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Functional significance of subjective response to alcohol across levels of alcohol exposure

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ABSTRACT

Pre-clinical neurobiological models of addiction etiology including both the allostatic model and incentive sensitization theory suggest that alcohol consumption among alcohol-dependent (AD) individuals will be dissociated from hedonic reward as positive reinforcement mechanisms wane in later stage dependence. The aims of this study are to test this claim in humans by examining the relationship between dimensions of subjective responses to alcohol (SR) and alcohol craving across levels of alcohol exposure. Non-treatment-seeking drinkers ($n = 205$) completed an i.v. alcohol challenge (final target breath alcohol concentration = 0.06 g/dl) and reported on SR and craving. Participants were classified as light-to-moderate drinkers (LMD), heavy drinkers (HD) or AD. Analyses examined group differences in SR and craving response magnitude, as well as concurrent and predictive associations between SR domains and craving. At baseline, LMD and AD reported greater stimulation than HD, which carried over post-alcohol administration. However, stimulation was dose-dependently associated with alcohol craving in HD only. Furthermore, lagged models found that stimulation preceded craving among HD only, whereas this hypothesized pattern of results was not observed for craving preceding stimulation. Sedation was also positively associated with craving, yet no group differences were observed. In agreement with the prediction of diminished positive reinforcement in alcohol dependence, this study showed that stimulation/hedonic reward from alcohol did not precede craving in AD, whereas stimulation was dose-dependently associated with and preceded craving among non-dependent HD.

Keywords Alcohol challenge, alcohol dependence, subjective response to alcohol.

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INTRODUCTION

Alcoholism is a chronic and relapsing condition with substantial economic and public health consequences (Rehm *et al.* 2009). Both animal and human theories of alcoholism etiology have focused on biobehavioral response to alcohol, albeit from different perspectives. While the human laboratory represents an integral component of addiction research (Ray *et al.* 2010), ethical and methodological considerations limit the level of experimental control and neurobiological precision of clinical research. Thus, studies aiming to translate neurobiologically precise pre-clinical theories of alcoholism etiology to human populations stand to bridge the gap between pre-clinical and clinical research and provide much needed clarity about the development and maintenance of alcoholism. The aim of the present study is to position subjective response to alcohol (SR), a well-characterized phenotype in the clinical literature on alcoholism risk (King *et al.* 2014; Newlin & Thomson 1990; Schuckit 1994, 1984), as a biobehavioral marker of disease progression. In doing so, we aim to test central claims from several well-established pre-clinical models, namely, diminished positive reinforcement mechanisms in later alcohol dependence versus earlier non-dependent drinking.

The seminal research on SR from Schuckit *et al.* posited that a blunted response to alcohol intoxication was

a risk-factor for future alcohol misuse and dependence (Schuckit 1994, 1984; Schuckit & Smith 1996). Termed the low-level of response (LR) model, this theory examined response to alcohol as a unidimensional construct with a particular focus on the more sedative, or motor intoxication components of SR (Ray *et al.* 2009; Schuckit *et al.* 1997; Schuckit & Gold 1988). Conversely, the differentiator model was built on a two-dimensional conception of SR consisting of both stimulatory and sedative dimensions (King *et al.* 2011; Newlin & Thomson 1990). While in broad agreement with the LR model in terms of sedative effects, the differentiator model adds enhanced stimulation as a risk factor for later dependence. Both models address important questions about alcoholism risk and have garnered considerable empirical support in terms of prospectively predicting alcohol dependence (King *et al.* 2014, 2011; Quinn & Fromme 2011; Schuckit 1994). However, neither model addresses a complementary question concerning the function of SR in promoting escalation of alcohol consumption, including escalation to binge drinking levels, within a given drinking episode. Furthermore, with few exceptions (e.g. Bujarski & Ray 2014; Hobbs *et al.* 2005), no major studies have examined whether the function of SR domains in promoting alcohol use is responsive to, or even causally related to the pathophysiology of alcohol dependence. This gap in the literature is particularly salient as dynamic changes in the function of SR over levels of alcohol exposure is a central tenet of several prominent pre-clinical models of alcoholism etiology including the allostatic model (Koob & Le Moal 1997) and incentive sensitization theory (IST; Robinson & Berridge 1993, 2001).

Both the allostatic model and IST support a waning of positive reinforcement mechanisms in substance dependence. The allostatic model proposes a cycle of progressive neurobiological dysregulation, beginning with preoccupation and anticipation (reflecting positive reinforcement) and ending with withdrawal-mediated alcohol use (reflecting negative reinforcement) (Koob & Kreek 2007; Koob & Le Moal 1997; Koob & Volkow 2009). In IST, hedonic reward 'liking', and motivational salience, 'wanting', are both neurobiologically and phenotypically dissociated (Berridge 2007; Robinson & Berridge 2001). IST proposes drug dependence to be the result of neuro-adaptation that potentiates, or 'sensitizes', the motivational salience of the drug and associated cues. In most depictions of IST, hedonic reward from drug administration is blunted in drug dependence (Robinson & Berridge 2000, 1993); however, the strong predictions of IST lie in the sensitization of incentive motivation systems. Thus, according to IST, while the magnitude of hedonic reward decreases or remains unchanged in dependence, the function of these hedonic responses in promoting drug taking (i.e. positive reinforcement) decreases in dependence as the sensitization of incentive salience systems comes dominate motivated drug-taking behavior. While these influential models differ in terms of their conception of late-stage addiction maintenance factors, namely, withdrawal/negative reinforcement versus potentiated motivational salience of drug-associated cues, both suggest that alcohol consumption among dependent individuals will be dissociated from hedonic reward.

