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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; CS_2) predicted onset of an olfactory cue (first-order conditional stimulus; CS_1). 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 CS_2 across its conditioning and extinction, and to the CS_1, provided neurophysiological indices of incentive salience (IS). **Findings** The IS of the alcohol CS_1 was higher among participants low in alcohol sensitivity (LS), relative to their higher-sensitivity (HS) peers. The IS of the CS_2 paired with the alcohol CS_1 increased across the CS_2 conditioning phase among LS but not HS participants. Also, LS (but not HS) individuals also experienced increases in alcohol craving following alcohol CS_1 exposure, and this change was correlated with increases in the IS of the CS_2 paired with the alcohol CS_1. **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.

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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

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]. Secondly, 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, CS_2) predicted onset of an olfactory cue (the first-order conditional stimulus, CS_1), such that three novel associations (alcohol CS_2–CS_1, sweet CS_2–CS_1 and NCAppC CS_2–CS_1) 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 CS_2 and olfactory CS_1 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 CS_1 exposure (H1). In addition, because alcohol odor (but not sweet odor) probably has more pre-existing incentive salience for LS individuals, the alcohol CS_1 should support more effective conditioning of the alcohol CS_2 among LS than HS individuals. Hence, the alcohol CS_2 (but not sweet CS_2) was expected to elicit larger P3 amplitude among LS versus HS participants during CS_2 acquisition (H2). More specifically, the P3 elicited by the alcohol CS_2 was expected to increase across pairings among LS but not HS individuals (H3). The P3 elicited by the newly conditioned alcohol CS_2 was expected to remain larger among LS versus HS individuals during CS_2 extinction (CS_1 omission) (H4), reflecting short-term maintenance of incentive salience for the alcohol CS_2. Also, given evidence for associations between neurophysiological and craving responses to drug-related cues [61,62], we predicted that alcohol CS_1-induced craving would correlate with P3 response to the alcohol CS_2 (H5).

Following previous demonstrations of larger neurophysiological response to alcohol but not to other appetitive visual CS_1 among LS versus HS individuals [31,32,36], we

expected larger LPC amplitude for alcohol but not for other olfactory CS_1 among LS compared to HS individuals (H6). Because alcohol CS_2–CS_1 pairings involved presentation of alcohol CS_1 without subsequent alcohol ingestion [unconditional stimulus [US)], alcohol CS_2 acquisition involved *de-facto* alcohol CS_1 extinction (US omission). Thus, LS versus HS LPC amplitude differences were expected to be largest at the beginning of CS_2 acquisition (H7). Finally, given that ST is resistant to cue extinction [22,23], less extinction of CS_1 response—i.e. less decrease in LPC amplitude across non-reinforced CS_1 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

	LS (n = 49)		*HS (n = 47)*		*Group difference*	
	n (%)		*n (%)*			
	10 (20)		*12 (25)*		χ^2 *0.12*	*P 0.723*
AUD FH+	*Mean (SD)*	*Median (IQR)*	*Mean (SD)*	*Median (IQR)*	U	P
Past-month drinks/week	12.46 (10.67)	9.00 (11.50)	5.93 (7.66)	3.75 (4.56)	584	< 0.001
Past-month binges	1.15 (0.87)	0.62 (0.87)	0.37 (0.23)	0.25 (0.62)	514	< 0.001
Past-month maximum drinks/hour	2.50 (1.19)	2.17 (1.65)	1.97 (1.19)	1.75 (1.08)	793	0.008
Negative consequences	7.47 (6.40)	5.90 (8.8)	4.54 (5.09)	2.30 (6.0)	778.5	0.006
AUD-related negative consequences	4.17 (4.34)	2.00 (6.00)	2.42 (3.11)	1.00 (2.75)	867.5	0.037

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 (CS_1: alcohol, sweet and NCAppC) with a previously unassociated visual cue (CS_2: red, green and blue 2″ squares) while EEG was recorded. The task was divided into three blocks, two for acquisition of CS_2–CS_1 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 CS_2 (1000 ms), which was followed 3 sec later by presentation of a CS_1 (2000 ms). CS_1 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 CS_2; and (2) the CS_1 was always omitted. CS_2–CS_1 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 CS_2-locked ERP (Fig. 2a), we quantified P3 mean amplitude over parieto-occipital and occipital electrodes. In the CS_1-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

