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Brain correlates and functional connectivity linking stress, autonomic dysregulation, and alcohol motivation

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ABSTRACT

High stress is a key risk factor for alcohol use disorder (AUD) and often accompanied by physiological dysregulation including autonomic nervous system (ANS) disruptions. However, neural mechanisms underlying drinking behaviors associated with stress and ANS disruptions remain unclear. The current study aims to understand neural correlates of stress, ANS disruptions, and subsequent alcohol intake in social drinkers with risky drinking. Using functional magnetic resonance imaging (fMRI), we investigated brain and heart rate (HR) autonomic responses during brief exposure to stress, alcohol, and neutral cues utilizing a well-validated, individualized imagery paradigm in 48 social drinkers of which 26 reported high-risk drinking (HD) while 22 reported low-risk drinking (LD) patterns. Results indicated that HD individuals showed stress and ANS disruptions with increased basal HR, stress-induced craving, and decreased brain response to stress exposure in frontal-striatal regions including the ventromedial prefrontal cortex (VmPFC), anterior cingulate cortex, striatum, insula, and temporal gyrus. Furthermore, whole-brain correlation analysis indicated that greater basal HR was associated with hypoactive VmPFC, but hyperactive medulla oblongata (MOb) responses during stress, with an inverse association between activity in the VmPFC and Mob (whole-brain corrected (WBC), $p < 0.05$). Functional connectivity with the MOb as a seed to the whole brain indicated that HD versus LD had decreased functional connectivity between the VmPFC and MOb during stress (WBC, $p < 0.05$). In addition, those with more compromised functional connectivity between the VmPFC and MOb during stress consumed greater amount of alcohol beverage during an experimental alcohol taste test conducted on a separate day, as well as in their self-reported weekly alcohol intake. Together, these results indicate that stress-related, dysfunctional VmPFC control over brain regions of autonomic arousal contributes to greater alcohol motivation and may be a significant risk factor for hazardous alcohol use in non-dependent social drinkers. Findings also suggest that restoring VmPFC integrity in modulating autonomic arousal during stress may be critical for preventing the development of AUD.

1. Introduction

High-risk alcohol consumption is a serious public health problem (Anderson, et al., 2023; Esser et al., 2021; Volpicelli and Menzies, 2022). However, it remains unclear why some individuals are more susceptible to high-risk drinking and the development of alcohol use disorder (AUD). High stress is a key risk factor for AUD, which is often accompanied by physiological dysregulation including autonomic nervous system (ANS) disruptions (Sinha, et al., 2009). Studies have shown that stress-motivated drinking is a major contributor to alcohol problems (Perkins, 1999; Sebena et al., 2012), and early alcohol drinking in conjunction with stressful life experiences is a significant risk factor for the development of AUD (Blomeyer, et al., 2011). Stress response involves autonomic nervous system (ANS) arousal (Thayer, et al., 2012) and it is well documented that alcohol misuse and AUD are associated with stress and ANS disruptions, including high stress sensitivity, negative emotions, increased blood pressure and basal heart rate (HR), sustained phasic HR acceleration, and reduced heart rate variability (Karpyak, et al., 2013; Sinha et al., 2009; Stormark et al., 1998; Vaschillo et al., 2008). Specifically, our previous studies have shown that AUD is characterized by impaired autonomic regulation marked by elevated basal heart rate (Hwang, et al., 2022; Seo et al., 2013; Sinha et al., 2009). Basal ANS upregulation in AUD has been regarded as ANS hyperarousal resulting from hazardous alcohol use (Cheng, et al., 2019)

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or hyperactive basal-state physiology in AUD (Breese, et al., 2011; Hwang et al., 2022). It is also suggested that neural regulation over ANS systems may be compromised in those with hazardous drinking patterns. Yet, the exact neural mechanisms underlying the link between stress, ANS disruptions, and the development of AUD remain unclear.

Although neural evidence in this area is limited, available literature indicates that ANS activity is modulated in the central-peripheral, neuro-feedback circuit involving the medulla oblongata (MOb) of the brain stem, a region involved in autonomic arousal (Gordan, et al., 2015; Mitchell and Victor, 1996). The ventromedial prefrontal cortex (VmPFC) plays a key role in controlling this circuit (Thayer, et al., 2012; Thayer and Lane, 2000). The VmPFC is involved in the modulation of stress, reward, and emotional decision-making partly via its control over ANS and hypothalamic-pituitary-adrenal (HPA) axis systems (Bechara, 2004; Radley et al., 2006; Urry et al., 2006). Given that the VmPFC is known to guide adaptive behaviors during stress by modulating emotional and ANS responses, dysfunctional VmPFC control over stress and ANS systems may lead to risky alcohol use by increasing alcohol craving and dysfunctional ANS arousal under stress.

Consistent with this hypothesis, previous studies have pointed to the critical role of the VmPFC in AUD. It has been suggested that the VmPFC is a key regulatory region that engaged in alcohol craving and relapse (Seo and Sinha, 2014). In particular, the role of the VmPFC in AUD has been indicated by multiple studies with different paradigms including the VmPFC dysfunction associated with high alcohol craving (Myrick, et al., 2004) and decision making impairments in AUD (Bechara, 2005). Our previous study also showed that hypoactive VmPFC response to stress is associated with alcohol craving and the severity of future relapse measured by more days of alcohol use after treatment (Seo, et al., 2013). These findings suggest a potential role of the VmPFC dysfunction in stress-related high-risk drinking and ANS disruption in alcohol misusing populations. However, thus far, no study has directly assessed the relationship between the VmPFC and autonomic arousal regions (e.g., medulla oblongata (Mob)) in AUD risk, specifically whether functional connectivity in the VmPFC-MOb feedback circuit contributes to ANS disruption and increased alcohol intake in risky drinkers. If specific connectivity patterns in the VmPFC-MOb circuit are associated with risk for AUD, such dysfunction may serve as novel neural markers to identify individuals who are at most risk for stress-related hazardous drinking and the development of AUD.