The aim of this study is to test this claim in humans by examining the relationship between SR and alcohol craving as a function of alcoholism development. Based on previous studies that have shown significant correlations between craving and alcohol self-administration over and above SR variables (McKee *et al.* 2009, 2008; O'Malley *et al.* 2002) and on the content of craving scales that position craving as a theoretically more proximal variable to actual alcohol consumption (e.g. 'All I want to do now is have a drink'; Bohn *et al.* 1995), alcohol craving was selected as the primary dependent variable of interest. Thus, in this study, craving serves as a proxy for escalation of drinking, although future studies should examine these questions utilizing alcohol self-administration paradigms.

The present study aims to replicate and extend previous work by our group (Bujarski & Ray 2014) examining the relationship between SR and craving by addressing several key limitations and using a new, large and well-balanced sample of drinkers. Utilizing data from i.v. alcohol challenge sessions with 91 subjects (42 with current alcohol dependence), we previously demonstrated that stimulatory SR were strongly and positively correlated with craving among heavy drinkers, whereas craving was dissociated from SR among dependent drinkers. These results were consistent with the purported waning of positive reinforcement-mediated drinking in alcohol dependence; however, several limitations in our prior study dampened the inferences that could be drawn. For example, our prior drinking groups were recruited from separate locations, thus increasing their heterogeneity. In this study, we recruited a larger sample of drinkers from the same location, thus limiting the influence of unmeasured demographic confounds. This study represents a replication effort insofar as we utilized an identical alcohol challenge paradigm with an independent subject pool and aim to address the same conceptual question as our prior paper, namely, whether alcohol dependence is characterized by changes in the function of SR in motivating continued alcohol use.

This study also aims to extend our prior findings. First, this study examines the function of SR in a wider range of alcohol use/misuse, from light-to-moderate drinkers (LMD) to those meeting criteria for current alcohol

dependence. Furthermore, this study aims to provide initial evidence of directionality between SR and craving via the application of lagged analysis.

Based on the allostatic model and IST, we hypothesize that stimulation/hedonic reward will be dose-dependently associated with and predictive of craving to a greater extent in non-dependent HD as compared with AD. This result would represent diminished positive reinforcement in dependence (i.e. the positive hedonic value gained from alcohol administration is associated with craving for additional alcohol in HD, but not AD). Furthermore, based on the allostatic model, we hypothesized that negative reinforcement would be more prominent in alcohol dependents (Koob & Kreek 2007; Koob & Le Moal 1997; Koob & Volkow 2009), which would be evidenced by a stronger negative association between tension and craving in dependent versus non-dependent drinkers. In sum, the present study will examine the coupling of positive and negative reinforcement-related SR with craving, thus providing insight about the role of alcohol dependence on the potential drivers of continued alcohol use.

METHODS AND MATERIALS

Participants

This study was approved by the Human Research Review Committee at the University of New Mexico. Non-treatment-seeking drinkers with a range of alcohol use/problems ($n = 205$) were recruited for a study on the biobehavioral responses to alcohol from the community through fliers and advertisements targeting drinkers over the age of 21 years. In order to reduce the possibility of adverse events in the alcohol challenge, participants were required to drink at least three or more drinks (two for women) twice per week. Participants with a history of depression with suicidal ideation or a lifetime psychotic disorder were excluded. Based on drinking participants' drinking history and a structured clinical interview (First 2005), participants were stratified as LMD (< 14 drinks per week [<7 for women]), *heavy drinkers* (HD; ≥ 14 drinks per week [≥7 for women]), or *alcohol-dependent* (AD: met DSM-IV criteria for past month alcohol dependence).

Screening procedure

Initial eligibility was conducted via telephone screening, and eligible participants were then invited to a laboratory session. After providing written informed consent, participants were breathalyzed to ensure no recent alcohol consumption, provided urine for urinalysis and completed a battery of self-report questionnaires and interviews (see section on Measures). All participants were required to test negative on a urine drug screen (except marijuana); otherwise, they were rescheduled. Female participants were required to test negative for pregnancy. This in-person assessment visit took approximately 2 hours after which participants traveled with the experimenter to a university-based hospital for the alcohol administration procedure.