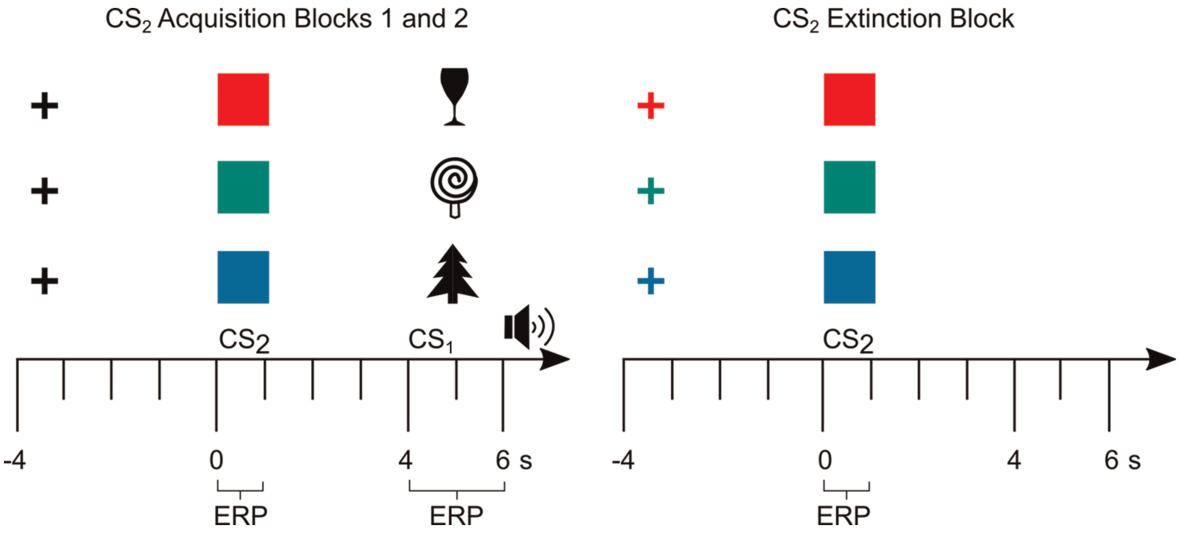

Figure 1 Structure of second-order appetitive conditioning and extinction task. During the two conditional stimulus $(CS)_2$ acquisition blocks, each color square was presented for 1 sec and followed consistently after 3 sec by presentation for 2 sec of one of three odor CS_1. Pairing of CS_2 color square with CS_1 odor category was counterbalanced across participants; the schematic illustrates one example pairing (red square CS_2 with alcohol odor CS_1, green square CS_2 with sweets odor CS_1 and blue square CS_2 with NCAppC odor CS_1). Odorant release was signaled on the computer screen by the word 'sniff', instructing participants to inhale. After 2 sec, an auditory tone alerted participants to cease inhalation. During the CS_2 extinction block, odor CS_1 were never presented, and the fixation cross-color signaled the identity of the subsequent CS_2. CS_2- and CS_1-locked event related potentials (ERPs) were derived from the scalp electroencephalogram (EEG) to measure CS_2-elicited P3 and CS_1-elicited late positive complex (LPC). [Colour figure can be viewed at wileyonlinelibrary.com]

