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Irregular Autonomic Modulation Predicts Risky Drinking and Altered Ventromedial Prefrontal Cortex Response to Stress in Alcohol Use Disorder

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Abstract

Aims: Autonomic dysfunction has been associated with risky drinking and alcohol use disorder (AUD). Although autonomic nervous system (ANS) activity has been attributed to the ventromedial prefrontal cortex (VmPFC)-limbic-striatal regions, the specific role of ANS disruption in AUD and its association with these regions remain unclear. Using functional magnetic resonance imaging (fMRI) and concurrent electrocardiogram (ECG), the current study examined neural correlates of ANS activity in AUD and its role in AUD pathology.

Methods: Demographically matched 20 AUD patients and 20 social drinkers (SD) completed an fMRI task involving repeated exposure to stress, alcohol-cue and neutral-relaxing images in a block design. Based on the known VmPFC-limbic-striatal functions involved in emotions, reward and the ANS, we performed a regions of interest (ROI) analysis to examine the associations between ANS activity and neural responses in the VmPFC, amygdala, and ventral striatum.

Results: Across conditions, AUD patients showed significantly higher levels of overall heart rate (HR) and approximate entropy (ApEn) compared to SD (Ps < 0.05). In all participants, increased HR was associated with greater drinking volume ($P < 0.05$). In addition, higher ApEn levels were associated with greater drinking volume ($P < 0.05$) and decreased right VmPFC response to stress ($P < 0.05$).

Discussion: Our findings demonstrate ANS disruption in AUD indexed by high overall HR and ApEn. The association between ApEn and rVmPFC response suggests that ApEn may play a role in modulating drinking via interactions with neural regions of emotion regulation. These findings provide insight into patterns of ANS disruption and their relevance to AUD pathology.

INTRODUCTION

Individuals with alcohol use disorder (AUD) display disruptions in the autonomic nervous system (ANS) including elevated heart rate (HR) and diminished heart rate variability (HRV), which may result from complex interacting factors at the level of the brain and body (Ralevski *et al.* 2019). Despite numerous studies on AUD-related ANS disruption, its role in alcohol pathology and underlying neural mechanisms remain unclear. In previous studies, we found basal group differences with higher HR in AUD patients than in controls (Sinha 2009; Seo *et al.* 2013). Unlike other response measures such as brain activity and emotion ratings (which differentially react to stress and alcohol-cue exposure), ANS dysfunction in AUD was characterized by overall, noncondition-specific HR dysregulation (Seo *et al.* 2013). This overall ANS disruption in AUD has been interpreted

as long-term ANS hyperarousal resulting from repeated alcohol use (Cheng *et al.* 2019). This interpretation is also consistent with the basal state hyperactive physiology hypothesis in AUD (Breese *et al.* 2011). Although these concepts present viable explanations, further investigation into different components of ANS measures may provide useful insight into complex AUD pathology.

Emerging evidence indicates that nonlinear characteristics of HRV may uncover distinct aspects of pathology that are not fully understood by conventional linear metrics (Karmakar *et al.* 2017). Classic HRV indices, such as root mean square of successive differences (RMSSD) and high-frequency power (HF), characterize parasympathetic activation and vagal tone in healthy and AUD populations (Cheng *et al.* 2019), yet few studies have examined nonlinear HRV indices such as approximate entropy (ApEn). ApEn is known to detect complex ANS patterns by quantifying the influence of ANS and other physiological factors (e.g. thermoregulation, endocrine factors) on HRV, thereby providing an index of the complex network of nonlinear systems in ANS regulation (Young and Benton 2015). Therefore, examining ApEn may help capture autonomic complexity related to AUD pathology.

In addition, investigating neural systems underlying ANS modulation is necessary to further elucidate AUD pathology. Recent studies suggest that the central autonomic network plays a crucial role in addictive behaviors by regulating and receiving feedback from ANS via neurocardiovascular pathways (Eddie *et al.* 2020) and modulating neural responses via breathing (Bates *et al.* 2019). Although these studies highlight the importance of brain-ANS interactions, few studies have investigated autonomic and brain functions simultaneously (Napadow *et al.* 2008), limiting our comprehension of AUD pathology.