Alcohol administration paradigm

Alcohol was administered intravenously to assess participants' pharmacological response to alcohol and to allow for precise control over breath alcohol concentration (BrAC) (Li *et al.* 2001; Plawecki *et al.* 2008). The alcohol challenge followed an established infusion protocol (Ray *et al.* 2013; Ray & Hutchison 2004). Participants were seated in a recliner chair with an i.v. placed in their non-dominant arm. Alcohol was administered using a 6 percent alcohol solution. Participants were infused at a rate of 0.166 ml/min × body weight in kilograms (0.126 ml/min × body weight for women). The alcohol infusion started at half target rate, to ensure safety, and was then escalated to the full rate after 5 minutes of monitoring. BrAC was measured via Breathalyzer every 3 to 5 minutes. Target BrACs were 0.02, 0.04 and 0.06 g/dl. Upon reaching each target BrAC, infusion rates were reduced by half to maintain BrAC during testing. During the consenting process, participants were informed they would receive alcohol, but remained blinded to the target dose and their BrAC throughout the experiment. The i.v. alcohol administration resulted in very highly controlled BrAC levels at each assessment (mean BrAC (SD): 0.020 (0.001), 0.040 (0.002) and 0.060 (0.002), respectively).

Measures

During the in-person screening, participants completed a demographics questionnaire as well as the Beck Depression Inventory-II (BDI-II) and the Beck Anxiety Inventory to assess for affective symptomatology, both of which are widely used and well-validated measures (Beck *et al.* 1996; Beck & Steer 1990; Fydrich *et al.* 1992; Sharp & Lipsky 2002).

Alcohol use measures

The Timeline Follow-Back (TLFB) was administered in interview format to capture daily alcohol use over the 60 days prior to the visit (Sobell *et al.* 1988). From the TLFB, we computed drinks per drinking day (DPDD) and number of drinking days (Drinking Days). The Structured Clinical Interview for DSM-IV was also administered by a masters' level clinician to assess for current (i.e. past month) alcohol dependence (First 2005).

Participants also completed the Alcohol Dependence Scale (ADS; Skinner & Allen 1982).

Subjective response measures

The following self-report measures were selected based upon previous alcohol challenge research, which provided empirical support for a three-factor model of SR consisting of stimulation/hedonic reward, sedation and tension relief dimensions (Ray *et al.* 2009). The Biphasic Alcohol Effects Scale was used to capture self-reported feelings of stimulation and sedation in response to alcohol and is a reliable and valid measure of SR (Erblich & Earleywine 1995; Martin *et al.* 1993; Roche *et al.* 2014). The Subjective High Assessment Scale assessed subjective feelings of alcohol intoxication. This measure was adapted from Schuckit (1984) and has been widely used in alcohol challenge studies (e.g. Courtney *et al.* 2013; Ray *et al.* 2012, 2009). The Profile of Mood States has four dimensions: positive mood, negative mood, vigor and tension, and it has been shown to be valid in the context of alcohol administration at the doses examined in this study (Ray *et al.* 2009). The Alcohol Urge Questionnaire (AUQ) assessed alcohol craving across the alcohol challenge. The AUQ has demonstrated high reliability in experimental studies of state-level alcohol craving (Bohn *et al.* 1995; MacKillop 2006). In the present sample, the AUQ was found to be a reliable measure in all drinking groups and at all BrAC timepoints ($\alpha \geq 0.72$).

Data analysis

Initially, a series of principal component analyses were conducted, which validated the three-factor model of SR (Supporting Information Table S1), and thus, sum scores within each factor were computed to produce reliable indicators of SR.

To examine SR and craving magnitude over the course of the alcohol administration, a series of mixed ANOVA models were conducted in SPSS version 20. BrAC was a within-subject factor with four levels (baseline, 0.02, 0.04 and 0.06 g/dl), and drinking group was a between-subject factor with three levels (LMD, HD and AD). *Post hoc* tests were conducted using Tukey's honest significant difference (HSD) test on all three possible drinking group pairs, and/or pairwise mixed ANOVA models.

Tests of the association between SR and craving utilized a multi-level modeling (MLM) framework using the **lme** function in the **multilevel** package (Bliese 2008) in R version 2.13.1. The first series of MLM models tested the associations between SR domains and craving at concurrent timepoints across rising BrAC. These models tested the dose-dependent associations between SR and craving and whether these effects were moderated by drinking group. In all MLM analyses, omnibus interactions were tested via a likelihood ratio test using the **anova** function. *Post hoc* tests examining differences between each pair of drinking groups were conducted through a dummy coding scheme. Standardized βs are presented as effect sizes in all MLM models.

The final series of statistical analyses examined the possible mechanistic pathways between SR and craving via testing lagged associations between SR and craving. These models tested (1) whether SR at a previous timepoint was predictive of craving at the subsequent timepoint, and (2) whether this prediction was moderated by drinking group. Subsequently, a series of models were conducted to see whether craving (lagged) was predictive of SR. While these analyses are not orthogonal to the concurrent analyses described above they do allow for initial testing of directionality, which is an important element of the study hypotheses derived from reinforcement models. Furthermore, to increase the independence of these lagged analyses compared with the concurrent analyses, additional models were run, which examined lagged effects over and above the effect of concurrent timepoint measures entered as covariates.