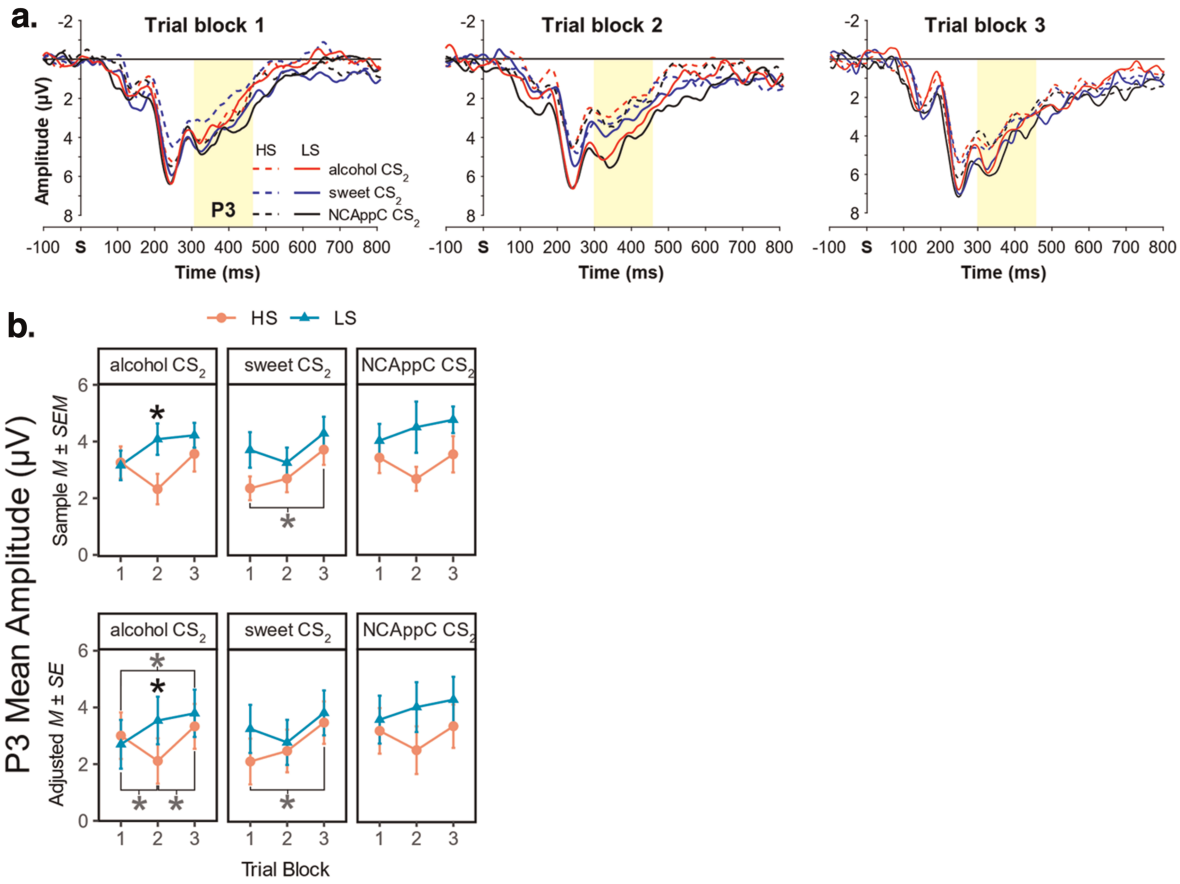

Figure 2 Visual conditional stimulus $(CS)_2$-event-related brain potential (ERP) and P3 component mean amplitudes as a function of trial block, paired odor CS_1 category and alcohol sensitivity group. (a) Visual stimulus-locked ERP waveform for the alcoholic beverage odor, sweet food odor and non-comestible appetitive control (NCAppC) odor CS_1-paired CS_2 across CS_2 conditioning (trial blocks 1 and 2) and CS_2 extinction (CS_1 omission; trial block 3). 'S' on the *x*-axis denotes time of stimulus onset. Window (300–450 ms) for P3 component mean amplitude quantification denoted by yellow rectangle. (b) Mean amplitude (μV) of the P3 component for the alcohol-, sweet- and NCAppC CS_1-paired CS_2 across trial blocks. Top row: sample mean and standard error of the mean (SEM). Bottom row: model-estimated marginal population mean and standard error (SE) adjusted for effects of age, sex, race and typical alcohol use. Black asterisk indicates $P < 0.05$ for between-group mean comparisons. Gray asterisk indicates $P < 0.05$ for within-group mean comparisons. (a,b) 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. [Colour figure can be viewed at wileyonlinelibrary.com]