To better understand autonomic dysfunction in AUD, the current study examined both nonlinear HRV (i.e. ApEn) and conventional HRV (i.e. HF, LF, RMSSD) metrics via electrocardiogram (ECG) signal data in conjunction with blood-oxygen-level-dependent (BOLD) responses to stress and alcohol cues obtained from a well-validated emotion provocation task using functional magnetic resonance imaging (fMRI) (Sinha *et al.* 2016). Using this fMRI-ECG method, we examined concurrent brain-ANS responses with baseline drinking measures in AUD patients and social drinkers (SD). We used a regions-of-interest (ROI) approach to specifically study associations between the ANS and the ventromedial-limbic-striatal region, a well-known neural system involved in emotion, reward, and ANS function (Hiser and Koenigs 2018). Evidence consistently points to the ventromedial prefrontal cortex (VmPFC) as an underlying neural correlate of emotional control (e.g. stress, reward), ANS modulation (Thayer *et al.* 2012), and alcohol-related pathology, such as high craving and early relapse (Seo *et al.* 2013; Blaine *et al.* 2020). In addition, limbic-striatal pathways involved in emotion and reward processing, especially the amygdala and ventral striatum (VS), are known to play important roles in ANS modulation (Rajmohan and Mohandas 2007). These studies suggest that ANS activity in AUD can be differentially modulated by activity in ventromedial-limbic-striatal regions represented by the VmPFC, amygdala, and VS. Accordingly, our ROI approach specifically examined the role of *a priori* regions (VmPFC, amygdala, VS) in modulating ANS activity in AUD. Based on prior literature, we expect AUD patients to exhibit greater overall ANS hyperactivity across conditions. We further hypothesize that nonlinear HRV metrics, such as ApEn, will additionally explain autonomic disruption in AUD patients and be associate with altered activity in these regions.

METHODS

Participants and study procedure

Forty adults (20 treatment-seeking AUD, 20 SD) were recruited from the Greater New Haven area. Given the high rates of comorbidity between AUD and other psychiatric disorders (Castillo-Carniglia *et al.* 2019), those with comorbid conditions were included to model real-world AUD pathology. Controls (SD) were regular, low-risk drinkers based on NIAAA guidelines (NIAAA 2009). The AUD group was demographically matched to the SD group on age, sex, intelligence, and comorbid conditions (Table 1). To remove the effects of physiological disorders, those with somatic and cardiovascular diseases were excluded. Blood pressure levels were at normal range in all participants. For further information on our sample, please refer to Supplemental Information (SI). Prior to the fMRI session, baseline demographic, psychiatric, and cognitive measures were collected from all participants. The Structured Clinical Interview for DSM-5 (First 2015) was administered to determine AUD and assess any additional psychiatric diagnoses. Participants also completed the Alcohol Use Disorders Identification Test (AUDIT) (Saunders *et al.* 1993) and Quantity-Frequency Variability (QFV) Index (Cahalan and Cisin 1968) as measures of problematic drinking and alcohol consumption patterns over time. For all appointments and fMRI sessions, breathalyzer and urine screenings confirmed drug and alcohol abstinence. All study procedures were approved by the Human Investigation Committee of the Yale University School of Medicine.

fMRI session

Overview All fMRI-ECG sessions were conducted in the morning (8–10 AM) to control for the potential effects of circadian rhythm and diurnal hormone variations. During the scan, participants underwent a block-designed, sustained emotion provocation (SEP) task (Sinha *et al.* 2016) (Fig. 1). There were three blocks, each displaying visual stimuli from one of three conditions (stress [S], alcohol-cue [A], neutral-relaxing [N]). As part of a larger published study (Blaine *et al.* 2020) reporting brain activity in AUD, the current study examined the subset of individuals with available ECG data and primarily focused on ANS activity associated with neural responses using concurrent ECG-fMRI data collection.

Visual stimuli The visual stimuli were 66 highly stressful, 66 alcohol-cue, and 66 neutral-relaxing images; all images were either selected from the International Affective Picture System (IAPS) (Lang *et al.* 2008) or developed by the Yale Stress Center as previously described (Sinha *et al.* 2016; Blaine *et al.* 2020). The stressful images consisted of photographs depicting scenes of terror, violence, disgust, threat, etc. The alcohol-cue pictures included images of various alcoholic beverages (e.g. beer, wine, liquor) by themselves or within a range of social scenarios (e.g. bars, social drinking). The neutral-relaxing images contained neutral-relaxing content (e.g. relaxing meditation poses, natural landscapes, etc.).

Each condition started with 3 baseline runs, consisting of blank gray images with a centered fixation cross, followed by 6 provocation runs containing picture content pertaining to the applied condition. Each run consisted of 11 discrete visual stimuli. To achieve equivalent levels of emotional intensity of pictures throughout the task, we ensured that there were no statistical differences in valence/arousal ratings or in picture content across the 6 provocation runs within each condition, as described previously (Sinha *et al.* 2016; Blaine *et al.* 2020). For detailed information on the valence and arousal ratings of

Table 1. Demographic and clinical characteristics

Subject variable	AUD patients ($N = 20$)	Social drinkers (SD) ($N = 20$)	*P*-values
Age (s.d.)	31.4 (10.4)	29.4 (9.3)	n.s.
Sex (% female)	10 (50.0%)	10 (50.0%)	n.s.
Race (N%)			
Caucasian	10 (50.0%)	10 (50.0%)	n.s.
Cognitive information, s.d.			
Education, in years	14.2 (2.1)	15.4 (2.4)	n.s.
Shipley, test score	110.2 (5.7)	111.5 (5.1)	n.s.
Baseline alcohol assessment, s.d.			
AUDIT total score	19.3 (5.4)*	3.6 (3.0)	$P < 0.001$
Drinks per week, QFV Index	27.7 (16.9)*	3.3 (3.7)	$P < 0.001$
Years of regular use	9.9 (9.8)	6.2 (8.0)	n.s.
Monthly drink frequency	28.2 (32.2)*	5.7 (4.6)	$P < 0.001$
Maximum drinks in one sitting	10.1 (6.4)*	4.2 (2.6)	$P = 0.001$
Psychiatric history			
PTSD (current)	2	1	n.s.
Depression (current)	1	1	n.s.

