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Heart rate variability, cue-evoked ventromedial prefrontal cortical response, and problem alcohol use in adult drinkers

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Abstract

Background—Many studies employed cue exposure paradigms to investigate the neural processes underlying cue-elicited alcohol craving. Cue exposure elicits robust autonomic reactivity. However, whether or how cue-elicited autonomic response relates to the severity of alcohol misuse and the neural bases underlying the potential relationship remain unclear.

Methods—We examined cue-related brain activations in association with heart rate variability (HRV), as indexed by the Root Mean Square of the Successive Differences (RMSSD), during alcohol vs. neutral cue blocks in 50 adult alcohol drinkers (24 men). Imaging and HRV data were collected and processed with published routines. Mediation analyses were conducted to examine the inter-relationship between regional activities, cue-elicited changes in RMSSD, and the severity of problem alcohol use, as assessed with the Alcohol Use Disorder Identification Test (AUDIT).

Results—The results showed higher RMSSD during alcohol than during neutral cue exposures, with alcohol (vs. neutral) cue-evoked RMSSD positively correlated with AUDIT score. Further, alcohol (vs. neutral) cue-elicited activity in the ventromedial prefrontal cortex (vmPFC) was negatively correlated both with increases in RMSSD and with AUDIT score. Mediation analyses suggested that the RMSSD mediated the relationship between vmPFC cue activity and AUDIT score.

Conclusions—These findings substantiate the neural correlates of the presumably parasympathetic response during alcohol cue exposure and the inter-relationship between vmPFC activity, autonomic response and problem alcohol use.

Keywords

alcohol; cue; autonomic; HRV; imaging; ventromedial PFC

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Introduction

Drug craving is associated with changes in interoceptive responses and physiological arousal (1–10). Many imaging studies have examined the neural correlates of craving (11) and of physiological arousal during cognitive and affective challenges (12). For instance, as compared to light drinkers, individuals abusing alcohol demonstrated greater cue-related activation of the parietal and temporal cortices (13). We recently showed higher alcohol vs. neutral cue responses in the occipital, retrosplenial, and medial orbitofrontal cortex and left caudate head in non-dependent drinkers (14). A study of male drinkers demonstrated a positive correlation between cue-evoked craving as well as thalamic cue activities and skin conductance response (SCR) (15). Further, mediation analyses suggested that thalamic activity mediated the correlation between craving and SCR. The latter findings suggested higher physiological arousal during cue-evoked craving and a specific role of the thalamus in supporting physiological changes during cue-evoking craving.

Skin conductance manifests via sympathetic innervations of the sweat glands and SCR is elevated during heightened arousal as one would experience during exposure to stress or a salient environment (16, 17). Another aspect of autonomic responses, as supported by the parasympathetic system, can be evaluated by heart rate variability (HRV). Heart rate is controlled by the balancing acts of the sympathetic and parasympathetic systems, each serving to accelerate and decelerate heart beat. HRV quantifies the variation of inter-beat-intervals and, as with heart rate, is regulated by the autonomic system. The sympathetic and parasympathetic systems modulate HRV each at the low (0.04–0.15 Hz) and higher (0.15–0.4 Hz) frequency range of the heart rate (18). Heightened sympathetic and parasympathetic tone decreases and increases HRV, respectively. Decreases in HRV are known to co-exist with or precede a number of medical conditions, including impending myocardial infarction (19), and have been associated with diminished cognitive performance (20).

Cerebral control of cardiovascular responses is supported by the limbic circuits. In particular, the medial prefrontal cortex (mPFC) has been implicated in numerous studies in the regulation of autonomic and cardiovascular responses (21–27). For instance, an fMRI study showed that functional connectivity of the perigenual anterior cingulate cortex covaried with individual variation in high-frequency HRV during resting state (22). During isometric hand grips the time course of heart rate was associated with decreases in MPFC activation and individuals with higher physical fitness demonstrated greater deactivation of the MPFC (28). An earlier study showed that heart rate responses to evaluation of social threat were mediated by opposing signals in two distinct sub-regions of the MPFC – activations in rostral dorsal anterior cingulate cortex and de-activations in ventromedial prefrontal cortex (29).

The root mean squared difference of successive intervals (RMSSD) has been widely used as a time-domain HRV measure to reflect parasympathetic activity (30) in basic neurophysiological studies and investigations involving clinical populations, including those with substance misuse. A recent meta-analysis showed that HRV, as indexed by the RMSSD, was significantly lower in individuals with alcohol use disorders, as compared to healthy controls (31). Another review also showed decreased HRV in association with heavy

drinking (32). Further, heavy drinkers showed higher HRV in response to alcohol or emotional cues, when compared to controls, and the reactivity was associated with craving, negative mood, and faster relapse (32). A number of studies have specifically examined HRV as a physiological marker of cue exposure in drug addicted individuals (33–40). Alcohol attentional bias was positively associated with parasympathetically mediated HRV (39). Alcohol-dependent patients who relapsed exhibited a significantly greater high-frequency HRV in response to alcohol cues, as compared to those who did not relapse (38). Overall, previous studies have consistently reported increases in HRV during alcohol cue exposures in heavy drinkers. On the other hand, no studies to our knowledge have examined the cerebral processes underlying the changes in HRV or whether these neural processes may relate to the severity of alcohol use.