Therefore, we conducted a functional magnetic resonance imaging (fMRI) study investigating neural correlates of basal ANS activity and related functional connectivity patterns in social drinkers using a well-validated individualized guided imagery paradigm (e.g. (Sinha, 2009)). We hypothesized that high-risk drinkers would show stress and ANS disruptions including higher basal heart rate and altered neural responses to stress. We also expected that ANS disruptions would be associated with compromised connectivity between the VmPFC and ANS modulatory regions (e.g., MOb) during stress exposure. Finally, we evaluated whether altered connectivity in the VmPFC-Mob circuit is associated with higher alcohol intake, as measured through an experimental alcohol taste test (ATT) and self-reported alcohol intake levels.

2. Materials and method

Participants. Participants were 48 social drinkers (age = 28.31(SD = 6.9)), consisting of demographically-matched 26 high-risk drinkers (HD) and 22 low-risk drinkers (LD) (see Table 1). Participants were recruited from the Greater New Haven area through flyers, advertisements in local newspapers, and social media. Drinking group status was determined using the criteria established by the National Institute on Alcohol Abuse and Alcoholism Guideline (NIAAA, 2005). Specifically, participants who reported consuming 8 or more drinks per week for women, or 15 or more drinks per week for men, or engaging in binge drinking episodes at least once/month were selected for the high-risk drinking (HD) group. Binge drinking is defined as consuming 4 or more drinks for women and 5 or more drinks for men within 2 h occurring at least one day within the past month. Control participants were selected for the low-risk drinking (LD) group, if they reported consuming 7 or fewer drinks per week for women, or 14 or fewer drinks per week for men without engaging in binge drinking episodes. Eligible participants were in good health, were able to read and speak in English, and provide written informed consent. Participants had no current DSM-IV-TR diagnosis for alcohol or substance dependence and also did not meet criteria for current alcohol abuse or mild alcohol use disorder; had no cerebral, cardiovascular, renal, thyroid, hepatic pathologies, or pregnancy; had no current use of psychiatric medications; and had no severe psychiatric disorders (including psychotic or Axis I disorders).

Table 1
Demographic characteristics.

Variable	High-risk (n = 26)	Low-risk (n = 22)
Demographics		
Age (in years)	27.6 (6.6)	29.1 (7.4)
Sex (% Male)	85%	77%
Race (% Caucasian)	69%	82%
Education (in years)	15.7 (2.3)	15.9 (1.7)
Body Mass Index (BMI; kg/m2)	26 (3.6)	27.9 (5.2)
Alcohol Use* **		
AUDIT Total Score	11.3 (4.1)	3.95 (1.8)
Drinks per Week	17.8 (8.5)	4 (3.1)
Days alcohol consumed/week	3.1 (1.4)	1.8 (1.4)
Binge episodes per month	2.3	0
Alcohol Taste Test (mL) (Average amount consumed)	471.5 (208.9)	266.2 (156.2)

Note. None of the high-risk drinkers met criteria for alcohol abuse or alcohol dependence (DSM-IV TR) equivalent of any DSM-5 alcohol use disorder (mild, moderate, severe) diagnoses. AUDIT = Alcohol Use Disorders Identification Test.
*** $p < 0.001$.

Study Procedure. General study procedures consist of 1) baseline assessments, 2) guided imagery script development and training, 3) an experiment assessing responses to an alcohol taste task (ATT) (reported in Blaine et al., 2019), and 4) fMRI session. Prior to fMRI, participants underwent several intake sessions to complete self-report assessments as well as guided imagery script development training. Then, participants were engaged in the ATT task, and the fMRI session occurred approximately two weeks after. All participants were instructed to abstain from alcohol consumption for a minimum of 72 h before the MRI session. To confirm drug and alcohol abstinence, breathalyzer and urine toxicology screenings were administered at each intake and scanning session. On the day of the MRI scan, participants underwent a 1.5-h functional magnetic resonance imaging (fMRI) session. The Human Investigation Committee of the Yale University School of Medicine granted approval for the study protocol and procedures.

Alcohol Measures. Alcohol motivation was measured using an experimental alcohol taste test (Blaine, et al., 2019; Thomas et al., 2011) as well as self-report assessments including Alcohol Use Disorders Identification Test (AUDIT) (Babor, et al., 2001) and the Cahalan Quantity-Frequency Variability (QFV) index (Cahalan, et al., 1969).

Alcohol Use Disorders Identification Test (AUDIT). The AUDIT is a 10-item screening instrument developed based on the World Health Organization (WHO) collaborative data to assess alcohol use, drinking-related behaviors, and hazardous alcohol consumption in the past year (Babor, et al., 2001). For each item, the response options range from 0 (no evidence of symptom) to 4, and a score of 8 or more is regarded as hazardous alcohol use.

Quantity-Frequency Variability (QFV) Index. The QFV index (Cahalan, et al., 1969) is a commonly used to measure real world drinking patterns, including measures of drinking volume, drinking frequency, and quantity-frequency variability of alcohol intake.

Guided Imagery Script Development and Training. For provocation of stress, alcohol-cue, and neutral conditions, a well-validated individualized imagery script procedure was implemented as previously described (Sinha, 2009). The individualized imagery task has been widely applied in numerous laboratory and neuroimaging studies to induce emotion, stress, and craving (Cooney, et al., 1997; Fox et al., 2012; Fox et al., 2007; Hommer et al., 2012; Jastreboff et al., 2011; Li et al., 2005; Potenza et al., 2012; Seo et al., 2011; Sinha et al., 2009; Sinha et al., 2011). Preceding the fMRI scan session, six individualized imagery scripts were developed based on participants' descriptions of two alcohol-related cues, two stressful, and two neutral-relaxing experiences. The Scene Construction Questionnaires (Sinha, 2009) were employed to construct these scripts using standardized methods outlined in previous literature (Sinha, 2009). For the stress scripts, participants were instructed to recall "a situation that made them sad, mad, upset and which in the moment they could do nothing to change it." Examples of highly stressful situations included job loss, conflicts with significant others, and the loss of important relationships. To ensure the efficacy of the stress-inducing stimuli, participants rated the situations on a 10-point Likert scale (1 = not at all stressful and 10 = the most stressful). Only scenarios rated eight or above were used for script development. Alcohol context cue scripts were developed from personal experiences of anticipating and consuming alcohol, excluding scenarios involving negative affect or psychological distress. Participants were prompted to describe a recent situation in which they strongly desired an alcoholic drink and subsequently indulged in response to the query "please tell us about a recent situation when you really wanted an alcoholic drink and then you went ahead and had one." Neutral scripts involved mundane neutral-relaxing experiences, such as relaxing in the backyard or going for a walk in the park. Each script was tailored to personal experiences, but maintained a consistent script style including stimulus and response content specific to experience, content format, and length in a standardized manner across conditions and participants, as previously detailed (Sinha, 2009). In addition, all participants underwent a structured relaxation and imagery-training protocol designed to reduce variability in imagery ability (refer to (Sinha, 2009) for specifics) prior to the scanning session. Each imagery script, lasting 2 min, was randomly ordered and audio-taped for presentation during the scanning session. All research staff and fMRI technicians were kept blind to the content, order, and condition type throughout the scanning process.