RESULTS

Sample characteristics

Sample characteristics are reported in Table 1. Seventy-eight subjects were classified as LMD, 57 as HD and 57 as AD. No demographic differences were observed between drinking groups ($P \geq 0.23$). Drinking groups differed significantly in terms of BDI-II total score ($F(2, 183) = 9.48$, $P < 0.001$), which was driven by greater depressed mood among ADs as compared with LMD and HD (Tukey's HSD $P < 0.001$). Of note, while the AD group did report greater levels of depression, only two subjects met the criteria for current major depression on the Structured Clinical Interview. Similarly, a trend towards significantly greater anxiety among ADs was also observed ($F(2, 176) = 2.94$, $P = 0.06$). As expected, drinking groups differed significantly in terms of drinking quantity, frequency, binge drinking frequency and ADS score ($P < 0.001$).

Subjective response and craving magnitude

Stimulation increased over the alcohol administration ($F(3, 555) = 53.29$, $P < 0.001$, $\eta_p^2 = 0.22$, Fig. 1a), and a main effect of group was observed ($F(2, 185) = 5.70$, $P < 0.01$, $\eta_p^2 = 0.06$). Unexpectedly, HD reported lower stimulation than LMD and AD (Tukey's HSD $P < 0.05$). The drinking group × BrAC interaction was not significant ($P = 0.15$). On *post hoc* analysis, these group

Table 1 Sample demographics, affective symptomatology and drinking quantity and frequency (taken from 60-day timeline follow-back).

	Light-to-moderate drinkers ($n = 78$)	*Heavy drinkers* ($n = 57$)	*Alcohol-dependent* ($n = 57$)	*Statistical test*
Age (SD)	25.46 (4.07)	25.77 (4.31)	25.63 (4.42)	$F(2, 188) = 0.09$, $P = 0.92$
Years of education (SD)	14.72 (2.65)	15.07 (2.17)	14.31 (1.99)	$F(2, 183) = 1.47$, $P = 0.23$
Sex (percent Male)	63 percent	61 percent	61 percent	X^2 $(2) = 0.04$, $P = 0.98$
Ethnicity (percent Caucasian)[a]	46 percent	54 percent	53 percent	Fisher exact $P = 0.38$
Beck Depression Inventory-II (SD)	4.53 (5.60)	4.89 (5.69)	9.55 (9.39)	$F(2, 183) = 9.48$, $P < 0.001$
Beck Anxiety Inventory (SD)	4.83 (5.49)	4.60 (5.62)	7.17 (7.45)	$F(2, 176) = 2.94$, $P = 0.06$
DPDD (SD)	3.48 (1.62)	5.55 (2.40)	7.08 (3.14)	$F(2, 189) = 38.42$, $P < 0.001$
Drinking days (SD)	17.14 (9.91)	30.56 (10.74)	34.88 (13.10)	$F(2, 189) = 47.36$, $P < 0.001$
Heavy drinking days (SD)	3.67 (3.38)	16.88 (9.77)	25.04 (12.81)	$F(2, 189) = 96.47$, $P < 0.001$
ADS (SD)	6.96 (4.18)	8.49 (4.20)	12.77 (4.93)	$F(2, 182) = 28.11$, $P < 0.001$

ADS = Alcohol Dependence Scale; DPDD = drinks per drinking day; All drinking groups differed from each other in terms of DPDD and Heavy drinking days (All Tukey's HSD $P < 0.001$). HD and AD groups were found to differ from LMD in terms of Drinking days (Tukey's HSD $P < 0.001$) yet HD and AD did not differ significantly ($P = 0.10$). AD differed from both LMD and HD on ADS score (Tukey's HSD $P < 0.001$), yet LMD and HD did not differ significantly from each other ($P = 0.13$). [a]Ethnicity was analyzed as a seven-level categorical variable, but for ease of presentation, percent Caucasian is reported.

differences on stimulation appeared to be baseline differences that were carried forward to the post-alcohol timepoints. Specifically, groups differed significantly at baseline on stimulation ($F(2, 187) = 7.33$, $P < 0.001$; Tukey's HSD: HD versus LMD/AD: $P < 0.01$, LMD versus AD: $P = 0.97$) and the main effect of drinking group was not significant when looking at post-alcohol timepoints controlling for baseline ($F(2, 184) = 1.47$, $P = 0.23$). To confirm that these group differences were not due to depression or anxiety differences, these analyses were repeated controlling for BDI-II and Beck Anxiety Inventory, and all results were unchanged by the inclusion of these affective covariates.

Sedation also increased over BrAC ($F(3, 552) = 87.35$, $P < 0.001$, $\eta_p^2 = 0.32$, Fig. 1b). Neither drinking group nor the BrAC × group interaction was significant ($P \geq 0.37$). On average, tension decreased over BrAC ($F(3, 558) = 7.18$, $P < 0.001$, $\eta_p^2 = 0.04$). No main effect of drinking group was observed ($P = 0.86$), yet there was a trend towards a BrAC × drinking group interaction ($F(6, 558) = 1.99$, $P = 0.07$, $\eta_p^2 = 0.02$). As seen in Fig. 1c, this interaction was driven by a decrease in tension from baseline to 0.02 among AD subjects only (*post hoc* BrAC × group interaction: $F(2, 188) = 3.56$, $P < 0.05$, $\eta_p^2 = 0.04$).