Here, fifty adult drinkers participated in clinical assessments and an fMRI study of alcohol cue reactivity in conjunction with physiological recordings. We addressed the following aims. Firstly, is HRV altered during alcohol vs. neutral cue exposure and, if yes, are changes in HRV related to individual differences in the severity of alcohol use? As described earlier, the literature has largely supported cue-elicited increases in HRV and its relationship with the extent of alcohol misuse and treatment outcomes, and we expected to replicate these findings. Secondly, what are the neural processes underlying cue-elicited changes in HRV and their relationship with problem alcohol use? Considering the imaging literature implicating the mPFC in autonomic control, we hypothesized cue-evoked mPFC responses in association with changes in HRV. We also explored the inter-relationships between cue-related mPFC activity and HRV and the severity of alcohol use. As studies have reported gender and age effects on HRV (41–44), we analyzed the data with age and gender as covariates.

Materials and methods

Experimental procedures are largely identical to those described in our previous study (15).

Subjects and assessments

Candidates were recruited from the greater New Haven, CT area. Fifty adult drinkers met eligibility criteria and participated in this study. All subjects were required to be physically healthy with no major medical conditions. Those with current use of prescription medications or with a history of head injury or neurological illness were excluded. Other exclusion criteria included current or past dependence on a psychoactive substance (except alcohol and nicotine) and current or history of Axis I disorders according to the Structured Clinical Interview for DSM-IV [SCID; (45)]. Four men and four women met the DSM criteria for an alcohol use disorder and none met criteria for a nicotine use disorder. Candidates who reported current use of illicit substances or tested positive for cocaine, methamphetamine, opioids, marijuana, barbiturates, or benzodiazepines were not invited to participate. The Human Investigation committee at Yale University School of Medicine approved the study procedures. All participants signed an informed consent prior to the study.

Participants were evaluated with Alcohol Use Disorders Identification Test [AUDIT; (46)], an instrument widely used to assess alcohol use behavior and related problems. Participants were assessed with the Fagerström Test for Nicotine Dependence [FTND; (47)] and averaged 1.1 ± 2.4 (mean \pm SD) in FTND score, suggesting low dependence. Table 1 summarizes the demographic and clinical characteristics of the participants. Men and women did not differ in any of the demographic and clinical characteristics or outcome measures except for cue-elicited craving. However, cue-elicited craving was not correlated with AUDIT score or with physiological responses to cues (see Results). Thus, we noted these results on sex differences and, considering the moderate sample size, combined men and women in data analyses.

Behavioral tasks

We employed a cue-induced alcohol craving task for fMRI in the current study. Participants viewed alcohol-related or neutral pictures and reported alcohol craving in alternating blocks. Briefly, a cross was used to engage attention at the beginning of each block. After 2 s, six pictures displaying alcohol related cues (alcohol block) or neutral visual scenes (neutral block) were shown for 6 s each. Participants were asked to view the pictures and ponder how they might relate to the scenes. The pictures were collected from the Internet and independently reviewed by two investigators. Alcohol pictures included bar scenes, individuals or a group of people holding or drinking alcoholic beverages, and images of a variety of alcoholic drinks, such as beer, wine, and vodka. Neutral pictures comprised natural sceneries. Participants were asked at the end of each block to report how much they craved for alcohol from 0 (no craving) to 10 (highest craving ever experienced) on a visual analog scale. Each block lasted about 45 s, including time for craving rating. A total of 6 alcohol and 6 neutral blocks took approximately 9 m to complete. Each participant completed two runs of the task.

Heart rate variability: data acquisition and analysis

The photoplethysmography (PPG) was a non-restraining and non-invasive method widely used to detect pulse and oxygen saturation (48). The PPG of heart rate was collected during fMRI scan using a pressure transducer which adhered to the participant's left thumb (all used right hand to respond). A Biopac MP150 pressure transduction system, running AcqKnowledge 4.1 software (Biopac Systems, USA), was used for acquiring heart rate data at a sampling rate of 1000 Hz (49). With AcqKnowledge 5.0.2 software (Biopac Systems, USA), the raw signal was bandpass filtered at 0.5–35 Hz. Independent component analysis was applied to separate the PPG signal from respiration and scanner signals. A template-correlation function was then applied to identify the waveform that matched the time course of the template, a “good” heart beat (27). Peaks were identified from the waveforms that closely matched the template to define the inter-beat or R-R intervals (30).

