial structure during extinction was similar, except: (1) the fixation cross was color-matched to the subsequent CS2; and (2) the CS1 was always omitted. CS2-CS1 mappings were consistent throughout the task within participants but counterbalanced across participants. Each block consisted of 72 trials (n = 24 per odor category). Within each block, trial types were randomized such that there were no more than three consecutive trials of any one type.

Electrophysiological recording, data reduction and analysis

EEG recording and processing details are presented in the Supporting information. In the CS2-locked ERP (Fig. 2a), we quantified P3 mean amplitude over parieto-occipital and occipital electrodes. In the CS1-locked ERP (Fig. 3a), we quantified LPC mean amplitude over fronto-central, central and centro-parietal electrodes. All ERP component amplitudes were analyzed using linear mixed-effects models (LMMs). LMMs are known to handle the nested structure of psychophysiological repeated-measures data and unequal numbers of observations per subject more effectively than traditional repeated-measures analysis of variance (ANOVA) [67,68]. Technical details concerning LMM fitting procedures are presented in the Supporting information. In each LMM, we controlled for factors that could affect ERP component amplitudes (age, sex, race...
and recent alcohol use; see Table 1). To obtain type III sums of squares, ANOVA F-tests for effects of interest in each LMM, we used Satterthwaite’s method [69]. Predicted mean differences (estimated marginal population means) were tested using either asymptotic Z-tests (when > 3000 observations were considered) or t-tests with Kenward–Roger [70] estimated degrees of freedom (d.f.) (when fewer observations were considered).

Procedure

Participants were told the purpose of the study was to assess brain responses to odors. Upon arriving at the laboratory, participants provided informed consent. Next, participants were seated in an EEG recording room, 30 cm from a 25” LED monitor. A nasal cannula, affixed to adjustable plastic tubing, was placed beneath the participant’s nostrils. Participants then completed craving measures (AUQ-alcohol, AUQ-sweets), were assessed for olfactory acuity [71] (see Supporting information), and rated the odorants’ different perceptual qualities (pleasantness, valence, arousal, intensity and representativeness; see Supporting information), after which they were prepared for EEG recording. Participants then completed two blocks of CS2 conditioning trials (separated by a 10-minute break), followed by completion of craving measures again and then the block of CS2 extinction trials. After the task the EEG cap was removed, participants completed self-report measures of alcohol use and related experiences, were debriefed and dismissed.

RESULTS

Craving response to CS1

Predictions concerning differential craving responses (H1) were tested with a 2 (group: HS, LS) × 2 (craving type: alcohol, sweets) ANOVA with repeated-measures on the
latter factor. The predicted group × craving type interaction was significant, $F_{(1,94)} = 6.06$, $P = 0.016$, which was decomposed via simple effects (Table 2, Fig. 4). The results supported H1.

P3 response to CS2

Predictions concerning differential acquisition of P3 response to the alcohol CS1–paired CS2 across CS2 acquisition and extinction (H2–4) were tested with a 2 (group: HS, LS) × 3 (CS1 category: alcoholic beverage odors, sweet odors, NCAppC odors) × (trial block: 1, 2, 3) LMM adjusting for age, typical alcohol use, race and sex. CS2-elicited ERPs are shown in Fig. 2a; mean CS2-elicited P3 amplitudes are given in Fig. 2b. The predicted three-way interaction involving the alcohol sensitivity group, trial block and CS1 category was significant, $F_{(4,7107.7)} = 15.47$, $P < 0.001$. This interaction was deconstructed via planned comparisons of the model-estimated, covariate-adjusted marginal population means (Table 3, Fig. 2b). Results supported H2 and H3, but only partially supported H4.

Acquired P3 response to alcohol CS2 is associated with craving response to alcohol CS1

To test the prediction that P3 response to the newly trained alcohol CS2 covaried with craving induced by the alcohol CS1 (H5), we computed a CS2-elicited P3 residual variable, reflecting the change in P3 amplitude across acquisition (trial blocks 1–2), and correlated this variable with the residual alcohol AUQ score for each participant. The correlation was small but significant, $r_{(91)} = 0.23$, $P = 0.029$ (Fig. 5), supporting H5.