There were no significant differences in age, sex ratio, race, or intelligence as measured by both years of education and by the Shipley Institute of Living Scale (range: 98–123) (Zachary *et al.* 1985). Alcohol Use Disorders Identification Test (AUDIT) scores were used to measure problematic alcohol use and drinking volume, and the Cahalan Quantity-Frequency Variability (QFV) Index was used to measure drinking volume and frequency. Compared to social drinkers (SD), AUD participants reported significantly higher drinking volume, drinking frequency, maximum number of drinks in one sitting, and alcohol-related problems. s.d. = standard deviation; n.s. = nonsignificant; $^*P \leq 0.001$.

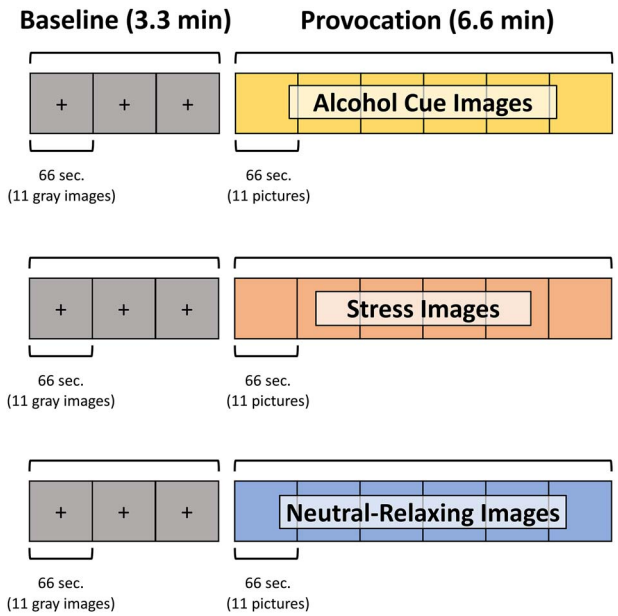

Fig. 1. Task design. The sustained emotion provocation (SEP) task contained three blocks, each displaying visual stimuli from one of the three conditions (stress [S], alcohol-cue [A], neutral-relaxing [N]). Examples of pictures shown during each condition are depicted above. Each condition presented three baseline runs of blank gray images with a centered fixation cross followed by six provocation runs containing picture content representative of the condition. Each run consists of 11 visual stimuli. All visual stimuli were presented through a liquid crystal display panel using E-Prime software (Psychology Software Tools, Inc., PA, USA).

the visual stimuli, refer to SI. All visual stimuli were presented with E-Prime software (Psychology Software Tools, Inc., Pennsylvania, USA) on a liquid crystal display panel mounted on the fMRI head coil.

fMRI experimental task On the fMRI scan day, all individuals received training on the fMRI experimental procedure. Participants completed a practice task consisting of 4 runs using neutral visual stimuli that were not used during the actual fMRI scan task. After a 15-minute adaptation period supplemented by a 4-minute guided relaxation exercise to create standardized baseline conditions across participants, the participant entered an MRI suite at approximately 8:00 AM and underwent the SEP task.

In this task, participants were asked to view a series of images in the stress [S], alcohol-cue [A], and neutral-relaxing [N] conditions. Participants were shown a total of 198 pictures (66 pictures per condition). The order of stimuli and conditions were randomized and counterbalanced across subjects. For each baseline/provocation run, the blank or visual image was presented for 5 seconds with a 1-second interstimulus interval, and each image was presented only once. At the end of each run, participants rated their stress and alcohol craving using a scale displayed on the screen, with the scales consisting of numbers 1 (not at all) through 9 (very much). The 'stress' rating asked participants to rate their level of tension, anxiety, and distress. The 'craving' rating asked participants to rate their level of desire to drink alcohol while viewing the visual stimuli. Participants selected their ratings using a nonferromagnetic button box in each hand.

Data acquisition

MRI data were acquired on a 3-T Siemens Prisma MRI system, equipped with a standard quadrature head coil, using a T2*-sensitive gradient-recalled single-shot echo-planar pulse sequence. For fMRI data, 75 axial slices parallel to the AC-PC line covering the whole brain were acquired with the following parameters: TR = 1000 ms, TE = 30 ms, bandwidth = 1894 Hz/pixel, flip angle = 55°, field of view = 220x220 mm, slice thickness = 2 mm with no gap. A high-resolution 3D magnetization-prepared rapid gradient-echo (MP-RAGE) sequence was used to acquire sagittal anatomical

images. fMRI data were acquired with a 64-channel head coil with multiband accelerated echo-planar imaging sequence. To address potential susceptibility artifact issues in gradient-echo-planar imaging, we used microscopic voxel size, high bandwidth, and advanced registration methods (see SI for further details). Throughout the fMRI session, ECG signals were concurrently acquired by Siemen's MRI-compatible 4-lead EKG system.