We estimated mean heart rate (HR) on the basis of the R–R intervals each for the alcohol and neutral cue blocks using the Kubios software (Kubios.com). We distinguished low- (0.04–0.15 Hz; LF-HRV) and high- (0.15–0.4 Hz; HF-HRV) frequency components of the HRV. The root mean squared difference of successive intervals (RMSSD) reflects the high-frequency dynamics of HRV (30, 50) and is considered a reliable measure of vagal

parasympathetic activity (27, 30). The RMSSD was computed as follows. Assuming the successive differences are $RR1 - RR2 = D_1$; $RR2 - RR3 = D_2$; ..., $RR_{n-1} - RR_n = D_{n-1}$,

$$RMSSD = \sqrt{\frac{\sum_{i=1}^{n-1} D_i^2}{n-1}}$$

where i = interval index, n = the total number of intervals.

Imaging protocol and data analyses

Brain images were collected using multiband imaging (multiband factor = 3) with a 3-Tesla MR scanner (Siemens Trio, Erlangen, Germany). We acquired conventional T1-weighted spin echo sagittal anatomical images for slice localization. Anatomical 3D MPRAGE image were obtained with spin echo imaging in the transverse plane parallel to the anterior commissure – posterior commissure or AC–PC line with TR = 1900 ms, TE = 2.52 ms, bandwidth = 170 Hz/pixel, field of view = 250×250 mm, matrix = 256×256 , 176 slices with slice thickness = 1 mm and no gap. Functional, blood oxygenation level-dependent (BOLD) signals were then acquired with a single-shot gradient echo echoplanar imaging (EPI) sequence. A total of 51 transverse slices parallel to the AC–PC line covering the whole brain were acquired with TR = 1000 ms, TE = 30 ms, bandwidth = 2290 Hz/pixel, flip angle = 62° , field of view = 210×210 mm, matrix = 84×84 , and slice thickness = 2.5 mm with no gap.

We analyzed the imaging data with Statistical Parametric Mapping or SPM12 (Wellcome Department of Imaging Neuroscience, University College London, U.K.). We aligned (motion corrected) and corrected for slice timing of images of each individual subject, and constructed a mean functional image volume for each subject per run from the realigned volumes. We co-registered these mean images with the high-resolution structural image and segmented the images for normalization with affine registration and nonlinear transformation (51–53). We applied the normalization parameters as determined for the structure volume to the corresponding functional images for each subject. The resampled voxel size was $3 \times 3 \times 3$ mm³. Finally, we applied a Gaussian kernel of 8 mm at Full Width at Half Maximum for smoothing.

In modeling of imaging data, we distinguished alcohol and neutral cue blocks for each individual subject using a general linear model (GLM) that included the realignment parameters in all six dimensions. We corrected for serial autocorrelation caused by aliased cardiovascular and respiratory effects by a first-degree autoregressive model. The GLM was used to estimate the component of variance explained by each of the regressors. We constructed for individual subjects a contrast of alcohol vs. neutral blocks to evaluate regional activities that differentiated viewing of alcohol and neutral pictures. We used the contrast or *con* (difference in β) images of the subject-level analysis for group-level, random-effects analyses.

We performed a linear regression of *con* images against the differences in RMSSD between alcohol and neutral blocks across subjects, with age and sex as covariates. In a separate

model, we performed a linear regression of the *con* images against individual AUDIT score, with age and sex as covariates. To identify the neural correlates that overlapped between the two regressions, we employed a $p < 0.05$, uncorrected to cover brain regions as extensively as possible. Shared correlates were designated as the regions of interest (ROI). In ROI analysis, we used MarsBaR (<http://marsbar.sourceforge.net/>) to derive for each individual subject the β contrast or alcohol – neutral cue activity for the ROIs. We showed all voxel activations in the Montreal Neurological Institute (MNI) coordinates.

Mediation analyses

For shared correlates, we performed mediation analyses to evaluate the inter-relationship of cue activity (β contrast), differences in RMSSD (alcohol – neutral) and AUDIT score.

We performed mediation analyses (54) with the toolbox M3 (<http://wagerlab.colorado.edu/tools>), as in our previous work (55). In a mediation analysis, one examines if the relation between the independent variable X and dependent variable Y (i.e. $X \rightarrow Y$) is significantly mediated by a variable M . Three regression equations were performed for the mediation test (54):

$$Y = i_1 + cX + e_1$$

$$Y = i_2 + c'X + bM + e_2$$

$$M = i_3 + aX + e_3$$

where a represents $X \rightarrow M$, b represents $M \rightarrow Y$ (controlling for X), c' represents $X \rightarrow Y$ (controlling for M), and c represents $X \rightarrow Y$, the constants i_1 , i_2 , i_3 are the intercepts, and e_1 , e_2 , e_3 represent the residual errors. In the literature, a , b , c and c' are referred to as path coefficients or simply paths (54, 56). We followed this notation. Variable M is said to be a mediator of the correlation $X \rightarrow Y$ if $(c - c')$, mathematically equivalent to the product $a*b$ of the path a and b , differs significantly from zero (54). If the product $a*b$ and the paths a and b are both significant, one concludes that $X \rightarrow Y$ is mediated by M . Further, if path c' is not significant, one concludes that there is no direct connection from X to Y and that $X \rightarrow Y$ is completely mediated by M .