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 CS_2 conditioning trials (separated by a 10-minute break), followed by completion of craving measures again and then the block of CS_2 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 CS_1

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

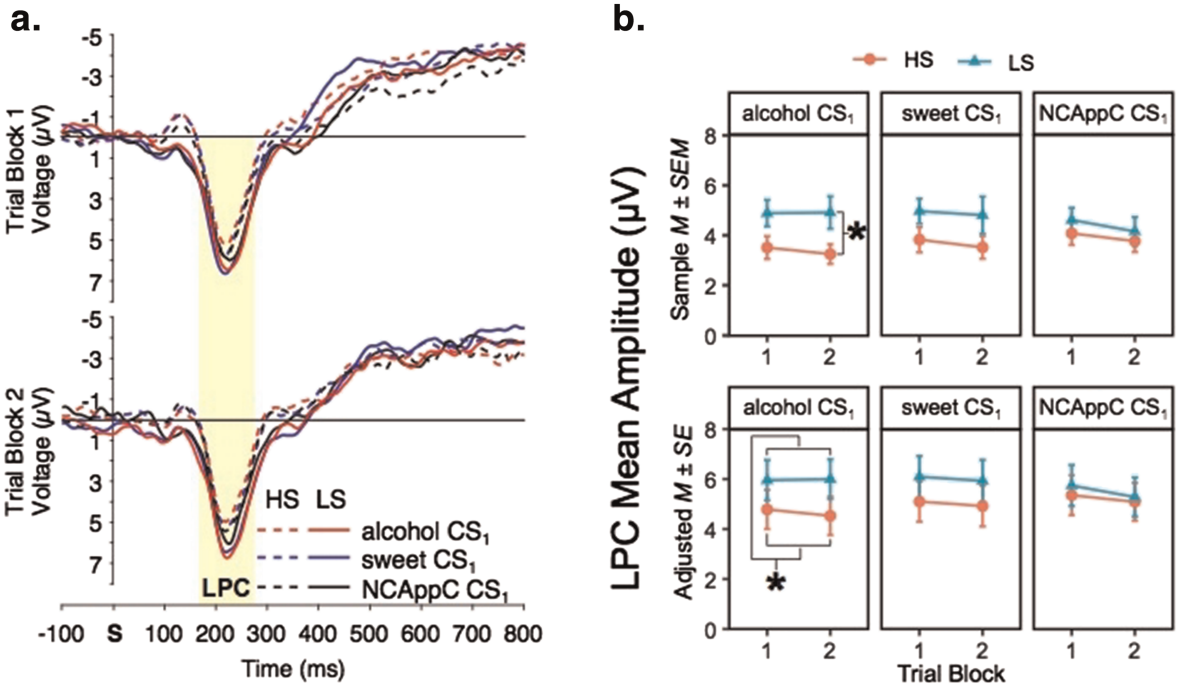

Figure 3 Odor conditional stimulus $(CS)_1$-elicited event-related brain potential (ERP) and late positive complex (LPC) component mean amplitudes as a function of trial block, odor CS_1 category and alcohol sensitivity group. (a) Odor stimulus-locked ERP for the alcoholic beverage odors, sweet food odors and non-comestible appetitive control (NCAppC) odor CS_1 across CS_2 conditioning (trial blocks 1 and 2). As the putative US was never presented, CS_2 conditioning was *de-facto* CS_1 extinction. 'S' on the *x*-axis denotes time of stimulus onset. Window (180–280 ms) for LPC component mean amplitude quantification denoted by yellow square. (b) Mean amplitude (μV) of the LPC for alcohol, sweet and NCAppC CS_1 across trial blocks. Top row: sample mean and standard error of the mean (SEM). Bottom row: model-estimated marginal population mean and standard error (SE) adjusted for effects of age, sex, race and typical alcohol use. Black asterisk indicates $P < 0.05$ for between-group mean comparisons. (a,b) HS = participants ($n = 47$) with high sensitivity to alcohol, as defined by alcohol sensitivity questionnaire (ASQ) scores. LS = participants ($n = 49$) with low sensitivity to alcohol as defined by ASQ scores. [Colour figure can be viewed at wileyonlinelibrary.com]