Experimental Alcohol Taste Test (ATT) protocol. All participants also engaged in an adapted version of a laboratory-based alcohol taste test to assess alcohol motivation on separate days as described previously (Blaine, et al., 2019). The adapted version of a widely used Alcohol Taste Test (ATT) (Marlatt, et al., 1973; Thomas et al., 2011) was designed to measure implicit behavioral motivation for alcohol. In the ATT, participants were first presented with two 12-Oz beer mugs filled with 375 ml of Bud Light chilled beer (Anheuser–Busch) of 4.2% alcohol, along with a glass of water with ice. Participants were then instructed to taste those beers and determine whether they were different or the same type of beer. The instructions provided were the following: 'There are 2 glasses in front of you, each containing beer. You are to taste each beer and tell us whether you think they are the same or different. You can drink as much as you need to make your decision. If you are correct, you will be paid $10. You have 10 min to decide'. Participants underwent the ATT task following imagery exposure to alcohol, stress, neutral conditions respectively on 3 consecutive days. There was high reliability in the amounts consumed allowing for this determination across conditions (Blaine, et al., 2019). Therefore, the average amount of beer consumed (in ml) across three imagery conditions was measured and interpreted as an index of implicit motivation for consuming alcohol. The average amounts of beer consumed in each group were presented in Table 1. Most participants from the Blaine et al. (2019) study reporting on responses to the ATT also participated in the current fMRI experiment with a 90% overlap in participants between the two studies. However, different imagery scripts were employed for the ATT and fMRI tasks to guarantee that participants encountered separate imagery scripts in each experiment.

fMRI task. In an fMRI block design, participants completed six 5.5-min blocks with two blocks per imagery condition (stress, alcohol-cue, neutral) as described previously (Fox, et al., 2012; Seo et al., 2013; Seo et al., 2014). Each block consisted of a 5.5-min fMRI run, which included a 1.5-min quiet baseline period, followed by a 2.5-min imagery period (comprising 2 min of read-imagery and 0.5 min of quiet-imagery), and concluded with a 1-min quiet recovery period. During the baseline period, participants were instructed to refrain from any mental or imagery activities. During the recovery period, they were instructed to stop imagining and stay still for an additional minute. The presentation order of each imagery condition was counter-balanced and randomized across participants. Each imagery script was presented only once per participant, and scripts from certain condition were never presented consecutively. Behavioral Ratings. Participants provided their anxiety and craving ratings before and after each fMRI trial using a 10-point verbal analog scale (ranging from 1 = "not at all" to 10 = "extremely high"). Anxiety rating pertains to the participants' self-reported feelings of being "tense, anxious, and/or jittery," and craving ratings indicate their "desire to drink alcohol at that moment." To mitigate any potential carryover effects, participants listened to a 2-min progressive muscle relaxation audio in between each trial without engaging in any mental imagery.

fMRI data acquisition. MRI data were acquired using a 3 T Siemens Trio MRI system equipped with a standard quadrature head coil. Anatomical images were obtained using spin echo imaging in the axial plane parallel to the AC-PC line with the following parameters: TR = 300 ms, TE = 2.5 ms, bandwidth = 300 Hz/pixel; flip angle = 60°, FOV = 220 × 220 mm, matrix size = 256 × 256, and 32 slices of 4 mm thickness with no gap. For functional MRI, whole-brain images were acquired using a T_2*-sensitive gradient-recalled single-shot echo planar pulse (EPI) sequence with the following specifications: TR = 2000 ms, TE = 25 ms, bandwidth = 2004 Hz/pixel, flip angle = 85°, FOV = 220 × 220 mm, matrix size = 64 × 64, and 32 axial slices of 4 mm thickness with no gap, aligned parallel to the AC-PC line. In addition, sagittal anatomical images were acquired for multi-subject registration using a high-resolution 3D magnetization-prepared rapid gradient-echo (MP-RAGE) sequence with the following parameters: TR = 2530 ms, TE = 3.34 ms, bandwidth = 180 Hz/pixel, flip angle = 7°, slice thickness = 1 mm, FOV = 256 × 256 mm, and matrix = 256 × 256. ANS activity as indexed by heart rate was measured throughout the fMRI task using a Siemens MRI-compatible pulse oximeter built into the 3 T scanner (Siemens Healthineers, Erlangen, Germany).

2.1. Data analytic approach

Behavioral ratings during fMRI task. To examine the significance of anxiety and stress ratings as a function of drinking group (HD, LD) and imagery script condition (stress, alcohol-cue, neutral-relaxing), general linear models (GLM) were employed using SPSS Statistics software (IBM, SPSS Inc). The main effects of drinking group (LD, HD) and imagery script condition (stress, alcohol-cue, neutral-relaxing), as well as the interaction between these two factors were analyzed to assess the significance of model parameters at the $p < 0.05$ level. Heart rate during fMRI task. The heart rate data were processed via Siemens Physio software (Siemens Healthineers, Erlangen, Germany), which manages the logging and processing of physiological signals during MRI scans. This includes artifact reduction and adaptive filtering to obtain clean physiological signals by filtering out noise from the MR signal. Heart rate data during each trial were processed for a 1.5-min baseline period and a 2-minnutes cue presentation period respectively, and then the data were averaged across two trials for each condition. First, baseline differences were examined using GLM with Group (HD, LD) as a between-subjects factor and Condition (Stress, Alcohol, Neutral) as a within-subjects factor using SPSS Statistics software. After determining baseline group

differences, data analysis for task-related HR responses utilized changes from baseline (imagery – baseline) values to account for the baseline difference. Subsequently, GLM for task-related HR responses was conducted with Group (HD, LD) as a between-subjects factor and Condition (Stress, Alcohol, Neutral) as a within-subjects factor.