Results

Cue-elicited craving

Subjects reported higher craving during alcohol (3.6 ± 3.0) as compared to neutral (2.4 ± 2.4) cue blocks ($t=5.16$, $p < 0.001$, two-tailed paired sample t test). Cue-elicited craving (alcohol – neutral) was not significantly correlated with AUDIT score without covariates ($r=0.10$; $p=0.4788$) or with age and gender as covariates ($r=0.09$; $p=0.5647$). Compared to women (0.73 ± 0.98), men (1.74 ± 2.07) showed higher cue-elicited craving ($t=2.22$, $p=0.031$; two-tailed independent sample t test).

Heart rate and heart rate variability

Men and women did not differ in alcohol vs. neutral cue-elicited HR ($t=0.93$, $p=0.359$). Across all subjects, the HR was not significantly different during alcohol and neutral cue blocks (29 of the 50 showed an increase: mean \pm SD increases: 0.41 ± 1.96 beats/s; $t=1.48$, $p=0.1442$, two-tailed paired sample t test). The mean heart rate (HR) during alcohol and neutral cue blocks are shown in Figure 1A. The block differences in HR were not significantly correlated with the AUDIT score ($r=0.09$, $p=0.519$, without covariates; $r=0.08$, $p=0.585$, with age and gender covariates; Figure 1B). The difference in cue-induced craving was not correlated with the difference in HR between alcohol and neutral cue blocks ($r=0.24$, $p=0.0972$, without covariates; $r=0.26$, $p=0.0751$, with age and gender as covariates).

Men and women did not differ in alcohol vs. neutral cue-elicited RMSSD ($t=0.47$, $p=0.643$). The RMSSD showed a small but significant increase during alcohol as compared to neutral cue blocks across participants (32 of the 50 showed an increase: mean \pm SD increases: 0.738 ± 2.1 ms; $t=2.49$, $p=0.0164$, two-tailed paired sample t test). The RMSSD during alcohol and neutral cue blocks are shown in Figure 1C. The differences in RMSSD between the alcohol and neutral cue blocks was positively correlated with the AUDIT score ($r=0.53$, $p<0.001$, without covariates, Figure 1D; or with age and gender covariates). The difference in cue-induced craving was not correlated with the difference in RMSSD between alcohol and neutral cue blocks ($r=0.11$, $p=0.4438$, without covariates; $r=0.03$, $p=0.8423$, with age and gender as covariates).

Cue-elicited regional responses in relation to AUDIT score and changes in RMSSD

The AUDIT score and RMSSD (alcohol – neutral) was positively correlated across subjects. We performed regression analyses to identify shared cue-elicited activities - activities in correlation with both AUDIT score and RMSSD (alcohol – neutral). We evaluated the results at a liberal threshold ($p<0.05$, uncorrected) in order to identify all shared voxels. In whole-brain regressions of alcohol – neutral blocks each with the AUDIT score and RMSSD (alcohol – neutral) as predictors and age and sex as covariates, two distinct clusters were positively correlated with both predictors, each in the right rostral anterior cingulate cortex or rACC (mean coordinates: $x=11$, $y=39$, $z=4$, 267 mm³) and right thalamus ($x=11$, $y=-17$, $z=8$, 342 mm³). A cluster in the ventromedial prefrontal cortex or vmPFC ($x=-2$, $y=34$, $z=-12$, 933 mm³) showed negative correlation with both predictors. These clusters are shown in Figure 2A.

We plotted the linear regressions between the regional activities (β contrast) and predictor to visualize the correlations. The β contrasts of the rACC and thalamus clusters were highly correlated ($r=0.48$, $p<0.001$), we thus combined the two clusters as a single region of interest. As expected, the β contrast of the rACC-thalamus cluster was positively correlated both with the differences in RMSSD between the alcohol and neutral cue blocks ($r=0.39$, $p=0.005$; $r=0.39$, $p=0.006$ with age and gender as covariates) and with AUDIT scores ($r=0.38$, $p=0.007$; $r=0.37$, $p=0.009$ with age and gender as covariates) (Figure 2B). Likewise, the β contrast of vmPFC was negatively correlated both with the differences in RMSSD between the alcohol and neutral cue blocks ($r=-0.39$, $p=0.0050$; $r=-0.41$, $p=0.0038$, with

age and gender as covariates) and with the AUDIT scores ($r=-0.34$, $p=0.0142$; $r=-0.36$, $p=0.0109$, with age and gender as covariates) (Figure 2C).

Mediation analysis:

AUDIT score, rACC-thalamus cue response, and RMSSD were pair-wise correlated across participants. We performed mediation analyses for all six models with age and gender as covariates and none of the mediation effects were significant (all p 's > 0.062). AUDIT score, vmPFC cue response, and RMSSD were also pair-wise correlated across participants. Thus, we examined their inter-relationships using mediation analyses with age and gender as covariates. As shown in Figure 3, of the six models, model 1 showed that the RMSSD significantly mediated the correlation between vmPFC cue response and AUDIT score ($p<0.05$). None of the other five models showed significant mediation.