ECG data processing and analysis

ECG signals were preprocessed with a bandpass Butterworth filter, and RR intervals were detected, artifact-corrected, and analyzed for linear (HR, HF, LF, RMSSD) and nonlinear (ApEn) HRV measures with Kubios HRV software. Before conducting analyses, raw ECG data were checked for normalcy and for outliers, and we removed univariate outliers for HR (±2 standard deviations from the group mean) as well as HRV data points associated with those HR outliers (3.4% of the data set) to ensure that data results were not impacted by ECG signal artifacts. Linear HRV indices were log-transformed. ApEn and HR were not log-transformed, as the data were normally distributed (see SI for details).

fMRI data processing and analysis

fMRI data were converted from DICOM to Analyze format and motion-corrected for three translational and three rotational directions. On the individual level, we performed a GLM analysis with a regressor specific to each fMRI task condition using BioImage Suite (Duncan *et al.* 2004). For each run, functional images were spatially smoothed, and individually normalized beta maps were generated. To account for individual variations in brain anatomy, we calculated three sequential registrations and applied them to the individually normalized beta maps.

To examine the associations of ANS activity and specific functions in the ventromedial-limbic-striatal regions, we used a regions of interest (ROI) approach to focus on the role of these hypothesized regions. Based on prior literature (Lane *et al.* 2009; Thayer *et al.* 2012; Hiser and Koenigs 2018), three structured brain regions related to emotion, reward, and ANS modulation were defined as *a priori* ROI, which included the VmPFC (BA 10), ventral striatum (nucleus accumbens) and amygdala. The structural ROIs for these regions were anatomically defined using the Yale-Brodmann atlas from BioImage Suite (www.bioimagesuite.org). For each region, beta weights were extracted from mean blood–oxygen-level-dependent (BOLD) signal (averaged across all voxels within each ROI per participant) in Stress relative to Neutral-Relaxing (S–N) and Alcohol-Cue relative to Neutral-Relaxing (A–N) contrasts. Then, these signals were associated with both ANS and drinking measures in our sample using regression analyses to examine the functional associations of these ROIs. Outliers in regression analyses were evaluated by Cook's distance and visual inspection of each scatterplot.

RESULTS

Demographic and clinical characteristics

Table 1 summarizes basic demographic and background information for AUD and SD groups. No group differences were found in sex, age, race, intelligence and comorbid psychiatric disorders, except for alcohol use (see Table 1).

Alcohol craving and stress ratings

Mixed factorial ANOVA analyses (2 × 3) were conducted on alcohol craving and stress ratings with Group (AUD, SD) as a between-subjects factor and Condition (A, N, S) as a within-subjects factor (Fig. 2). These models produced significant Group and Condition main effects, but no interaction (Group × Condition) effects. Group main effects were found for craving ($F_{1,36} = 18.32$, $P < 0.05$) and stress ratings ($F_{1,36} = 5.37$, $P < 0.05$), indicating that AUD patients reported higher overall craving ($t = 4.57$, $P < 0.01$) and higher stress levels ($t = 2.38$, $P < 0.05$) compared to SD. Condition main effects were also found for craving ($F_{1,35} = 6.86$, $P < 0.05$) and stress ratings ($F_{1,35} = 38.44$, $P < 0.05$). Craving ratings were higher during the alcohol-cue condition compared to stress and neutral-relaxing conditions (all Ps < 0.05). Stress ratings were higher during the stress condition compared to alcohol-cue and neutral-relaxing conditions (all Ps < 0.05).

Group differences in ANS activity

Mixed factorial ANOVA analyses were conducted on HR and HRV measures including Group (AUD, SD) as a between-subjects factor and Condition (A, N, S) as a within-subjects factor (Fig. 3). These models produced significant Group main effects for HR ($F_{1,36} = 4.69$, $P < 0.05$) and ApEn ($F_{1,34} = 6.46$, $P < 0.05$), indicating that AUD patients reported higher overall HR ($t = 2.16$, $P < 0.05$) and higher ApEn levels ($t = 2.51$, $P < 0.05$) during the provocation period. There were no significant effects of Condition or Group × Condition in HR and ApEn values. No significant effects were found for Group, Condition, or Group × Condition on linear HRV measures (HF, LF, RMSSD).

As only Group main effects were found for HR and ApEn, we averaged each ANS measure across conditions and used them as overall ANS measures. The overall HR and ApEn measures were computed by averaging values across provocation runs (6 per condition) and the three task conditions. Then, the overall HR and ApEn measures were associated with drinking measures and BOLD responses to stress and alcohol cues within the AUD group, SD group and both groups. In preliminary analysis, we also averaged the data per condition and examined their associations with drinking and BOLD responses. However, the results were consistent with the results from the overall measure.