LPC response to CS1

Predictions concerning differential LPC response to the alcoholic CS1 (H6–8) were tested with a 2 (group: HS, LS) × 3 (CS1 category: alcoholic beverage odors, sweet odors, NCAppC odors) × 2 (trial block: 1, 2) LMM. Mean LPC amplitude values are given in Fig. 3b. The predicted three-way interaction of alcohol sensitivity group, trial block and CS1 category was not significant, $F_{(2,4752)} = 1.12$, $P = 0.324$, but there was a significant group × CS1 category interaction, $F_{(2,959.0)} = 3.27$, $P = 0.042$. We decomposed the latter by comparing the model-estimated, covariate-adjusted marginal population means collapsing trial block (Table 4, Fig. 3b). The results supported H6, but not H7 or H8.
DISCUSSION

ISTA has received considerable pre-clinical support as a neurobiological theory of addiction [10,12]. However, like pre-clinical models of other psychiatric conditions, pre-clinical models of addiction have been strongly criticized for limited translational value to clinical experience [72,73]. The current study represented an attempt at translation of some of the ISTA tenets—including its emphasis on individual differences [7,74,75]—into a human laboratory model. Improving upon previous studies [31–36], the current study adopted a Pavlovian conditioning paradigm such as those used in pre-clinical tests of ISTA and tested for individual differences in the incentive value of a novel cue across its conditioning and extinction. Given these similarities, the current study provides a strong first test of the ISTA in humans with a known phenotypical risk for development of AUD.

LS has been associated with hazardous alcohol use and development of AUD [27,76], but the mechanisms by which it confers this risk are not well understood. Previous work has posited social–environmental (affiliation with heavy-drinking peers) and cognitive–motivational factors (positive alcohol outcome expectancies; drinking to cope with stress) as potential mechanisms [77,78]. The current study represents the most direct test to date, to our knowledge, of a proposed neurobiological mechanism—inscentive sensitization—through which LS might increase risk for AUD [14].

Among LS individuals, a previously neutral stimulus became an incentivized alcohol-related cue (alcohol CS2) after repeated pairing with an existing, incentivized alcohol-related cue (alcohol CS1). Given the voluminous literature associating P3 amplitude with the incentive–motivational value of eliciting stimuli [48,49], increased P3 response to the alcohol CS2 across conditioning (trial blocks 1 and 2) is consistent with acquisition of a conditioned appetitive response in LS individuals [13,79]. Importantly, the increased P3 response to the alcohol CS2 was retained among LS participants during the extinction block (CS1 omission), partially supporting the idea (H4) that acquired incentive salience for the alcohol CS2 was maintained in the higher-risk group. Additionally, craving for

Table 2  Alcohol sensitivity group × craving type interaction effect on conditional stimulus (CS1)-elicited craving: simple effects analysis

<table>
<thead>
<tr>
<th>Simple effect of alcohol Sensitivity group (LS versus HS)</th>
<th>MeanLS</th>
<th>SDLS</th>
<th>MeanHS</th>
<th>SDHS</th>
<th>95% CI MeanD</th>
<th>t</th>
<th>d.f.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>1.71</td>
<td>4.96</td>
<td>−1.79</td>
<td>4.94</td>
<td>1.50–5.51</td>
<td>−3.47</td>
<td>94</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sweets</td>
<td>−0.22</td>
<td>8.49</td>
<td>0.31</td>
<td>8.49</td>
<td>−3.57–2.50</td>
<td>−0.35</td>
<td>94</td>
<td>0.729</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Simple effect of craving type</th>
<th>Mean</th>
<th>SD</th>
<th>95% CI mean</th>
<th>t</th>
<th>d.f.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS, alcohol</td>
<td>1.71</td>
<td>4.96</td>
<td>0.29–3.14</td>
<td>2.42</td>
<td>48</td>
<td>0.019</td>
</tr>
<tr>
<td>LS, sweets</td>
<td>−0.22</td>
<td>8.49</td>
<td>−2.66–2.22</td>
<td>−0.18</td>
<td>48</td>
<td>0.856</td>
</tr>
<tr>
<td>HS, alcohol</td>
<td>−1.79</td>
<td>4.94</td>
<td>−3.24 (−0.34)</td>
<td>−2.49</td>
<td>46</td>
<td>0.016</td>
</tr>
<tr>
<td>HS, sweets</td>
<td>0.31</td>
<td>8.49</td>
<td>−1.53–2.15</td>
<td>0.34</td>
<td>46</td>
<td>0.736</td>
</tr>
</tbody>
</table>

LS = participants (n = 49) with low sensitivity to alcohol as defined by alcohol sensitivity questionnaire (ASQ) scores. HS = participants (n = 47) with high sensitivity to alcohol, as defined by ASQ scores. Bold type indicates effects with P < 0.05. SD = standard deviation; CI = confidence interval; d.f. = degrees of freedom.