Although all 6 models are presented in Figure 3, the results of the mediation analyses can be more specifically evaluated for the role of the vmPFC in regulating autonomic functions. Thus, with the rationale that neural activities precede autonomic responses, only Models 1, 2 and 5 were physiologically plausible. The hypothesis was to test whether vmPFC activities contributed to problem alcohol use (AUDIT score) via cue-elicited changes in HRV (Model 1), whether vmPFC cue activities contributed to problem alcohol use and, in turn, to cue-elicited changes in HRV (Model 2), or whether problem alcohol use contributed to vmPFC cue activities and, in turn, cue-elicited HRV (Model 5). In particular, a contrast between Model 1 and 5 would provide evidence to address whether cue-evoked vmPFC activities and HRV are a result of problem alcohol use or whether problem alcohol use leads to these neural and physiological changes.

Discussion

Alcohol drinkers showed an increase in high-frequency heart rate variability (HRV), as reflected by the root-mean-square of successive differences (RMSSD) in heart beat, when exposed to alcohol as compared to neutral cues. This increase was correlated with the severity of problem alcohol use, as characterized by the AUDIT score, and decreases in ventromedial prefrontal cortical activity during alcohol vs. neutral cue exposure. Further, mediation analysis showed that decreases in cue-elicited vmPFC activities contributed to higher RMSSD during alcohol cue exposure and, in turn, higher AUDIT score. Together, these results substantiate the role of HRV in cue reactivity and problem drinking as well as the neural processes associating cue-evoked changes in HRV and the severity of alcohol misuse. We highlighted the main findings in the below.

Autonomic and neural responses to cue exposure

As described earlier, a number of studies have consistently reported increases in HRV during alcohol cue exposures in alcohol dependent or heavy drinking individuals. Here, we replicated this finding in adults with varying severity of problem alcohol use and showed that cue-evoked increases in HRV were positively correlated with the severity of alcohol misuse. This finding suggests HRV as an important physiological marker of habitual and heavy alcohol consumption.

Cue responses of the rostral anterior cingulate cortex (rACC) and thalamus showed a positive correlation both with differences in RMSSD and with AUDIT score across subjects, although their relationships could not be confirmed with mediation analyses. Another cluster in the ventromedial prefrontal cortex (vmPFC), in the area of the medial orbitofrontal frontal cortex showed a negative correlation both with differences in RMSSD and with AUDIT score across subjects. In mediation analysis, the model where vmPFC cue activities contributed to differences in RMSSD and, in turn, AUDIT score showed significant and complete mediation. Thus, the vmPFC may play a critical role in supporting cue-elicited differences in HRV in relation to the severity of alcohol misuse. Interestingly, the contrasting patterns of correlation between the rACC and vmPFC accorded with their opposing roles in regulating the cardiovascular and behavioral responses to emotional and other salient stimuli (29, 57).

The current findings are broadly consistent with many previous studies reporting vmPFC activity in relation to HRV or other autonomic responses (58–60). For instance, in a study of combat veterans exposed to alternating blocks of threats of electric shocks and safety, HRV was inversely correlated with the severity of PTSD symptoms (61). Further, an area in the vmPFC (though anterior to the vmPFC cluster in the current study) showed anticipatory activation (threat – safety) in negative correlation with the HRV. A study of aging showed age-related decreases in resting HRV and an age-dependent association between resting HRV and eigenvector centrality, a graph-theoretic measure of functional connectivity, in bilateral vmPFC (62). An earlier study demonstrated that patients with PTSD showed lower HRV and altered vmPFC connectivities with regions of emotional reactivity and motor readiness, as compared to healthy controls (63). Another study showed that HRV explained a significant portion of the individual variability in dietary self-control, and those with higher HRV were better able to downregulate their cravings in the face of taste temptations (64). Further, individuals with higher HRV showed attenuated taste representations in the vmPFC during self-control. The current findings thus extend this literature by showing an association of vmPFC activities with cue-elicited increases in HRV in adult drinkers. On the other hand, we did not observe a significant correlation between HRV and craving rating across alcohol and neutral blocks. Thus, the functional implications of the changes in HRV remained unclear.

Craving and autonomic response

We observed a small but significant increase in HRV during alcohol than during neutral blocks, consistent with previous reports of a moderate effect size of cue-evoked physiological responses (65). However, the differences in HRV and craving rating were not correlated, suggesting that subjective craving was not reflected by physiological reactivity (66, 67). Indeed, whereas some studies have reported a relationship between cue-induced craving and physiological reactivity, including changes in heart rate and HRV (68, 69), others did not reveal or examine such a relationship (67, 70–72). Other studies have reported the influences of various psychological factors on cue-elicited drug craving and autonomic reactivity. For instance, both adverse childhood experiences and duration of opioid use predicted blunted HRV during negative emotion regulation and increased negative emotional cue-elicited craving in women with chronic pain (34). Multi-level analysis suggested that

childhood abuse occasioned emotion dysregulation and appetitive responding toward opioids in negative affective contexts. It was not clear, however, whether cue-evoked differences in HRV and craving were correlated (34). Together, the literature appears less than consistent regarding the autonomic correlates of drug-induced craving. Substances of abuse, specific experimental manipulations and physiological measures need to be considered in reviewing this body of work and in conducting new studies to address this issue.