Craving increased over BrAC ($F(3, 558) = 89.77$, $P < 0.001$, $\eta_p^2 = 0.33$, Fig. 1d). A main effect of drinking group was observed ($F(2, 186) = 13.89$, $P < 0.001$ $\eta_p^2 = 0.13$), such that both HD and AD reported greater craving than LMD (Tukey's HSD $P < 0.01$; HD versus AD: $P = 0.20$). A BrAC× drinking group interaction was also observed ($F(6, 558) = 3.69$, $P = 0.001$, $\eta_p^2 = 0.04$). *Post hoc* analyses showed that LMD had a blunted increase in craving over the alcohol challenge as compared with both HD and AD ($P < 0.05$ $\eta_p^2 = 0.04$ and 0.03, respectively), yet HD and AD groups did not differ ($P = 0.35$).

Concurrent association between SR and craving

In terms of concurrent associations with alcohol craving, a three-way BrAC × drinking group × stimulation interaction was observed ($LR(2) = 13.41$, $P = 0.001$, Fig. 2a). Specifically, the association between stimulation and craving dose-dependently increase in HD only ($\beta = 0.16$, $P < 0.001$), whereas no dose-dependent association between stimulation and craving was apparent in either LMD ($\beta = -0.01$, $P = 0.74$) or AD ($\beta = 0.01$, $P = 0.85$). A significant BrAC × drinking group × sedation interaction was also observed ($LR(2) = 17.99$, $P < 0.001$, Fig. 2b). This interaction was such that overall, sedation was positively associated with craving; however, the association between sedation and craving was found to dose-dependently decrease in HD ($\beta = -0.21$, $P < 0.001$), which was blunted in both LMD and AD (LMD: $\beta = -0.01$, $P = 0.72$; AD: $\beta = -0.06$, $P = 0.06$). Lastly, a significant BrAC × drinking group × tension interaction was observed ($LR(2) = 7.31$, $P < 0.05$, Fig. 2c), such that LMD displayed a dose-dependent increase in the association between tension and craving ($\beta = 0.10$, $P < 0.001$), whereas no dose-dependent effect was observed in either HD ($\beta = 0.02$, $P = 0.52$) or AD groups ($\beta = -0.01$, $P = 0.73$).

In sum, both stimulation and sedation were positively associated with craving at concurrent timepoints, whereas tension was relatively unassociated with craving. Group differences were observed such that only HD displayed a dose-dependent association between stimulation and craving. Furthermore, the positive association between sedation and craving decreased over the alcohol

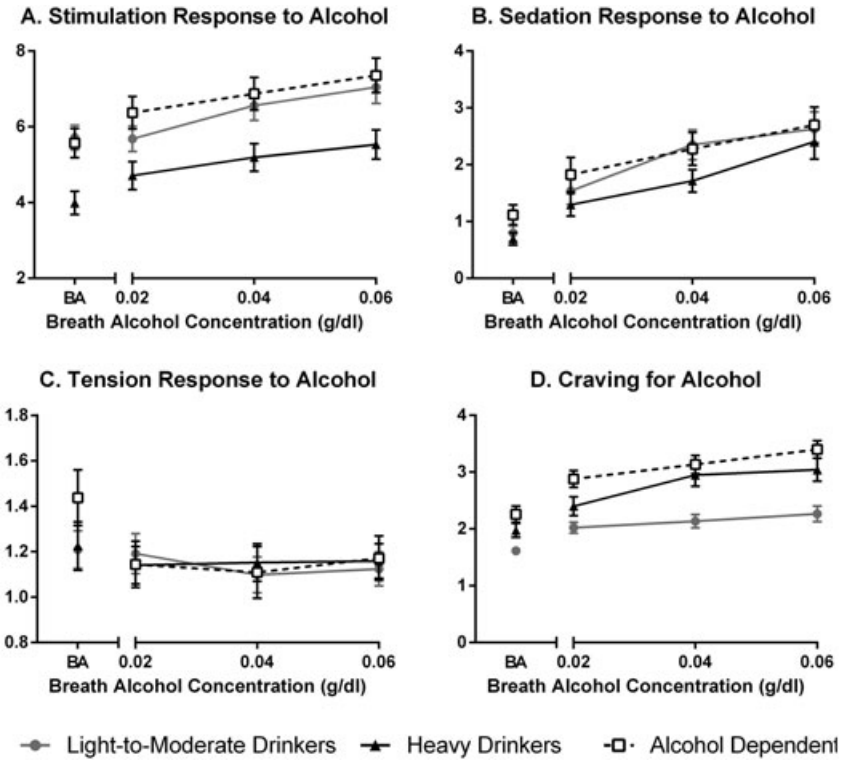

Figure 1 Magnitude of stimulation, sedation and tension responses to alcohol and alcohol craving. Means and standard error of the mean are presented from the raw data