Figure 4  Residualized alcohol urge questionnaire (AUQ) scores representing change in craving for alcohol and sweets as a function of alcohol sensitivity group. Positive values indicate greater craving following odor (conditional stimulus [CS1]) exposure than would be expected based on the baseline (pre-CS1 exposure) assessment. Sample mean and standard error of the mean (SEM) shown. HS = participants (n = 47) with high sensitivity to alcohol, as defined by ASQ scores. LS = participants (n = 49) with low sensitivity to alcohol as defined by ASQ scores. Black asterisk indicates P < 0.05 for between-group comparison of alcohol AUQ Δ. Gray asterisk indicates P < 0.05 for within-group test for non-zero AUQ Δ. [Colour figure can be viewed at wileyonlinelibrary.com]
Table 3 Between- and within- group comparisons of model-estimated, covariate-adjusted population marginal means for alcohol, sweet and NCAppC conditional stimulus (CS1)-elicited P3 mean amplitude (μV) across trial blocks

<table>
<thead>
<tr>
<th>LS versus HS</th>
<th>MeanLS</th>
<th>SE_LS</th>
<th>95% CI_LS</th>
<th>MeanHS</th>
<th>SE_HS</th>
<th>95% CI_HS</th>
<th>MeanD</th>
<th>SED</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1, alcohol CS2</td>
<td>2.70</td>
<td>0.86</td>
<td>1.01–4.39</td>
<td>3.00</td>
<td>0.82</td>
<td>1.40–4.39</td>
<td>–0.30</td>
<td>0.71</td>
<td>0.05</td>
<td>0.42</td>
</tr>
<tr>
<td>Block 2, alcohol CS2</td>
<td>3.54</td>
<td>0.84</td>
<td>1.89–5.18</td>
<td>2.11</td>
<td>0.80</td>
<td>0.55–3.68</td>
<td>1.43</td>
<td>0.69</td>
<td>2.06</td>
<td>0.039</td>
</tr>
<tr>
<td>Block 3, alcohol CS2</td>
<td>3.79</td>
<td>0.83</td>
<td>2.16–5.43</td>
<td>3.33</td>
<td>0.79</td>
<td>1.78–4.89</td>
<td>0.46</td>
<td>0.73</td>
<td>0.63</td>
<td>0.330</td>
</tr>
<tr>
<td>Block 1, sweet CS2</td>
<td>3.24</td>
<td>0.85</td>
<td>1.58–4.91</td>
<td>2.09</td>
<td>0.81</td>
<td>0.51–3.68</td>
<td>1.15</td>
<td>0.74</td>
<td>1.56</td>
<td>0.118</td>
</tr>
<tr>
<td>Block 2, sweet CS2</td>
<td>2.77</td>
<td>0.80</td>
<td>1.20–4.33</td>
<td>2.46</td>
<td>0.75</td>
<td>0.99–3.94</td>
<td>0.30</td>
<td>0.63</td>
<td>0.48</td>
<td>0.632</td>
</tr>
<tr>
<td>Block 3, sweet CS2</td>
<td>3.81</td>
<td>0.79</td>
<td>2.25–5.36</td>
<td>3.46</td>
<td>0.75</td>
<td>2.00–4.93</td>
<td>0.34</td>
<td>0.67</td>
<td>0.51</td>
<td>0.607</td>
</tr>
<tr>
<td>Block 1, NCAppC CS2</td>
<td>3.57</td>
<td>0.84</td>
<td>1.91–5.23</td>
<td>3.17</td>
<td>0.80</td>
<td>1.60–4.75</td>
<td>0.40</td>
<td>0.69</td>
<td>0.57</td>
<td>0.568</td>
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<tr>
<td>Block 2, NCAppC CS2</td>
<td>4.01</td>
<td>0.88</td>
<td>2.29–5.73</td>
<td>2.49</td>
<td>0.84</td>
<td>0.84–4.14</td>
<td>1.52</td>
<td>0.80</td>
<td>1.90</td>
<td>0.057</td>
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<tr>
<td>Block 3, NCAppC CS2</td>
<td>3.79</td>
<td>0.83</td>
<td>2.16–5.43</td>
<td>3.33</td>
<td>0.79</td>
<td>1.78–4.89</td>
<td>0.93</td>
<td>0.68</td>
<td>1.37</td>
<td>0.170</td>
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<table>
<thead>
<tr>
<th>LS versus LS</th>
<th>MeanB2</th>
<th>SEB2</th>
<th>95% CLI2</th>
<th>MeanB1</th>
<th>SEB1</th>
<th>95% CLI1</th>
<th>MeanD</th>
<th>SED</th>
<th>Z</th>