Even fewer studies have examined the neural processes linking craving and autonomic reactivity. In a recent study we showed in male but not female drinkers a positive correlation between cue-evoked craving as well as thalamic cue activities and skin conductance response (15). A previous work demonstrated higher cue-induced arousal, as indicated by a significant increase in skin conductance and a larger late positivity of visual event-related brain potential, although the physiological and neural responses were not correlated, in cannabis users (73). Likewise, more studies are needed to investigate this issue and experiments encompassing a variety of physiological measures would provide an opportunity to identify neural responses shared by physiological changes and a subjective psychological state.

Limitations of the study and conclusions

A number of limitations need to be considered for the study. First, the study comprised a moderate sample size. Although whole-brain analyses in correlation with AUDIT score and differences in RMSSD identified shared correlates in the mPFC and thalamus, a larger sample size may reveal other cortical and subcortical structures to support responses to cue-elicited changes in HRV and in link with the severity of alcohol misuse. Likewise, a larger sample would allow a direct comparison of women and men in the autonomic and neural markers of cue reactivity. Second, cue reactivity is a complex process, involving a multitude of other psychological processes. It remains unclear how alcohol cues may interact with other psychological constructs in eliciting changes in HRV or in determining future alcohol use. Further, other than rating their craving, the participants were passively involved in the task, which may have influenced the craving and imaging findings. A behavioral paradigm that better engages participants' attention (e.g., visual discrimination) would potentially be more powerful in eliciting craving and cue responses. Third, many other clinical variables, including depression and anxiety, weight and/or BMI, may influence cue-evoked regional activities and HRV. These variables were not accounted for here and more research is needed to address their potential confounding effects. Fourth, we employed a liberal threshold to explore how cue-evoked activities may relate to HRV and the severity of alcohol misuse for the whole brain. Notably, even at voxel $p < 0.05$ uncorrected, no regions other than the vmPFC showed cue activities that were correlated with both the HRV and AUDIT score. This finding may suggest a unique role of the vmPFC in supporting the changes in HRV during cue exposure and problem alcohol use, as substantiated by the mediation analysis. Further, we did not correct for the number of models tested in evaluating the results of mediation analyses. Contrasting the 3 different models is akin to model selection – Model 1 was significant but Model 2 and 5 was not. Nonetheless, more research is needed to replicate this finding and to investigate whether cue-elicited vmPFC activities and changes in HRV dispose individuals to problem drinking in a longitudinal setting.

To conclude, we showed that cue-evoked differences in RMSSD was negatively correlated with vmPFC activity and mediated the correlation between vmPFC cue activity and the severity of alcohol misuse. These findings add to the literature of the autonomic and neural markers of cue reactivity and substantiate the interacting roles of HRV and vmPFC activity during alcohol cue exposures.