ANS activity and drinking behaviors

Regression analyses were conducted including participants from both AUD and SD groups to determine whether overall HR and ApEn measures were associated with drinking behaviors (Fig. 3). HR was positively associated with AUDIT score ($\beta = 0.33$, $P < 0.05$) and alcohol volume defined as average drinks per week ($\beta = 0.36$, $P < 0.05$). Similarly, ApEn was positively associated with AUDIT score ($\beta = 0.38$, $P < 0.05$) and alcohol volume ($\beta = 0.33$, $P < 0.05$). No outliers were found in any of these associations. HR and ApEn levels were associated with weekly average drinking amount, but not with other types of drinking measures, such as maximum number of drinks, frequency or duration of drinking.

Neural correlates of ANS activity

Regression analyses were conducted to examine the associations between overall ApEn/HR measures, BOLD responses, and drinking behavior (Fig. 4). BOLD responses in the *a priori* brain regions (VmPFC, amygdala, VS) in S–N and A–N contrasts were used as

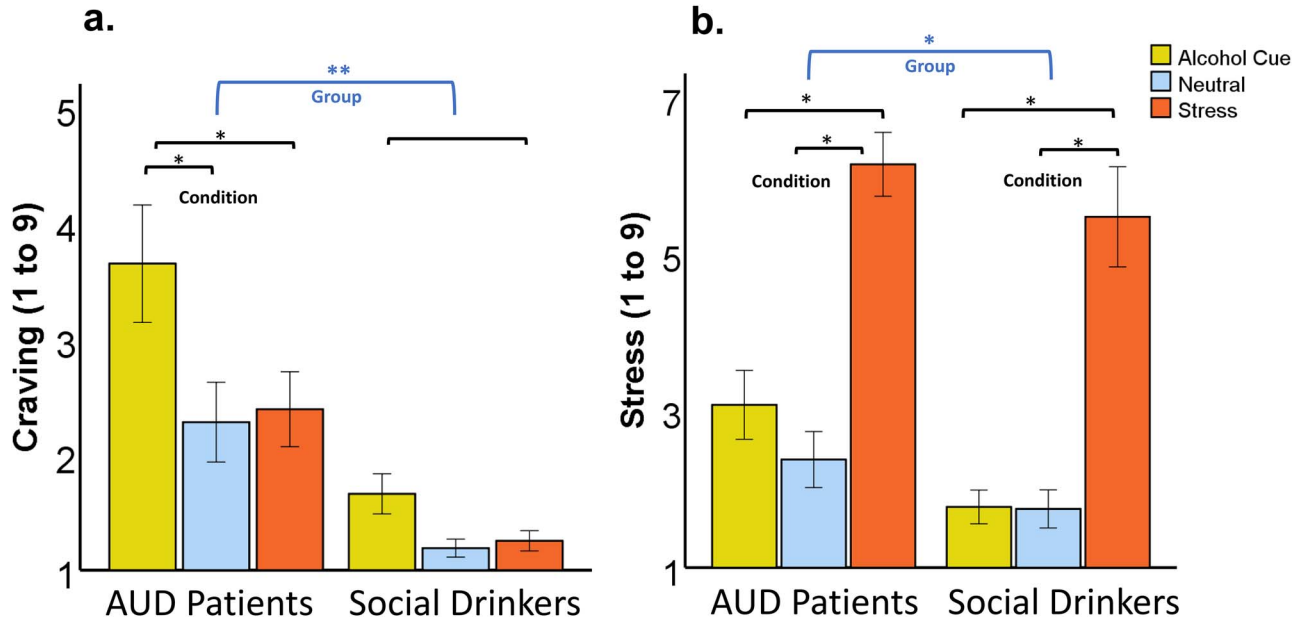

Fig. 2. Craving and stress ratings. Across groups, all participants reported **(a)** higher alcohol craving levels during the alcohol-cue condition than either the stress or neutral-relaxing conditions ($P < 0.05$) and **(b)** higher stress levels during the stress condition than either the alcohol-cue or neutral-relaxing conditions ($P < 0.05$). Group main effect was significant, such that AUD patients reported higher overall craving ($P < 0.01$) and higher overall stress levels ($P < 0.05$) than social drinkers (SD) across conditions. $^{*}P < 0.05$, $^{**}P < 0.01$.