fMRI data processing and analyses. fMRI data were pre-processed following the procedures described previously in fMRI studies using the individualized imagery paradigm (Fox, et al., 2012; Seo et al., 2013; Seo et al., 2014). First, raw fMRI data were converted from the Digital Imaging and Communication in Medicine (DICOM) format to the Analyze format using XMedCon (Nolfe, 2003). To allow the time for the MRI signal to reach steady-state equilibrium between radio-frequency pulsing and longitudinal relaxation, the initial 10 images from each fMRI run were discarded. Additionally, given the potential carryover effects from the imagery period, the 1-min recovery period were excluded from data analyses. fMRI images were slice time and motion corrected for 3 translational and 3 rotational directions (Friston, et al., 1996) using Statistical Parametric Mapping 5. fMRI data from runs containing head motion exceeding 1.5 mm or rotations surpassing 2 ° were excluded. To analyze the fMRI signal at the individual level, a general linear model (GLM) was implemented on each voxel in the entire brain volume using the Yale Bioimage Suite (www.bioimagesuite.org/ (Duncan, et al., 2004);). For each run per condition, a general linear model (GLM) was employed for individual-level analysis, utilizing a regressor to compare time during imagery to the baseline (stress–baseline, alcohol-baseline, and neutral–baseline). Drift correction was also applied in the GLM with drift regressors used to remove the mean time course, linear, quadratic, and cubic trends for each run. Each run was spatially smoothed with a FWHM 6 mm Gaussian kernel and then normalized to generate beta-maps in the acquired space (3.44mm × 3.44mm × 4 mm). To accommodate individual anatomical variations, a three-step registration procedure was implemented on the individual normalized β-maps using the Yale Bioimage Suite: (1) linear registration aligning the functional image with the T1 structural image within subjects, (2) linear registration aligning the T1 structural image with the 3D MPRAGE image (1 × 1 × 1 mm), and (3) non-linear registration to a reference 3D image. The Colin 27 Brain (Holmes, et al., 1998), a high-resolution anatomical image registered to the Montreal Neurological Institute space, served as the reference image. To examine group differences in neural responses to stress, alcohol-cue, and neutral-relaxing conditions, a whole-brain voxel-wise LME model was implemented using AFNI (/afni.nimh.nih.gov/ (Cox, 1996);) 3dLMEr program including drinking group (HD, LD) and imagery conditions (stress, alcohol-cue, neutral). To correct for multiple comparisons, all fMRI group-level analyses including correlation and connectivity analyses were whole-brain family-wise error (FWE) corrected at $p < 0.05$