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 CS_2

Predictions concerning differential acquisition of P3 response to the alcohol CS_1–paired CS_2 across CS_2 acquisition and extinction (H2–4) were tested with a 2 (group: HS, LS) × 3 (CS_1 category: alcoholic beverage odors, sweet odors, NCAppC odors) × (trial block: 1, 2, 3) LMM adjusting for age, typical alcohol use, race and sex. CS_2-elicited ERPs are shown in Fig. 2a; mean CS_2-elicited P3 amplitudes are given in Fig. 2b. The predicted three-way interaction involving the alcohol sensitivity group, trial block and CS_1 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 CS_2 is associated with craving response to alcohol CS_1

To test the prediction that P3 response to the newly trained alcohol CS_2 covaried with craving induced by the alcohol CS_1 (H5), we computed a CS_2-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 CS_1

Predictions concerning differential LPC response to the alcohol CS_1 (H6–8) were tested with a 2 (group: HS, LS) × 3 (CS_1 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 CS_1 category was not significant, $F_{(2,4752)} = 1.12$, $P = 0.324$, but there was a significant group × CS_1 category interaction, $F_{(2,95.9)} = 3.27$, $P = 0.042$. 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.

Table 2 Alcohol sensitivity group × craving type interaction effect on conditional stimulus $(CS)_1$-elicited craving: simple effects analysis

Simple effect of alcohol Sensitivity group (LS versus HS)	$Mean_{LS}$	SD_{LS}	$Mean_{HS}$	SD_{HS}	*95% CI* $Mean_D$	*t*	*d.f.*	*P*
Alcohol	**1.71**	**4.96**	**−1.79**	**4.94**	**1.50–5.51**	**−3.47**	**94**	**< 0.001**
Sweets	−0.22	8.49	0.31	8.49	−3.57–2.50	−0.35	94	0.729
Simple effect of craving type	*Mean*	*SD*	–	–	*95% CI mean*	t	*d.f.*	P
LS, alcohol	**1.71**	**4.96**			**0.29–3.14**	**2.42**	**48**	**0.019**
LS, sweets	−0.22	8.49			−2.66–2.22	−0.18	48	0.856
HS, alcohol	**−1.79**	**4.94**			**−3.24–(−0.34)**	**−2.49**	**46**	**0.016**
HS, sweets	0.31	8.49			−1.53–2.15	0.34	46	0.736

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.

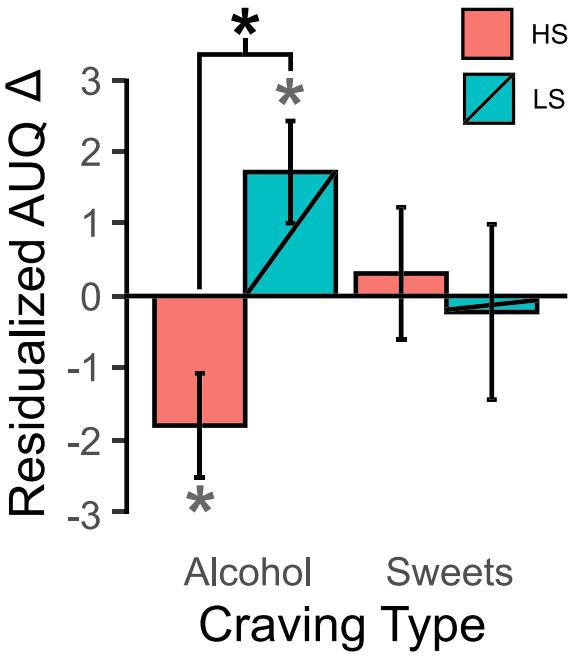

Figure 4 Residualized alcohol urge questionnaire (AUQ) scores representing change in craving for alcohol and sweets as a function of alcohol sensitivity group. Positive values indicate greater craving following odor [conditional stimulus (CS_1)] exposure than would be expected based on the baseline (pre-CS_1 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]

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's 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—incentive sensitization—through which LS might increase risk for AUD [14].