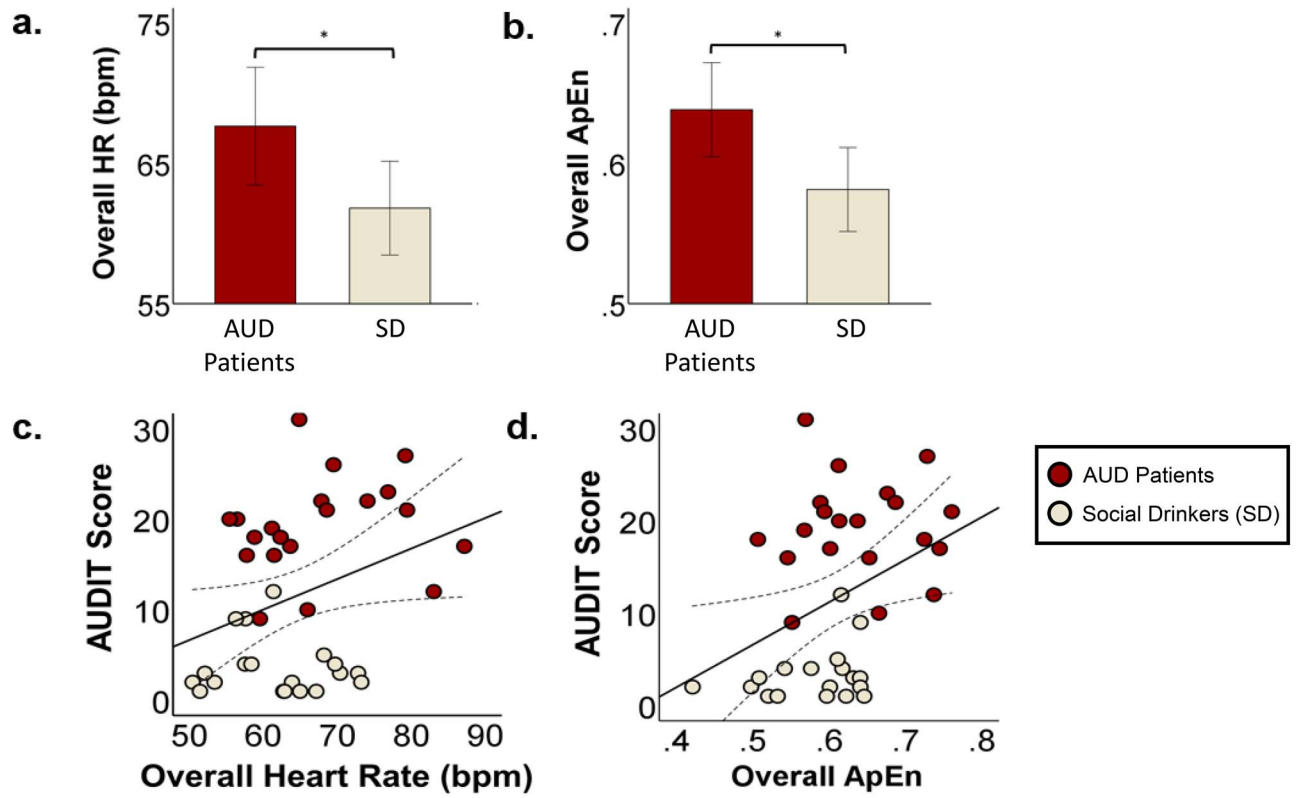

Fig. 3. Group differences in ANS activity. Across conditions, AUD patients exhibited higher **(a)** mean heart rate (HR; bpm) and **(b)** approximate entropy measures (ApEn; m, r, N) during the provocation period compared to social drinkers (SD) (Ps < 0.05). Higher AUDIT scores were associated with **(c)** higher HR (β=0.33, $P < 0.05$) and **(d)** higher ApEn scores (β=0.38, $P < 0.05$) in all participants. $^{*}P < 0.05$.

predictors, and either ApEn, HR or AUDIT scores were used as the outcome variable. A significant negative association was found between right VmPFC response to stress (S–N) and ApEn values ($\beta = -0.39$, $P < 0.05$) among all participants. In addition, decreased rVmPFC response to stress was associated with total AUDIT scores ($\beta = -0.37$, $P < 0.05$) and drinking volume ($\beta = -0.35$, $P < 0.05$).