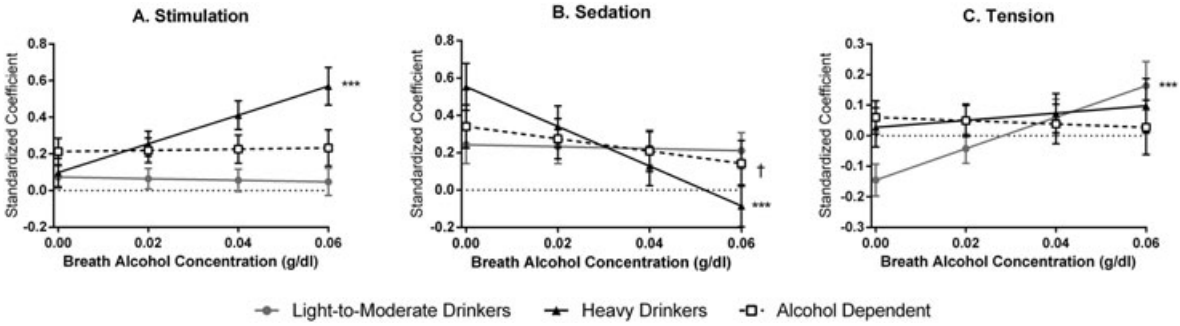

Figure 2 Associations between subjective responses to alcohol (SR) and craving at concurrent timepoints. Standardized simple effect coefficients are presented for (a) stimulation, (b) sedation and (c) tension. Significant breath alcohol concentration × SR interactions, representing a dose-dependent effect, are noted. Error bars represent the standard error of the simple effect coefficient. $^{\dagger}P < 0.10$ $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

administration in HD, whereas the association between tension and craving increased (and changed direction from negative to positive) over rising BrAC in LMD only.

Predictive associations between subjective response and craving

Lagged models were then conducted to provide initial evidence of directionality between SR and craving. In terms of SR predicting craving, a significant drinking group × stimulation interaction was observed (LR(2) = 6.37, $P < 0.05$, Fig. 3a), such that stimulation predicted craving in HD only ($\beta = 0.24$, $P < 0.001$), and not in LMD ($\beta = 0.03$, $P = 0.64$) or AD ($\beta = 0.04$, $P = 0.58$). Drinking groups did not differ in terms of sedation predicting craving (LR(2) = 0.88, $P = 0.64$), such that sedation significantly predicted craving in all groups (LMD: $\beta = 0.19$, $P = 0.001$; HD: $\beta = 0.29$, $P < 0.001$; AD: $\beta = 0.22$, $P < 0.01$; Fig. 3a). No group × tension interaction was observed (LR(2) = 1.28, $P = 0.53$), and in fact, tension was not found to predict craving for alcohol in any group ($P \geq 0.39$; Fig. 3a).

To ensure that these effects were not merely driven by the concurrent associations reported earlier, additional models were conducted, which included concurrent timepoint SR variables as covariates. As expected, craving was associated with concurrent timepoint stimulation

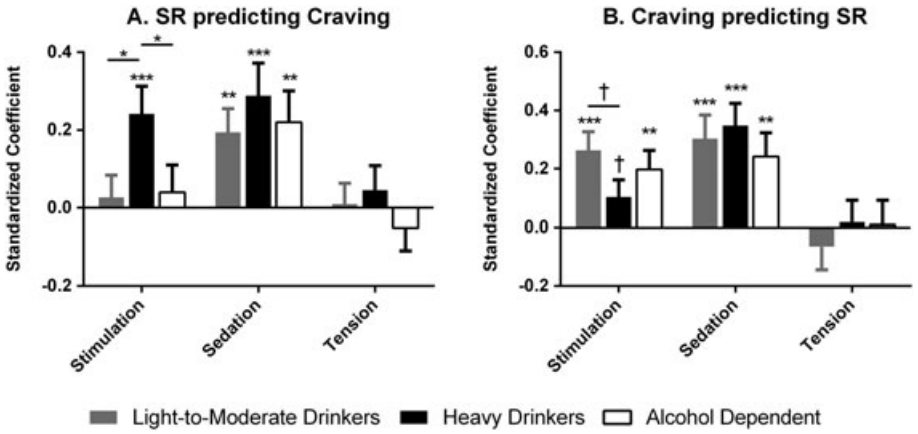

Figure 3 Results of lagged prediction models for (a) subjective responses to alcohol (SR) predicting craving at subsequent timepoints and (b) lagged craving predicting SR. Significant simple effects and drinking group differences are denoted. Error bars represent the standard error of the simple effect coefficient. $^{\dagger}P < 0.10$ $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

($\beta = 0.22$, $P < 0.001$) and sedation ($\beta = 0.10$, $P < 0.05$), but not tension ($\beta = 0.02$, $P = 0.63$). The inclusion of these concurrent timepoint SR variables only minimally affected the reported group differences. Specifically, in these models, the drinking group × lagged stimulation interaction was trending ($LR(2) = 5.70$, $P = 0.06$), and evidence of a positive predictive association between stimulation on craving was evidenced among HD only ($\beta = 0.12$, $P = 0.09$), not in LMD or AD ($\beta = -0.08$, and -0.05, respectively, $P \geq 0.17$). In these models, drinking group was not a moderator of lagged sedation or tension ($P \geq 0.49$).