Among LS individuals, a previously neutral stimulus became an incentivized alcohol-related cue (alcohol CS_2) after repeated pairing with an existing, incentivized alcohol-related cue (alcohol CS_1). Given the voluminous literature associating P3 amplitude with the incentive–motivational value of eliciting stimuli [48,49], increased P3 response to the alcohol CS_2 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 CS_2 was retained among LS participants during the extinction block (CS_1 omission), partially supporting the idea (H4) that acquired incentive salience for the alcohol CS_2 was maintained in the higher-risk group. Additionally, craving for

Table 3 Between- and within- group comparisons of model-estimated, covariate-adjusted population marginal means for alcohol, sweet and NCAppC conditional stimulus $(CS)_2$-elicited P3 mean amplitude (μV) across trial blocks

LS versus HS	$Mean_{LS}$	SE_{LS}	95% CI_{LS}	$Mean_{HS}$	SE_{HS}	95% CI_{HS}	$Mean_D$	SE_D	Z	*P*
Block 1, alcohol CS_2	2.70	0.86	1.01–4.39	3.00	0.82	1.40–4.39	−0.30	0.71	−0.42	0.671
Block 2, alcohol CS_2	**3.54**	**0.84**	**1.89–5.18**	**2.11**	**0.80**	**0.55–3.68**	**1.43**	**0.69**	**2.06**	**0.039**
Block 3, alcohol CS_2	3.79	0.83	2.16–5.43	3.33	0.79	1.78–4.89	0.46	0.73	0.63	0.530
Block 1, sweet CS_2	3.24	0.85	1.58–4.91	2.09	0.81	0.51–3.68	1.15	0.74	1.56	0.118
Block 2, sweet CS_2	2.77	0.80	1.20–4.33	2.46	0.75	0.99–3.94	0.30	0.63	0.48	0.632
Block 3, sweet CS_2	3.81	0.79	2.25–5.36	3.46	0.75	2.00–4.93	0.34	0.67	0.51	0.607
Block 1, NCAppC CS_2	3.57	0.84	1.91–5.23	3.17	0.80	1.60–4.75	0.40	0.69	0.57	0.568
Block 2, NCAppC CS_2	4.01	0.88	2.29–5.73	2.49	0.84	0.84–4.14	1.52	0.80	1.90	0.057
Block 3, NCAppC CS_2	3.79	0.83	2.16–5.43	3.33	0.79	1.78–4.89	0.93	0.68	1.37	0.170
Block 2 versus block 1	$Mean_{B2}$	SE_{B2}	95% CI_{B2}	$Mean_{B1}$	SE_{B1}	95% CI_{B1}	$Mean_D$	SE_D	Z	P
LS, alcohol CS_2	3.54	0.84	1.89–5.18	2.70	0.86	1.01–4.39	0.84	0.44	1.88	0.059
LS, sweet CS_2	2.77	0.80	1.20–4.33	3.24	0.85	1.58–4.91	−0.48	0.44	−1.07	0.283
LS, NCAppC CS_2	4.01	0.88	2.29–5.73	3.57	0.84	1.91–5.23	0.44	0.44	0.99	0.322
HS, alcohol CS_2	**2.11**	**0.80**	**0.55–3.68**	**3.00**	**0.82**	**1.40–4.39**	**−0.89**	**0.45**	**−1.98**	**0.047**
HS, sweet CS_2	2.46	0.75	0.99–3.94	2.09	0.81	0.51–3.68	0.37	0.45	0.83	0.409