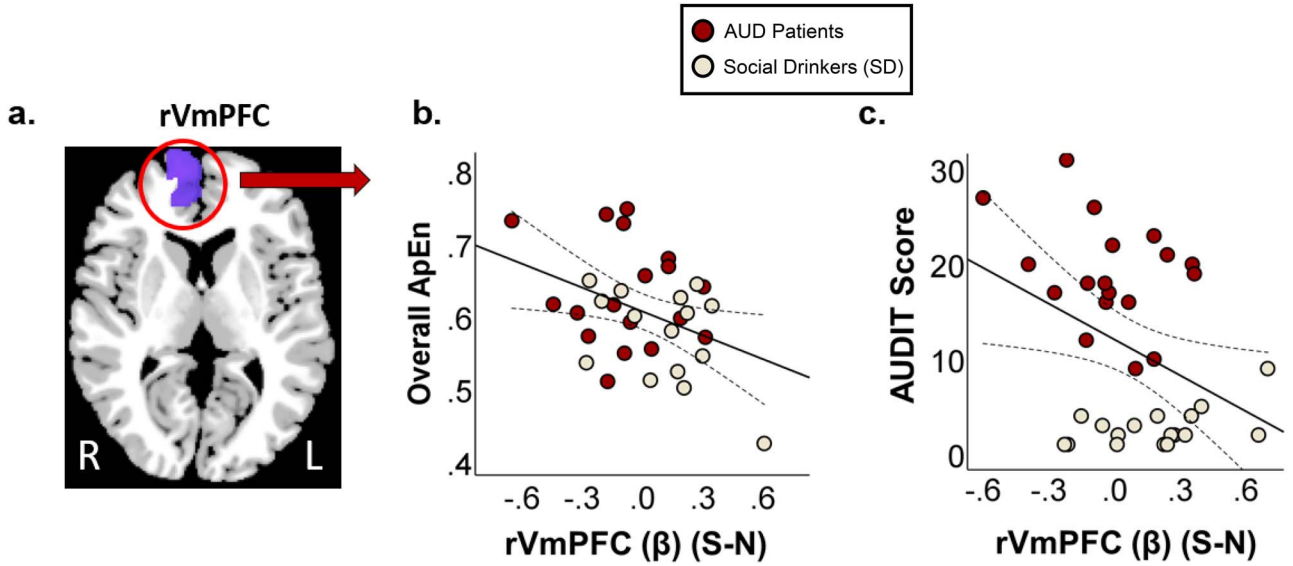

Fig. 4. Neural correlates of ANS activity. **(a)** ROI: right medial PFC (BA 10) from the Yale-Brodmann Atlas. Decreased activity in the right ventromedial prefrontal cortex (rVmPFC) during the stress condition, relative to neutral-relaxing, was associated with **(b)** higher ApEn levels ($\beta = -0.39$, $P < 0.05$) and **(c)** higher AUDIT scores ($\beta = -0.37$, $P < 0.05$) in all participants.

No outliers were found in these associations. No other statistically significant association was found for other brain regions or with the A–N contrast.

Controlling for psychiatric comorbidities

To evaluate the potential influence of comorbid conditions, we controlled for coexisting psychiatric disorders in all statistical analyses (see SI). Our findings remained significant, indicating that psychiatric comorbidity did not significantly impact the current findings.

DISCUSSION

The current study reports that AUD is characterized by high ANS arousal and irregular autonomic modulation indexed by high HR and ApEn values. Furthermore, increased ApEn was associated with greater alcohol consumption and a blunted rVmPFC response to stress, pointing to ApEn's crucial role in modulating drinking behaviors through interactions with key brain regions of emotion regulation. These results highlight the importance of disrupted autonomic function in AUD and its role in increasing vulnerability to risky drinking.

As expected, when comparing AUD and SD, we found that AUD patients exhibited higher overall HR and ApEn across conditions. This noncondition-specific group difference in overall ANS function is a replication of our prior findings (Seo *et al.* 2013) using HR collected concurrently with fMRI during stress, alcohol-cue and neutral-relaxing imagery. This is also consistent with prior studies noting heightened ANS function in AUD (Cheng *et al.* 2019), including a recent study that found no difference in HR between alcohol-cue and neutral conditions (Wang *et al.* 2020). Consistent with the concept of hyperexcitable physiological states associated with AUD (Breese *et al.* 2011), increased HR across conditions may indicate autonomic disruption resulting from long-term ANS hyperarousal. In addition, we observed elevated overall ApEn in AUD patients. To our knowledge, this is the first report of high ApEn as an index of ANS disruption in the AUD literature.

Although ApEn has not been widely studied in AUD, it has gained recognition in cardiovascular and psychiatric research (Bar *et al.* 2007; Byun *et al.* 2019). Emerging trends point to nonlinear HRV metrics, such as ApEn, for new features of cardiac modulation that are not captured by conventional linear HRV patterns. These studies propose that intact nonlinear HRV (e.g. ApEn) patterns reflect coordinated cardiac modulation by integrating information from a vast feedback network of spontaneity (spontaneous depolarization and repolarization of the sinoatrial node) and adaptability (ANS modulation) (Shi *et al.* 2017). This coordination is critical for adaptive ANS responses and flexible coping during stressful situations. Thus, the high ApEn values observed in our AUD patients may reflect maladaptive coping responses resulting from erratic ANS modulation.

Although the current study found significance in HR and ApEn associated with AUD, we did not find significance using linear HRV measures (e.g. LF, HF, RMSSD). However, some AUD studies using linear HRV measures report altered vagal modulation and heightened ANS activity indexed by reductions in HF and RMSSD, indicating decreased parasympathetic control and subsequent sympathetic dominance (Cheng *et al.* 2019). These discrepant results may be explained by a study reporting no linear HRV (LF, HF) differences between AUD patients and controls in supine positions while finding altered HRV in standing positions (Sucharita *et al.* 2012). The supine position of our fMRI participants may have hindered optimal evaluation of changes in linear HRV metrics (Cheng *et al.* 2019). In addition, task differences may also account for the discrepancies with the literature due to our specific task with stress, alcohol-cue and neutral-relaxing pictures. Supporting this, our results on HR elevation across conditions replicate the findings from a prior task using stress, alcohol-cue and neutral-relaxing imagery (Sinha *et al.* 2009; Seo *et al.* 2013).

Like its linear HRV counterparts, nonlinear HRV has been associated with vagal dysregulation in AUD (Bar *et al.* 2008). However, ApEn may further explain complex ANS irregularity resulting from compromised autonomic function, which can adversely impact brain–heart interactions (Shaffer and Ginsberg 2017). For

example, ApEn has been applied to electroencephalography (EEG), which shows significantly higher (more irregular) basal EEG ApEn values in AUD patients than controls (Shri *et al.* 2014). The higher ApEn observed in both ECG and EEG studies suggests that AUD is characterized by hyperactive, irregular electrical activity in the cardiovascular and central nervous systems (CNS), resulting in disrupted CNS–ANS interactions.