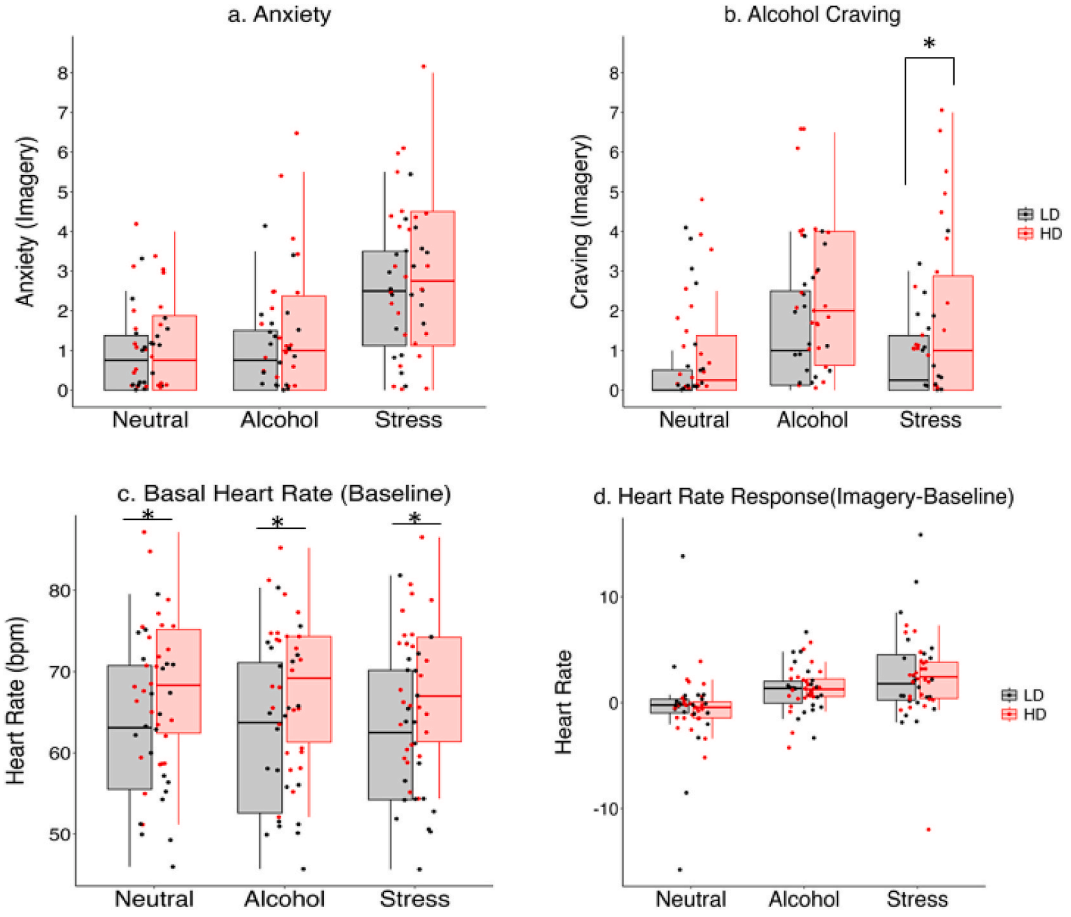

Fig. 1. Behavioral Ratings and Heart Rate

The figure illustrates patterns of ratings and heart rate in HD versus LD individuals and highlights only Group effects with asterisks. **(a) Anxiety Ratings.** During the task, a significant Condition main effect ($p < 0.001$) was found with greater anxiety ratings in the stress ($p < 0.001$) and alcohol-cue ($p < 0.05$) conditions compared to the neutral condition. **(b) Alcohol Craving**. Alcohol craving ratings were significantly higher in the stress ($p = 0.01$) and alcohol cue ($p < 0.001$) conditions compared to the neutral condition. During stress, HD individuals reported greater stress-induced alcohol craving relative to LD individuals ($p < 0.05$). **(c) Basal Heart rate (HR).** Elevated basal HR was found in HD individuals compared to LD individuals (all p's $< .05$). **(d) Heart Rate Response.** After adjusting for basal HR (provocation-baseline), no group differences were found in HR response during the task. A Condition main effect indicated higher heart rates during the stress and alcohol cue conditions than the neutral condition (p's < 0.001). bpm = beats per minute; HD = high-risk drinkers (in blue); LD = low-risk drinkers (in grey). Error bars represent ±1 standard error (SE) of the mean. *Note*: *$p < 0.05$.

and cluster-corrected at $\alpha < 0.05$ (6248 mm^3) using Monte Carlo simulations (Xiong, et al., 1995) with 10,000 iterations using AFNI.

fMRI correlation and connectivity analyses. To determine the neural correlates of ANS response, a whole-brain voxel-based correlation analysis with basal heart rate (HR) was conducted using Bioimage Suite in 48 social drinkers. In addition, to explore the brain connectivity patterns associated with ANS activity, a whole-brain, seed-based functional connectivity analysis was conducted with the medulla oblongata (MOb) as a seed region. The MOb was specially selected as a seed region, as this region has been regarded as a modulator of autonomic activity (Kumagai, et al., 2012; Mischel et al., 2015), and no prior studies investigated the functional connectivity of this region in AUD and risky drinkers. A seed-based connectivity analysis was conducted using Bioimagesuite as previously described (Power, et al., 2011; Seo et al., 2016). A seed region of the MOb was functionally defined from the whole-brain correlation map with basal HR, as we aim to identify specific connectivity patterns related to basal ANS activity in social drinkers. For connectivity analysis, the seed region was transformed into individual subject space using the inverse transforms from the GLM analysis. The time course of the inversely transformed reference region was computed as the average time-course across all pixels for each subject. Then the time-course was correlated with the time-courses of all the voxels in the entire brain during the homogeneous condition with seed region using a whole-brain voxel-wise Pearson correlation. This was then fisher transformed to z-values, averaged across runs, and spatially smoothed with a 6 mm Gaussian filter and a connectivity map was entered into two-sample t-tests to determine group differences.

Associations between brain connectivity and alcohol-related behavior. To examine the associations between the strength of functional connectivity between the VmPFC/ACC, MOb, and measures of alcohol consumption (i.e., beer amount and drinks per week), robust weighted squares regression models with robust M-estimation were estimated in R (R Core Team, 2022) using the MASS package (Venables and Ripley, 2002).

3. Results

Demographic characteristics. Demographic and health characteristics for HD and LD individuals are displayed in Table 1. There were no significant group differences in demographics in age, sex, race, education years, and BMI, except for alcohol use. The HD group reported greater average weekly alcohol consumption and severity indexed by the Cahalan QFV index and AUDIT scores as well as the average amount of beer consumed from the ATT task compared to the LD group (*ps* < 0.001). There were no participants who met the DSM criteria for AUD, including alcohol abuse or mild AUD cases.

Behavioral ratings and heart rate. Condition and Group effects on anxiety and craving ratings were examined using general linear models with Condition (Stress, Alcohol cue, Neutral) as within-subjects factor and Group (HD, LD) as a between-subjects factor (see Fig. 1). For anxiety ratings, significant Condition main effect was found ($F = 39.3$, $p < 0.001$) with no Group or Group X Condition effects. Anxiety ratings were significantly higher in stress ($t = 7.3$, $p < 0.001$) and alcohol-cue ($t = 2.5$, $p < 0.05$) conditions compared to the neutral condition. Anxiety