HS, NCAppC CS_2	2.49	0.84	0.84–4.14	3.17	0.80	1.60–4.75	−0.68	0.45	−1.51	0.130
Block 3 versus block 2	$Mean_{B3}$	SE_{B3}	95% CI_{B3}	$Mean_{B2}$	SE_{B2}	95% CI_{B2}	$Mean_D$	SE_D	Z	P
LS, alcohol CS_2	3.79	0.83	2.16–5.43	3.54	0.84	1.89–5.18	0.25	0.53	0.48	0.632
LS, sweet CS_2	3.81	0.79	2.25–5.36	2.77	0.80	1.20–4.33	1.04	0.53	1.95	0.051
LS, NCAppC CS_2	3.79	0.83	2.16–5.43	4.01	0.88	2.29–5.73	0.26	0.53	0.49	0.623
HS, alcohol CS_2	**3.33**	**0.79**	**1.78–4.89**	**2.11**	**0.80**	**0.55–3.68**	**1.22**	**0.54**	**2.27**	**0.023**
HS, sweet CS_2	3.46	0.75	2.00–4.93	2.46	0.75	0.99–3.94	1.00	0.54	1.86	0.063
HS, NCAppC CS_2	3.33	0.79	1.78–4.89	2.49	0.84	0.84–4.14	0.84	0.54	1.57	0.117
Block 3 versus block 1	$Mean_{B3}$	SE_{B3}	95% CI_{B3}	$Mean_{B1}$	SE_{B1}	95% CI_{B1}	$Mean_D$	SE_D	Z	P
LS, alcohol CS_2	**3.79**	**0.83**	**2.16–5.43**	**2.70**	**0.86**	**1.01–4.39**	**1.09**	**0.54**	**2.01**	**0.044**
LS, sweet CS_2	3.81	0.79	2.25–5.36	3.24	0.85	1.58–4.91	0.56	0.54	1.04	0.300
LS, NCAppC CS_2	3.79	0.83	2.16–5.43	3.57	0.84	1.91–5.23	0.71	0.54	1.30	0.195
HS, alcohol CS_2	3.33	0.79	1.78–4.89	3.00	0.82	1.40–4.39	0.33	0.55	0.60	0.548
HS, Sweet CS_2	**3.46**	**0.75**	**2.00–4.93**	**2.09**	**0.81**	**0.51–3.68**	**1.37**	**0.55**	**2.50**	**0.012**
HS, NCAppC CS_2	3.33	0.79	1.78–4.89	3.17	0.80	1.60–4.75	0.16	0.55	0.30	0.764
Linear trend	–	–	–	–	–	–	*B*	*SE*	Z	P
LS, alcohol CS_2							**1.09**	**0.54**	**2.03**	**0.044**
LS, sweet CS_2							0.56	0.54	1.04	0.299
LS, NCAppC CS_2							0.70	0.54	1.30	0.195
HS, alcohol CS_2							0.33	0.55	0.60	0.548
HS, sweet CS_2							**1.37**	**0.55**	**2.50**	**0.012**
HS, NCAppC CS_2							0.16	0.55	0.30	0.764
Quadratic trend	–	–	–	–	–	–	*B*	*SE*	Z	P
LS, alcohol CS_2							−0.58	0.82	−0.71	0.477
LS, sweet CS_2							1.52	0.82	1.85	0.064
LS, NCAppC CS_2							−0.18	0.82	−0.22	0.827
HS, alcohol CS_2							**2.11**	**0.83**	**2.55**	**0.011**
HS, sweet CS_2							0.63	0.83	0.76	0.446
HS, NCAppC CS_2							1.52	0.83	1.84	0.065

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. CS_2 conditioning (i.e. pairings with the alcohol, sweet or NCAppC CS_1) took place in trial blocks 1 and 2. CS_2 extinction (i.e. omission of CS_1) took place in trial block 3. Bold type indicates effects with $P < 0.05$. CI = confidence interval; SE = standard error.