This is consistent with our result showing an association between increased ApEn and blunted VmPFC response to stress. Decreased VmPFC function has been associated with emotion dysregulation and alcohol-related, maladaptive coping behaviors including high craving and early relapse (Seo *et al.* 2013; Sinha *et al.* 2016). Specifically, our VmPFC finding was localized in the right, but not the left VmPFC. The right VmPFC is known to be more involved in the affective and social aspects (Tranel *et al.* 2002) of emotion regulation, whereas the left VmPFC assumes a greater role in the cognitive aspects (Leopold *et al.* 2012). This suggests that the inverse association between the rVmPFC and ApEn reflects emotional and autonomic dysregulation in AUD patients. Consistent with this, the rVmPFC has been shown to be involved in regulating emotion via ANS modulation (Hansel and von Kanel 2008), and right VmPFC lesions led to disruption of sympathetic inhibition during processing of stressful stimuli (Hilz *et al.* 2006), highlighting the role of rVmPFC in emotion- and stress-related ANS dysregulation in AUD patients.

Supporting this, our finding of the neural-ANS relationship was more stress-specific, as we did not detect significance in the alcohol-cue condition. This is discrepant with a study showing that altered RMSSD HRV response to alcohol cues is associated with decreased VmPFC activity in SD (Wang *et al.* 2020). This discrepancy may be explained by the nature of our sample, which is predominantly composed by AUD patients. Addictive drinking is accompanied by a transition from positive to negative reinforcement (Koob and Volkow 2010), where negative affect and stress-induced craving play more prominent roles (Ramchandani *et al.* 2018). It has been suggested that negative reinforcement results from an altered stress system, which is closely related to the central autonomic network that integrates autonomic information (Koob and Volkow 2010; Eddie *et al.* 2020). This may explain the association between ANS dysregulation and VmPFC response to stress found in our study. Consistent with this idea, our prior studies showed that blunted VmPFC response to stress in AUD patients predicts more frequent heavy drinking and faster alcohol relapse (Seo *et al.* 2013; Blaine *et al.* 2020), highlighting the importance of stress-related neural response in AUD severity. The current study extends these findings by showing an association between stress-related rVmPFC hypoactivity and disrupted ANS function in AUD patients. Hypoactivity in the ANS-governing rVmPFC may reflect an insufficient recruitment of neural resources during stress, leading to irregular ANS modulation and subsequent dysregulation of drinking behaviors. Supporting this, both blunted rVmPFC and increased ApEn were associated with increased alcohol consumption in our data, suggesting the importance of ANS regulation in an adaptive stress response and drinking behaviors.

The limitations for the current study include a small sample size. However, our AUD and SD groups are well-matched on demographics and intelligence. Thus, despite this limitation, our findings add to the AUD–ANS literature by reporting AUD-related differences in ApEn that may account for risky drinking and an altered neural response during stress. Given the preliminarry nature of our findings it is worthwhile to extend current findings in future studies with a larger sample size and further explore the utility of ApEn as an autonomic marker of AUD.

It should be noted that ApEn can be a difficult index to interpret, given the lack of literature regarding its exact role in HRV-related contexts and definition of healthy range. Signal complexity (e.g. ApEn) sometimes indicates a functional response, and both increased and decreased ApEn values have been associated with pathological states including decreased ApEn in schizophrenia (Bar *et al.* 2007) and increased basal ApEn in anxiety disorder (Caldirola *et al.* 2004). Given that ApEn is associated with other mental disorders (Caldirola *et al.* 2004; Bar *et al.* 2007; Byun *et al.* 2019), global ANS irregularity may be a transdiagnostic ANS signature also present in AUD. However, in our study, high ApEn was specifically associated with drinking behaviors and stress-related vmPFC response, suggesting that high ApEn may constitute an autonomic marker associated with stress-related drinking in AUD. Future studies, especially neuroimaging studies, should further clarify the role of ApEn in AUD pathology and its underlying neural mechanisms. Neuroimaging research should also explore other neural regions, such as the anterior cingulate cortex and other medial-prefrontal cortical regions, that are known to be modulated by breathing regulation (Bates *et al.* 2019).

Taken together, our findings suggest that high ApEn activity may serve as a novel index of ANS dysfunction associated with AUD, signifying irregular autonomic modulations, stress-related dysregulation, and increased susceptibility to hazardous drinking behavior. Our study also highlights the potential clinical utility of ApEn as a noninvasive, cost- and time-efficient ANS measure of AUD. ApEn may serve as an affordable index of AUD by detecting early AUD risk and alcohol-related cardiovascular problems in clinical care.

DATA AVAILABILITY

Data is available upon request by directly contacting the authors of this work.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Alcohol and Alcoholism* online.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest has been declared by any of our authors for this work.

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