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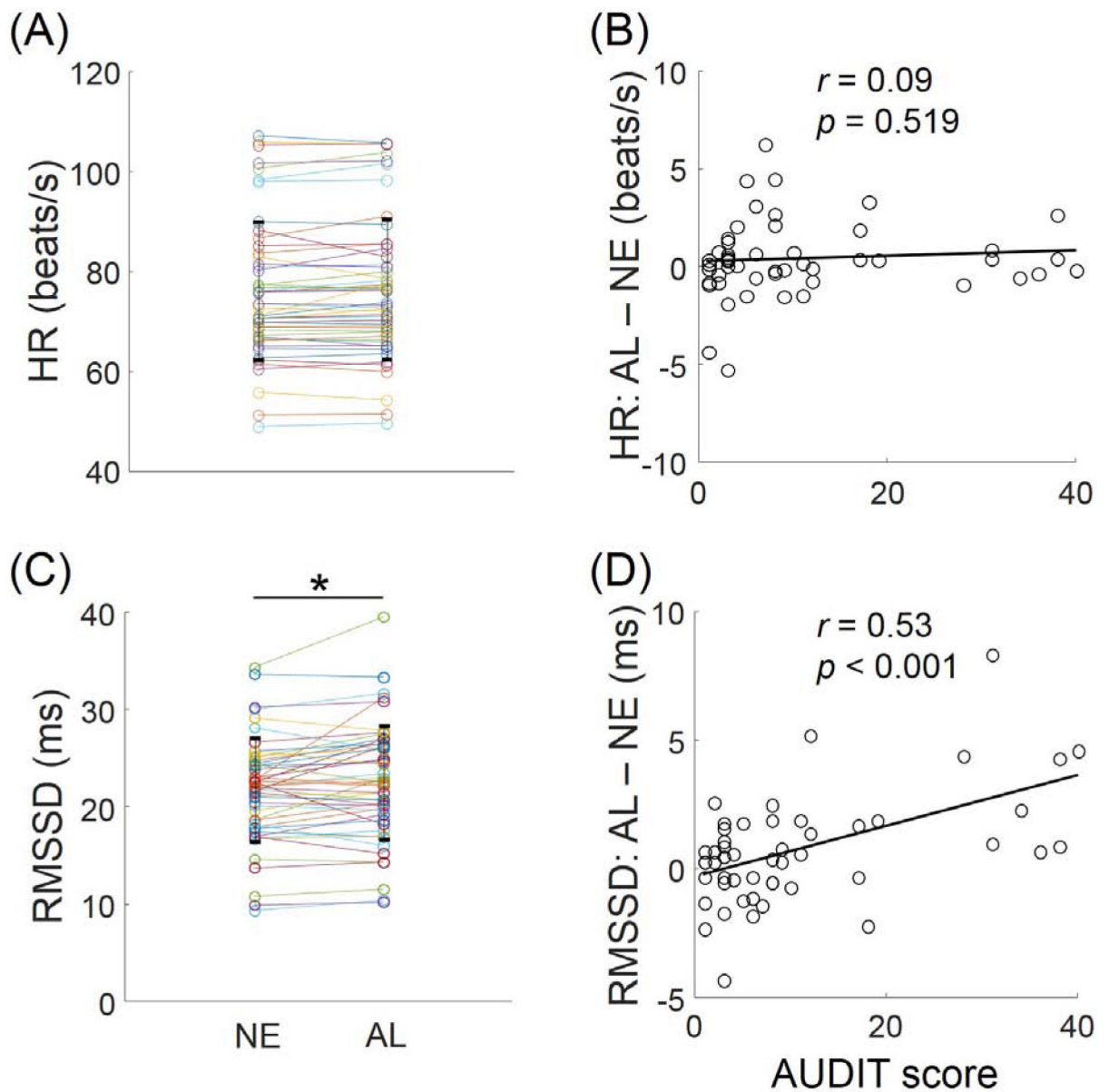


Figure 1.

Heart rate (HR) and heart rate variability. (A) HR did not show differences between alcohol (AL) and neutral cue (NE) blocks. Each pair of connected data points represents one subject. (B) The differences in HR (AL - NE) were not correlated with AUDIT score across subjects. (C) RMSSD showed a small but significant increase during AL as compared to NE blocks. $*p < 0.05$. Each pair of connected data points represents one subject. (D) The differences in RMSSD (AL - NE) were positively correlated with AUDIT score across subjects ($p < 0.001$).