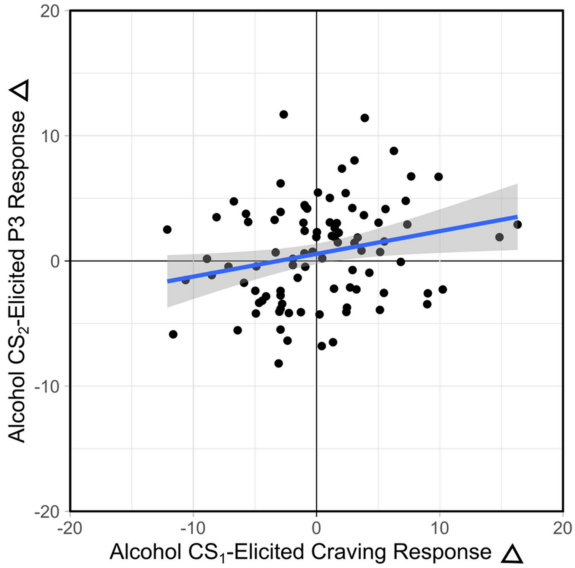

Figure 5 Association between acquired P3 response to alcohol conditional stimulus $(CS)_2$ and craving elicited by alcohol CS_1. P3 Response Δ = change in the P3 mean amplitude from CS_2 conditioning block 1 to block 2. Craving response Δ = change in the alcohol alcohol urge questionnaire (AUQ) sum score from baseline [before any alcohol beverage odor (CS_1) exposure] to after the CS_2 conditioning blocks (48 exposures total, 2 sec per exposure). Pairwise complete data from 91 individuals are shown. Association between acquired P3 response and degree of alcohol craving (Pearson's $r = 0.23$, $P = 0.023$) is illustrated on the plot by a simple linear regression line [B ± standard error (SE) = 0.18 ± 0.08 Δ P3/Δ craving, $P = 0.023$]. Shaded area around the regression line demarcates ± 95% confidence limits for prediction. NCAppC = non-comestible appetitive control odors. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 4 Between-group comparisons of model-estimated, covariate-adjusted population marginal means for alcohol, sweet and NCAppC conditional stimulus $(CS)_1$-elicited late positive complex (LPC) mean amplitude (μV) across CS_2 conditioning (collapsed trial blocks 1 and 2)

LS versus HS	$Mean_{LS}$	SE_{LS}	95% CI_{LS}	$Mean_{HS}$	SE_{HS}	95% CI_{HS}	$Mean_D$	*SE*	*Z*	*P*
Alcohol CS_1	**5.97**	**0.77**	**4.46–7.49**	**4.66**	**0.75**	**3.19–6.12**	**1.32**	**0.56**	**2.34**	**0.019**
Sweet CS_1	6.01	0.81	4.43–7.59	5.01	0.78	3.48–6.54	1.00	0.65	1.53	0.125
NCAppC CS_1	5.51	0.78	3.99–7.03	5.22	0.75	3.75–6.69	0.88	0.57	0.50	0.614

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.

alcohol increased during the course of the task for LS but not HS individuals—presumably as a result of alcohol CS_1 exposure—and individual differences in this craving change corresponded with changes in P3 amplitude elicited by the alcohol CS_2 during conditioning. Taken together, these findings show that alcohol odor, a proximal cue for alcohol consumption, supported conditioning of a novel CS_2 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_2 and CS_1 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).

Despite its strengths, the current study had several limitations. In particular, the paradigm differed in important ways from those used in pre-clinical tests of ISTA. For example, we used fewer than half the number of conditioning trials typically given to differentiate rodent STs

and GTs [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 human laboratory paradigms will need to parallel pre-clinical paradigms in order to successfully translate pre-clinical constructs into measurable human phenotypes [12–14]. Also, the use of cue-elicited P3/LPC amplitude as an index of motivational significance differs from the use of cue-directed behavioral approach measures typical of 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 CS_2 and CS_1 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 CS_2'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 CS_2. 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 GTs 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 CS_2 were observed after conditioning, this group difference did not persist into the CS_2 extinction (CS_1 omission) phase, owing to an increase in alcohol CS_2-elicited P3 amplitude among HS participants during extinction. The reasons underlying this increase are not clear, but the finding raises the possibility that alcohol CS_2 extinction may engage different psychological processes for HS and LS individuals, given acquired differences in alcohol CS_2 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.

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Author contributions

Kimberly Fleming: Conceptualization; formal analysis; funding acquisition; investigation; methodology; project administration. **Roberto Cofresí:** Conceptualization; data curation; formal analysis; visualization. **Bruce Bartholow:** 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.