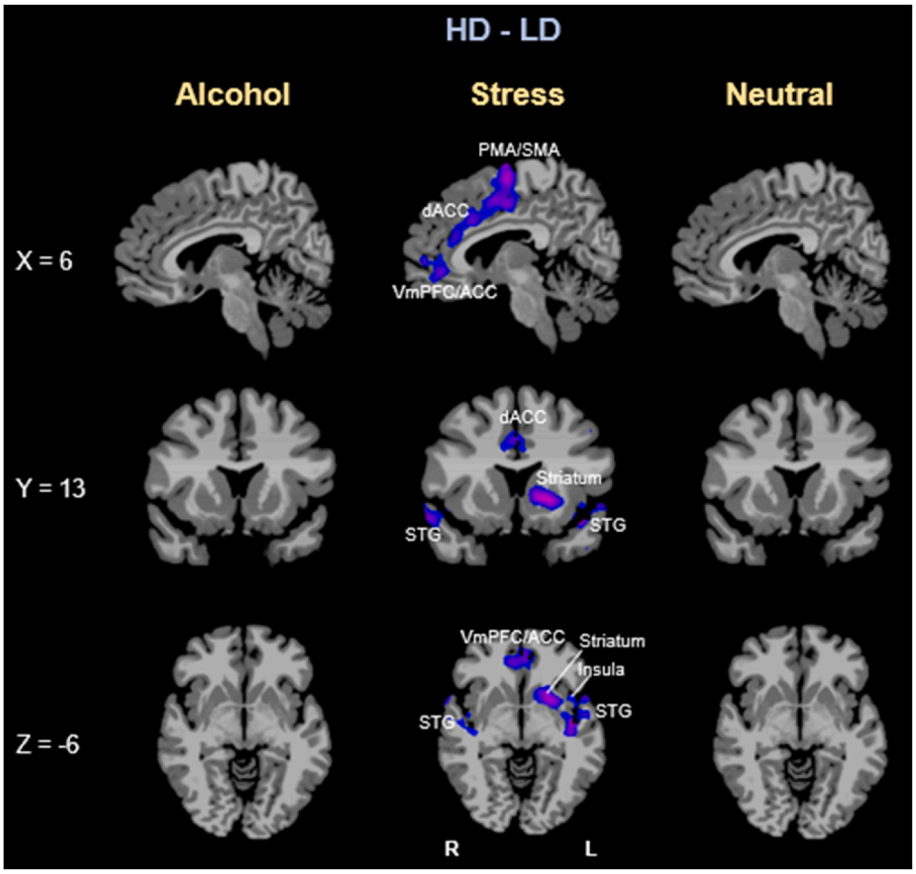

Fig. 2. Whole-brain voxel-wise map showing group differences.
Significant group differences were found in brain activity during stress exposure only, and there were no differences between groups in alcohol cue and neutral conditions. During stress exposure, HD individuals (n = 26) showed decreased activation in cortico-striatal regions, including the ventromedial prefrontal cortex (VmPFC), dorsal and rostral anterior cingulate cortex (dACC, ACC), insula, and striatum, and temporal gyrus compared to LD individuals ($n = 22$) ($p < 0.05$, whole-brain FWE corrected). Blue/Purple color indicate HD < LD. L = left; R = right; STG = superior temporal gyrus, PMA/SMA = primary/supplementary motor area. Coordinates (*x*, *y*, and *z*) represent Montreal Neurological Institute (MNI) anatomical coordinates.

during the stress condition was also higher than the alcohol-cue condition ($t = 6.0$, $p < 0.001$). For alcohol craving ratings, a significant Condition main effect was found ($F = 14.04$, $p < 0.001$). Alcohol craving was significantly higher in the stress ($t = 2.7$, $p = 0.01$) and alcohol ($t = 4.9$, $p < 0.001$) conditions compared to the neutral condition. Additionally, craving ratings in the alcohol-cue condition was higher than the stress condition ($t = 3.2$, $p < 0.01$). There was no Group main effect, but a trending Group × Condition effect ($F = 2.42$, $p = 0.09$), mainly driven by greater stress-induced alcohol craving in HD compared to LD individuals ($t = 2.1$, $p < 0.05$).

For heart rate, there was a significant main effect of Group in baseline heart rate ($F = 5.7$, $p < 0.05$) across conditions with significantly higher basal heart rate found in HD compared to LD individuals ($t = 2.4$, $p < 0.05$). To account for this difference, data analysis for HR responses used change from baseline (imagery – baseline) values. After adjusting for basal HR, there was no Group or Group X Condition effects. There was a Condition main effect ($F = 15.36$, $p < 0.001$), such that heart rate was higher in the stress ($t = 4.4$, $p < 0.001$) and alcohol-cue ($t = 4.0$, $p < 0.001$) conditions compared to the neutral condition. Heart rate during the stress condition was also higher than the alcohol cue condition ($t = 2.4$, $p < 0.05$).

3.1. fMRI results

Group Differences. Whole-brain voxel-wise analysis demonstrated significant group differences in cortico-striatal regions during stress exposure. The HD group exhibited lower neural responses to stress in the prefrontal cortex (PFC) including the ventromedial PFC as well as anterior cingulate cortex (ACC), motor cortex, insula, striatum (caudate, putamen), and temporal gyrus compared to the LD group. There were no group differences found in the neural responses to neutral or alcohol cue conditions (see Fig. 2, $p < 0.05$, whole-brain FWE corrected).

Basal Heart Rate and Stress-induced Neural Activity. In light of the significant effects observed in stress and the central focus of our study on stress and ANS regulation, we pursued further investigations into brain regions associated with ANS activity in the stress condition. A whole-brain correlational analysis correlating basal heart rate with stress-induced brain activity was conducted across the entire brain in 48 social drinkers (whole-brain FWE corrected at $p < 0.05$). The drinking group (HD, LD) was initially included as a predictor in this model. However, no significant group difference in associations with HR was observed that met the threshold for correction for multiple comparisons. Therefore, the correlation results were reported across all 48 participants regardless of group. The results indicated that elevated basal heart rate was significantly associated with altered brain responses during stress including hypoactive responses in the VmPFC/ACC ($r = -0.36$; center MNI coordinate: $X = -3$, $Y = 46$, $Z = -14$), but hyperactive response in the medulla oblongata ($r = 0.33$; MNI: $X = 2$, $Y = -29$, $Z = -44$) (see Fig. 3). Mean r values were obtained from the whole-brain correlation map generated from BioimageSuite. In addition, during the stress condition, VmPFC/ACC and medulla oblongata responses were inversely associated with each other ($\beta = -.43$, $p = 0.001$). No outliers