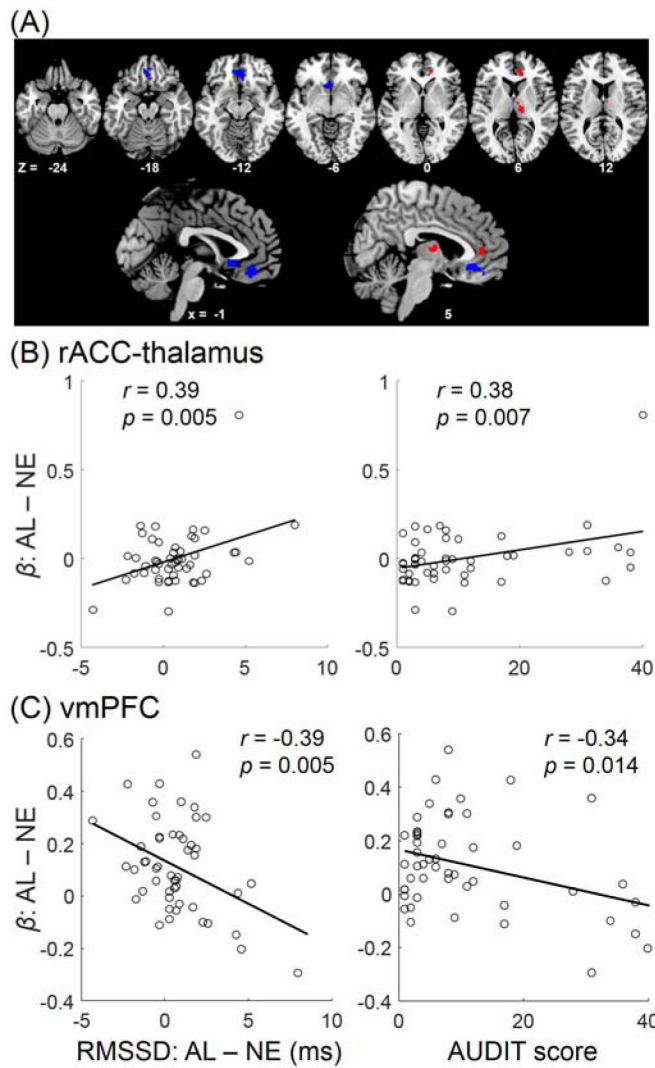
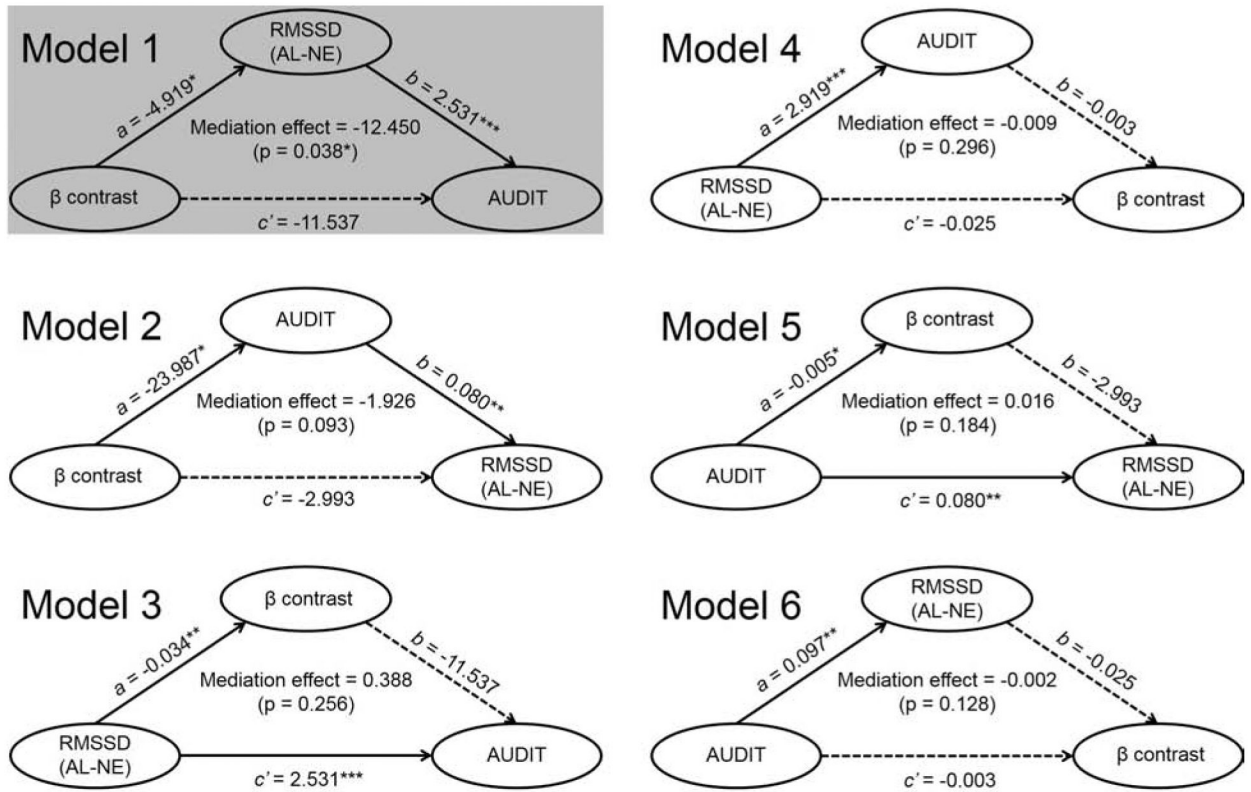


Figure 2.

AUDIT score and cue-elicited changes in HRV shared correlates in the medial prefrontal cortex and thalamus. (A) A cluster in the rostral anterior cingulate cortex (rACC) and right thalamus (red) and vmPFC (blue) showed cue responses (AL-NE) each in positive and negative correlation both with AUDIT score and with differences in RMSSD (AL-NE). These correlations are shown here for the (B) rACC-thalamus and (C) vmPFC.

**Figure 3.**

Mediation analyses of vmPFC cue response (β contrast), differences in RMSSD across alcohol (AL) and neutral (NE) blocks, and AUDIT score. All six models of mediation were tested with age and gender as covariates. Model 1 showed a significant and complete mediation: vmPFC activity → RMSSD (AL – NE) → AUDIT score. Solid lines represent significant correlation, dotted lines represent insignificant correlation, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 1:

Demographics and clinical measures of male and female participants

Subject characteristic	Men (n=24)	Women (n=26)	P value*
Age (yrs)	39.7 ± 12.7	40.4 ± 15.2	0.85
AUDIT score	11.8 ± 12.2	10.0 ± 11.0	0.57
Duration of alcohol use (yrs)	22.1 ± 13.5	21.8 ± 15.8	0.94
# of drinking days/mo, prior yr	9.2 ± 5.4	9.3 ± 6.3	0.95
# of drinks/per occasion	3.7 ± 2.5	3.2 ± 2.5	0.50
# of drinks/mo, prior yr	39.3 ± 42.4	38.9 ± 46.2	0.97
FTND score	0.7 ± 2.0	1.5 ± 2.6	0.26
Current smoker (yes/no)	5/24	10/26	0.32

Note: values are mean ± S.D.;

* two-tailed two-sample t test, except for smoker status which was based on Chi-square test; AUDIT: Alcohol Use Disorder Identification Test; FTND: Fagerström Test for Nicotine Dependence.

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