In terms of craving predicting SR, no drinking group × craving interaction was observed with respect to stimulation ($LR(2) = 3.44$, $P = 0.18$). In fact, craving was significantly predictive of stimulation in both LMD ($\beta = 0.26$, $P < 0.001$) and AD ($\beta = 0.20$, $P < 0.01$) and at a trend level in HD ($\beta = 0.10$, $P = 0.07$; Fig. 3b). Similarly, craving was found to predict sedation in all drinking groups (LMD: $\beta = 0.30$, $P < 0.001$; HD: $\beta = 0.35$, $P < 0.001$; AD: $\beta = 0.24$, $P < 0.01$; Fig. 3b), and groups did not differ significantly from each other ($LR(2) = 0.90$, $P = 0.64$). Lastly, craving did not predict tension in any group (LMD: $\beta = -0.06$, $P = 0.42$; HD: $\beta = 0.02$, $P = 0.81$; AD: $\beta = 0.01$, $P = 0.92$; Fig. 3b), with no differences between groups ($LR(2) = 0.75$, $P = 0.69$).

As with lagged SR models earlier, these models were reanalyzed covarying for concurrent timepoint craving. These results were modestly affected by the inclusion of the concurrent timepoint covariate. Specifically, the drinking group × stimulation interaction was now significant ($LR(2) = 6.24$, $P < 0.05$), such that craving significantly predicted stimulation among LMD ($\beta = 0.22$, $P < 0.001$), and AD ($\beta = 0.13$, $P < 0.05$), but not among HD ($\beta = 0.01$, $P = 0.87$). In these models, drinking group was not a significant moderator of the effect of lagged craving on either sedation ($LR(2) = 0.69$, $P = 0.71$), or tension ($LR(2) = 0.72$, $P = 0.70$).

Together, these lagged models extend the dose-dependent concurrent results reported earlier in suggesting that stimulation preceded craving in HD only. Importantly, this pattern of group differences was not observed with craving preceding SR, suggesting that the differences between drinking groups in the predictive utility of stimulation is unlikely a byproduct of a general association, but instead is potentially indicative of a mechanistic pathway where stimulation/hedonic reward leads to craving in HD, but not in AD or LMD. In contrast, sedation and craving were found to predict each other in a bi-directional manner with no differences observed between groups, and tension and craving were not found to predict one another in any drinking group. Importantly for disentangling concurrent from predictive associations in a paradigm with relatively small inter-trial intervals, these results were only modestly affected by the inclusion of concurrent timepoint data.

DISCUSSION

These results provide an important test of the behavioral predictions from pre-clinical models of alcoholism etiology using a large and well-characterized sample of drinkers representing varying levels of alcohol exposure and alcohol-related problems (Koob & Le Moal 1997; Robinson & Berridge 2001). Specifically, we found support for diminished salience of positive reinforcement mechanisms in alcohol dependence, a point of consilience between the allostatic model and IST. This claim was supported at several levels of analyses including a dose-dependent association between stimulation and craving in non-dependent HD only. Furthermore, stimulation preceded craving among HD only. Consistent with the claim that stimulation might lead to craving in HD only, reverse

lagged models where craving was allowed to predict stimulation revealed no such pattern of group differences. These data are consistent with the idea central to IST that hedonic reward and motivational value are phenotypically dissociable, particularly in dependence (Robinson & Berridge 2001). Moreover, these findings support the etiological claim that the functional significance of stimulation/hedonic reward in promoting continued alcohol use is diminished in dependence as compared to non-dependent heavy drinking.

While this central claim was supported, several ancillary hypotheses were not. First, as our group has reported previously (Bujarski & Ray 2014), we did not observe the hypothesized group differences in terms of the reported magnitude of stimulation/hedonic reward. Instead, in these data, HD reported lower levels of stimulation at baseline, which carried over to post-alcohol timepoints. To determine whether this effect may be a consequence of alcohol hangover, we examined baseline levels of selected items that might capture hangover (e.g. 'trouble concentrating', 'clumsy', 'sleepy', 'nauseated', 'muddles/confused', 'dizzy' and 'sluggish') and found no drinking group differences on any of these hangover-related items ($P \geq 0.12$). Thus, it seems unlikely that hangover effects are a substantial driver of the observed baseline differences on stimulation. Similarly, these baseline differences were not explained by group level differences in affective symptoms. Together, this suggests that the absolute level of stimulation may not be the operative factor in examining etiological changes in the function of SR. Instead, it appears that the mechanistic association between stimulation and craving may better capture SR as a marker of disease progression. While ADs were still reporting substantial stimulation and hedonic reward from alcohol, this hedonic response was relatively dissociated from their reported craving.

Further, in these analyses, we did not observe associations between tension and craving at any level of analysis, as a negative reinforcement mechanism would predict. With the exception of an initial reduction in tension among AD, alcohol administration was not found to produce marked reductions in tension and negative affect (Fig. 1c), thereby reducing the viability of tension reduction as an explanatory factor in subsequent analyses. Several possible explanations for this null finding should be explored in future studies. First, tension reduction mechanisms may require an acute state of negative affect (e.g. via stress induction techniques: Kirschbaum *et al.* 1993; Sinha 2009). Second, the negative affect/allostatic state produced by alcohol abstinence following chronic alcohol dosing and dependence described in the allostatic model may be features of late-stage dependence, of which the present sample was not representative (Table 1). Lastly, negative reinforcement mechanisms may depend upon a host of factors beyond alcohol's direct pharmacological effects (e.g. responses to alcohol cues or social context), which were suppressed in the i.v. alcohol administration paradigm. Future alcohol challenge studies will be required to further refine and test this negative reinforcement mechanism in alcohol dependence.