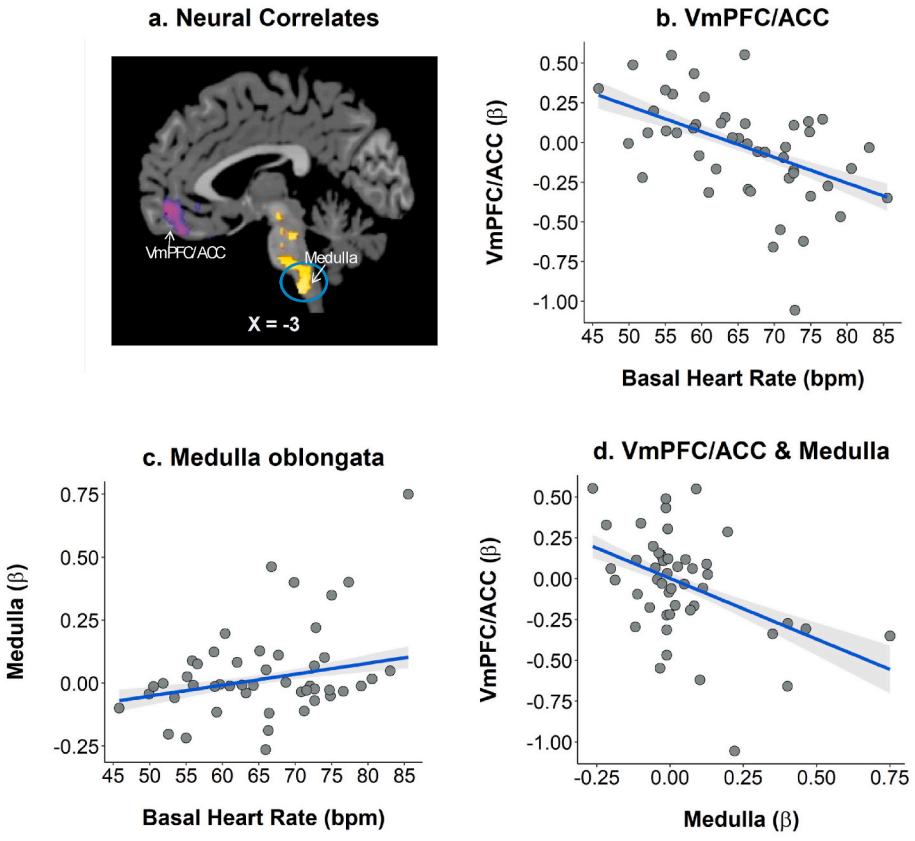

Fig. 3. Neural Correlates of Basal Heart Rate

(a) Whole-brain voxel-based correlation map showing significant associations between brain activity during stress and basal HR in all 48 individuals. During stress exposure, elevated basal HR was significantly associated with decreased activity in the VmPFC/ACC (shown in purple-blue) and increased activity in the medulla oblongata (shown in yellow-red). Corresponding scatterplots displays significant associations between basal heart rate and **(b)** VmPFC/ACC activity ($r = -.36$) as well as **(c)** the medulla oblongata ($r = 0.33$) during stress. Mean r values were obtained from the whole-brain correlation map generated from BioimageSuite ($p < 0.05$, whole-brain corrected). **(d)** An inverse relationship during stress was found between activities in the VmPFC/ACC and medulla oblongata ($\beta = -.43$, $p = .001$). No outliers were found in any of these associations. bpm = beats per minute; VmPFC = Ventromedial prefrontal cortex; ACC = Anterior cingulate cortex; Medulla = Medulla oblongata.

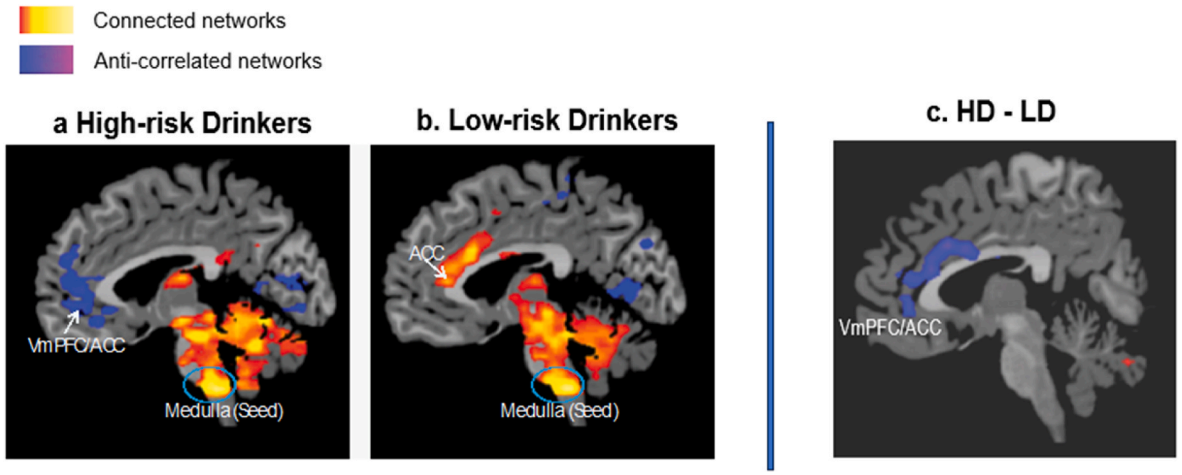

Fig. 4. Functional Connectivity with the Medulla Oblongata Seed
The figure illustrates whole-brain seed-to-voxel functional connectivity map with the medulla oblongata (MOb) as a seed region during stress exposure. The functional connectivity map showed that the MOb response to stress displayed a) decreased connectivity with the VmPFC/ACC in HD individuals, but **b)** increased connectivity with ACC in LD individuals. In addition, **c)** When the HD map is directly compared to the LD map using whole-brain voxel-based *t*-test, greater levels of decreased connectivity between the VmPFC/ACC and MOb during stress was found in HD individuals. Decreased connectivity is shown in blue/purple (anti-correlated network), while increased connectivity is shown in yellow/red (connected network). VmPFC = Ventromedial prefrontal cortex; ACC = Anterior cingulate cortex; Medulla = Medulla oblongata. ($p < 0.05$, whole-brain corrected).

were found in any of these associations as reflected in corresponding scatterplots in Fig. 3.

Functional Connectivity and Alcohol intake. To examine the brain connectivity patterns associated with ANS activity during the stress condition, whole-brain functional connectivity analysis was conducted with the medulla oblongata as a seed region. The seed region of the MOb was selected from the basal-heart rate correlation map during the stress condition (Fig. 3a) to specifically examine connectivity patterns related to basal ANS activity. Thus, in the connectivity map (Fig. 4), comparisons are made with the time course of the seed region, the medulla oblongata (MOb), in the stress condition. The results indicated that individuals with HD exhibited lower functional connectivity between the MOb (a seed region) and the VmPFC/ACC, whereas in LD individuals, functional connectivity between the MOb and the VmpFC/ACC increased during the stress condition (see Fig. 4; $p < 0.05$, whole-brain FWE corrected). When the HD group map (4a) was directly compared with the LD group map (4 b) during the stress condition using a whole-brain voxel-wise *t*-test, only the VmPFC/ACC was significantly more disconnected from the MOb throughout the entire brain regions in HD versus LD contrast (whole brain FWE corrected at $p < 0.05$; Fig. 4c). In addition, the strength of connectivity between the VmPFC/ACC and MOb during the stress condition was associated with future alcohol consumption (Fig. 5). Individuals with more decreased connectivity between the VmPFC and MOb during stress consumed greater amounts of alcoholic beverage during a laboratory alcohol taste test ($\beta = -.52$, $p < 0.001$; Fig. 5b). Further, the connectivity strength during stress was also associated with greater amounts of weekly alcohol consumption measured by Cahalan's QFV index ($\beta = -.43$, $p < 0.01$; see Fig. 5c) as well as AUDIT scores ($\beta = -.53$, $p < 0.001$). No outliers were found in any of these associations.