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<td>Block 2 versus block 1</td>
<td>MeanB2</td>
<td>SEB2</td>
<td>95% CLI2</td>
<td>MeanB1</td>
<td>SEB1</td>
<td>95% CLI1</td>
<td>MeanD</td>
<td>SED</td>
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<tr>
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<td>0.84</td>
<td>1.89–5.18</td>
<td>2.70</td>
<td>0.86</td>
<td>1.01–4.39</td>
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<td>0.44</td>
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<tr>
<td>LS, sweet CS2</td>
<td>2.77</td>
<td>0.80</td>
<td>1.20–4.33</td>
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<td>0.85</td>
<td>1.58–4.91</td>
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<th>SEB2</th>
<th>95% CLI2</th>
<th>MeanD</th>
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<th>Z</th>
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<td>MeanB2</td>
<td>SEB2</td>
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<td>1.20–4.33</td>
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<th>SE</th>
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<th>SE</th>
<th>Z</th>
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<tr>
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<td>–0.71</td>
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<td>0.82</td>
<td>1.85</td>
<td>0.064</td>
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<td>LS, NCAppC CS2</td>
<td>–0.18</td>
<td>0.82</td>
<td>–0.22</td>
<td>0.827</td>
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<td>2.55</td>
<td>0.011</td>
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<td>HS, sweet CS2</td>
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<td>0.83</td>
<td>0.76</td>
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<td>0.83</td>
<td>1.84</td>
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LS = participants (n = 49) with low sensitivity to alcohol as defined by alcohol sensitivity questionnaire (ASQ) scores. HS = participants (n = 47) with high sensitivity to alcohol, as defined by ASQ scores. NCAppC = non-comestible appetitive control odors, CS1 conditioning (i.e. pairings with the alcohol, sweet or NCAppC CS1) took place in trial blocks 1 and 2. CS2 extinction (i.e. omission of CS2) took place in trial block 3. Bold type indicates effects with P < 0.05. CI = confidence interval; SE = standard error.
alcohol increased during the course of the task for LS but not HS individuals—presumably as a result of alcohol CS exposure—and individual differences in this craving change corresponded with changes in P3 amplitude elicited by the alcohol CS during conditioning. Taken together, these findings show that alcohol odor, a proximal cue for alcohol consumption, supported conditioning of a novel CS in LS but not HS individuals, supporting the hypothesis that enhanced incentive salience attribution to alcohol-related cues may contribute to the LS phenotype [14,32,38] and may represent a neurobiologically based vulnerability for development of AUD [14]. That is, the heightened cue-reactivity responses consistently observed among individuals with substance use disorders [80] might reflect, in part, a trait-like vulnerability to attribute incentive salience to drug-related cues.