Unexpectedly, sedation was found to positively predict craving at all levels of analysis, suggesting that sedation was reinforcing. This result appears contrary to the predictions of the differentiator model wherein a robust sedation response negatively predicts the likelihood of future alcohol misuse (King *et al.* 2014; Newlin & Thomson 1990). This discrepancy may be explained by the contrasting timeframes in the differentiator model and the present study. Specifically, acute sedation may positively predict craving for alcohol within a given drinking episode, yet chronically heightened sedation may be protective in the long term. It is also possible that sedation on the descending limb, not the ascending limb, is protective against future dependence; however, longitudinal studies have shown sedation at peak BrAC to be as predictive of future dependence as descending limb sedation (King *et al.* 2014, 2011). Lastly, while the present results are in line with the differentiator model with respect to stimulation, the positive associations between SR and craving across both stimulation and sedation are more difficult to square with the LR model (Schuckit 1994).

Many studies on SR have been conducted with light-to-moderate drinkers; however, our results suggest that, at least for the translational questions being addressed here, LMD may provide only modest insight for several reasons. First, the LMD recruited in this, and similar studies, are likely 'over the hump' in terms of developmental risk for clinically significant alcohol-related problems (Wagner & Anthony 2002). Second, LMD displayed minimal craving for alcohol and did not demonstrate an alcohol-priming effect on craving, which has been argued to be a central characteristic of problematic alcohol use (de Wit, 2000, 1996).

While neurobiological models of alcoholism etiology can and do provide valuable insight into the pathophysiology of alcoholism, in order for animal-derived models to contribute optimal insights into human psychopathology, such theories must be validated in clinical samples. Translational validation studies such as this can then permit theory-driven inferences regarding both etiology and treatment development. For example, our results suggest that interventions targeting stimulation (such as opioid antagonism) may be better tailored for early-stage alcohol problems when stimulation still contributes to motivated alcohol consumption.

This study should be interpreted in light of its strengths and weaknesses. Strengths include the large sample size and a highly controlled i.v. alcohol challenge

paradigm. This study also benefits from a strong translational and clinical neuroscience perspective in its goals and hypotheses. Limitations of this study include the cross-sectional study design and the relatively low target dose as compared with participants' naturalistic drinking, which may limit ecological validity as HD and AD participants in this sample frequently experience BrACs that are substantially greater than 0.06 g/dl. Although these BrACs are lower than participants would frequently experience, work by several research groups including our own has utilized alcohol challenge paradigms to similar target BrACs to understand the phenomenology and treatment of alcohol dependence (e.g. Hendershot *et al.* 2014; O'Malley *et al.* 2002; Ramchandani *et al.* 2002; Ray *et al.* 2012; Ray & Hutchison 2007; Strang *et al.* 2014). Future alcohol challenge studies should examine these phenotypes at higher alcohol doses (e.g. 0.08 or 0.10 g/dl). Furthermore, the lack of a saline control condition limits our ability to distinguish time effects and expectancies. As with all controlled alcohol challenge studies, this study sacrifices external validity for greater experimental control, and thus, future research should examine these concepts using more naturalistic designs. That being said, we believe i.v. alcohol administration to be a useful experimental paradigm for understanding the potential role of disease-related neuroadaptation on the pharmacological effects of alcohol as dissociated from learned responses to cues (Plebani *et al.* 2012). The focus on pharmacological effects therefore may provide greater translational potential with pre-clinical research where cues (both discreet and contextual) are highly controlled. Lastly, because this study utilized alcohol craving as a proxy measure for alcohol-related motivation, future studies should examine the effect of SR in predicting actual alcohol consumption via self-administration paradigms.

As a whole, our findings extend the literature on SR and alcoholism etiology by examining predictive associations between SR and alcohol craving. In concordance with the prediction of diminished salience of positive reinforcement in alcohol dependence derived from both the allostatic model and IST, we found that stimulation/hedonic reward from alcohol was associated with and preceded craving in non-dependent HD, but not in AD. Further studies using a variety of alcohol challenge paradigms and longitudinal study designs are warranted in order to further refine or disconfirm the specific predictions about SR derived from prominent pre-clinical models of alcoholism etiology in human clinical samples.

Authors contribution

All authors have made substantial contributions warranting authorship on this manuscript. KEH conceptualized and was the principal investigator for this study. SB conducted all statistical analyses and was the primary author. LAR is the graduate mentor to SB, Co-I on the study, and contributed to manuscript conceptualization and interpretation. All authors contributed to manuscript preparation and revision. All authors critically reviewed this content and approved the final version for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Factor structure of subjective response to alcohol. Factor loadings and variance explained by the proposed 3-factor solution are presented from a series of principle component analyses at baseline, and BrAC's of 0.02, 0.04, and 0.06 g/dl.