Critically, differences in neurophysiological reactivity to the alcohol-associated CS and CS between LS and HS participants were robust to adjustment for recent alcohol use, suggesting that these differences do not merely reflect higher alcohol exposure (i.e. more CS–US pairings) in LS individuals. Furthermore, the differences cannot be attributed to greater sensory/perceptual sensitivity to alcohol odors among LS participants, because LS individuals perceived the alcohol odors as less intense and less representative of alcohol than did HS individuals (see Supporting information).

Table 4 Between-group comparisons of model-estimated, covariate-adjusted population marginal means for alcohol, sweet and NCAppC conditional stimulus (CS)-elicited late positive complex (LPC) mean amplitude (μV) across CS conditioning (collapsed trial blocks 1 and 2)

<table>
<thead>
<tr>
<th>LS versus HS</th>
<th>MeanLS</th>
<th>SE LS</th>
<th>95% CI LS</th>
<th>MeanHS</th>
<th>SE HS</th>
<th>95% CI HS</th>
<th>MeanD</th>
<th>SE</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol CS₁</td>
<td>5.97</td>
<td>0.77</td>
<td>4.46–7.49</td>
<td>4.66</td>
<td>0.75</td>
<td>3.19–6.12</td>
<td>1.32</td>
<td>0.56</td>
<td>2.34</td>
<td>0.019</td>
</tr>
<tr>
<td>Sweet CS₁</td>
<td>6.01</td>
<td>0.81</td>
<td>4.43–7.59</td>
<td>5.01</td>
<td>0.78</td>
<td>3.48–6.54</td>
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<td>0.65</td>
<td>1.53</td>
<td>0.125</td>
</tr>
<tr>
<td>NCAppC CS₁</td>
<td>5.51</td>
<td>0.78</td>
<td>3.99–7.03</td>
<td>5.22</td>
<td>0.75</td>
<td>3.75–6.69</td>
<td>0.88</td>
<td>0.57</td>
<td>0.50</td>
<td>0.614</td>
</tr>
</tbody>
</table>

LS = participants (n = 49) with low sensitivity to alcohol as defined by alcohol sensitivity questionnaire (ASQ) scores. HS = participants (n = 47) with high sensitivity to alcohol, as defined by ASQ scores. NCAppC = non-comestible appetitive control odors. Bold type indicates effects with P < 0.05. CI = confidence interval; SE = standard error.
and GIs [7,15]. This design choice represented a compromise between the need to provide sufficient trials for adequate associative learning and ERP measurement and the need to minimize participant burden. It is unclear how closely these different measures map onto typical pre-clinical tests of ISTA [7,75], and it remains unclear how closely these different measures map onto each other.

While this study provides evidence for differential salience of the alcohol CS2 and CS1 among LS versus HS individuals, it cannot address possible differences in the incentive value of the alcohol reward US. Moreover, although covarying recent alcohol use theoretically helps control the influence of CS–US pairings on differences in the CS2’s incentive value, it remains possible that different drinking histories contributed to the development of the LS phenotype and, by extension, LS participants’ P3 responses to the CS2. By definition, LS and HS individuals differ in the threshold for experiencing subjective responses to alcohol, and perhaps even in the profile of subjective responses [64]. Nonetheless, it is not clear whether the incentive value of alcohol reward differs for LS and HS individuals. Rodent STs and GIs generally attribute equivalent incentive value to food reward (US), despite attributing differential incentive value to a US-predictive CS [81,82]. Thus, to further the idea that the LS phenotype in humans resembles the ST phenotype in rodents, it will be important for future research to establish whether the reward value of alcohol consumption is equivalent for LS and HS individuals.

Additionally, the current study did not directly demonstrate enhanced cue-reactivity as a mechanism linking LS with heavy drinking. According to some accounts [83,84], the clinical relevance of Pavlovian-conditioned responses to alcohol cues hinges upon their ability to promote consumption. Thus, it will be important in future work to directly examine whether LS-related increases in reactivity to a novel alcohol-associated CS predict alcohol drinking behaviors (e.g. ad-libitum consumption in the presence of the CS) and can reinforce the learning of new actions (i.e. conditional reinforcement).

Also, although significant group differences in neurophysiological reactivity to the novel alcohol CS2 were observed after conditioning, this group difference did not persist into the CS2 extinction (CS1 omission) phase, owing to an increase in alcohol CS2-elicited P3 amplitude among HS participants during extinction. The reasons underlying this increase are not clear, but the finding raises the possibility that alcohol CS2 extinction may engage different psychological processes for HS and LS individuals, given acquired differences in alcohol CS2 neural reactivity and fewer real-world exposures to alcohol among HS individuals. Future research will benefit from additional levels of measurement, including behavioral assessment (e.g. approach tendency, attentional bias), physiological monitoring (e.g. heart rate variability, pupillometry, skin conductance) and self-report (e.g. affect, craving) during cue exposure.

In conclusion, the current findings are the first to demonstrate increased neurophysiological reactivity to a novel alcohol-associated CS among individuals at risk for AUD. These findings support the idea that faster or stronger appetitive conditioning of alcohol-related cues represents one mechanism by which the LS phenotype might confer increased AUD risk [14,84,85]. More broadly, the current work advances the difficult problem of translating pre-clinical models into human addiction-risk phenotypes, potentially suggesting avenues for intervention, such as decreasing the appetitive strength of conditioned alcohol stimuli among LS individuals.

Declaration of interests
None.

Acknowledgements
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Author contributions
KIMBERLY FLEMING: Conceptualization; formal analysis; funding acquisition; investigation; methodology; project administration. ROBERTO COFRESI: Conceptualization; data curation; formal analysis; visualization. BRUCE BARTHOLOM: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; visualization.

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Supporting Information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Supporting Information.