4. Discussion

The current study investigated the neural correlates and connectivity patterns associated with stress, ANS dysregulation, and alcohol intake in social drinkers exhibiting high-risk (HD) and low-risk (LD) intake behaviors. The findings revealed a link between ANS disruption, marked by elevated basal HR, and stress-induced brain activity in the VmPFC and MOb among social drinkers. Furthermore, diminished functional connectivity between the VmPFC and MOb during stress was associated

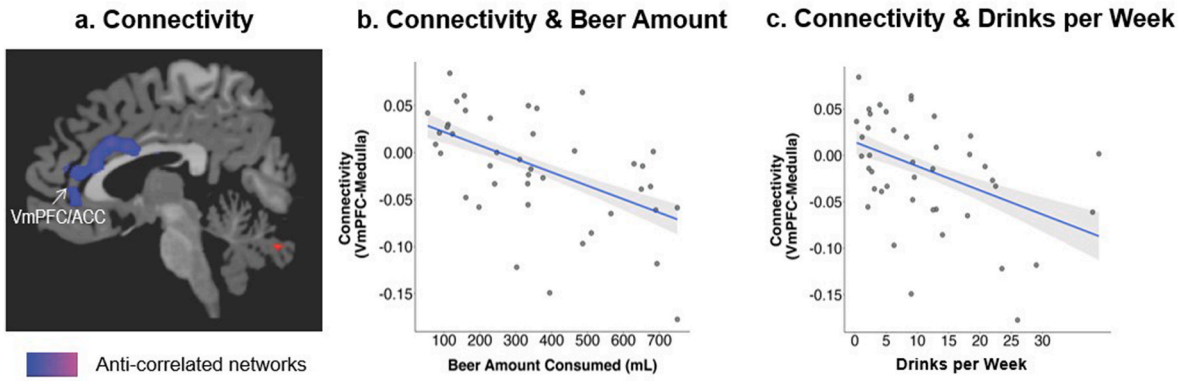

Fig. 5. Functional Connectivity and Alcohol Intake
The relationship between the strength of **(a)** connectivity between the VmPFC/ACC and MOb during stress and alcohol consumption. Enhanced connectivity strength between VmPFC/ACC and MOb during stress was associated with **(b)** greater amount of alcohol intake during a laboratory alcohol taste test ($\beta = -.52$, $p < 0.001$) as well as **(c)** greater real-world weekly alcohol consumption measured by Cahalan index ($\beta = -.43$, $p < 0.01$). No outliers were found in any of these associations. Note: VmPFC = Ventromedial prefrontal cortex; ACC = Anterior cingulate cortex; MOb = Medulla oblongata.

with increased alcohol consumption. These findings suggest that compromised functional connectivity between the VmPFC and the MOb during stress, key brain regions associated with autonomic arousal, may underlie the link between stress and ANS dysregulation, which in turn could enhance the propensity for high-risk alcohol consumption among social drinkers.

4.1. ANS and stress dysregulation in risky drinkers

In accordance with prior investigations with AUD (Hwang, et al., 2022; Seo et al., 2013), our study found ANS disruptions in high-risk drinkers characterized by heightened basal HR in comparison to low-risk drinkers. This aligns with our previous research involving individuals with AUD showing elevated basal HR in AUD patients relative to controls (Seo, et al., 2013) as well as an association between increased basal HR and the severity of alcohol consumption in individuals with AUD (Hwang, et al., 2022). Similar to individuals with a diagnosis of AUD, the consistent patterns of heightened basal state ANS activity observed in our data suggests prodromal patterns of autonomic dysregulation in non-dependent high-risk drinkers. This finding also corresponds with a study indicating a positive association between higher heart rate and alcohol intake (Ryan and Howes, 2002). Collectively, these studies suggest a continuum in the relationship between ANS dysregulation and alcohol misuse, indicating that autonomic disruption may serve as an early indicator for identifying individuals vulnerable to hazardous drinking behaviors.

In addition, HD drinkers showed hypoactive responses to stress in cortico-striatal regions including the VmPFC, ACC, motor cortex, insula, and striatum relative to low-risk drinkers. The VmPFC and ACC plays a crucial role in emotion regulation (Etkin, et al., 2011; Suzuki and Tanaka, 2021). The VmPFC regulates emotion and stress by modulating activities in the subcortical region associated with emotional reactivity, encompassing the striatum and amygdala (Etkin, et al., 2011; Urry et al., 2006). The ACC is engaged in the monitoring of these subcortical areas, thereby contributing to the regulatory function of the VmPFC (Ichikawa, et al., 2011). The striatum is associated with reinforcement learning and habitual responses (Cox and Witten, 2019) and often activated in conjunction with the motor cortex for behavioral actions as well as reward and habitual behaviors (Balleine, et al., 2007; Dias-Ferreira, et al., 2009). These studies suggest that hypoactivation in the VmPFC-striatum circuit during stress in high-risk drinkers indicate their compromised abilities to regulate stress and altered reward-related processing when faced with stress. This is consistent with our observations of elevated stress-related alcohol craving in HD individuals as well as prior research findings showing the associations between psychosocial stress, alcohol craving, and risky alcohol intake among social drinkers (Blaine, et al., 2019; Clay et al., 2018; Wemm et al., 2022), highlighting the important role of stress in risky alcohol use. Also, hazardous levels of alcohol consumption may exacerbate autonomic dysregulation via effects on neural regulatory regions, resulting in greater difficulties with regulating ANS arousal and resisting the urge to drink under stressful conditions.

4.2. Basal ANS dysregulation and the VmPFC-medulla circuit

Subsequent whole-brain correlation results further support the notion of stress-related neural alterations underlying ANS dysregulation. We found that disruptions in basal ANS functioning were associated with stress-related alterations in the VmPFC-MOb circuit. Specifically, during stress, hypoactive VmPFC/ACC, but hyperactive MOb responses were associated with increased basal HR.

The VmPFC and MOb are core regions of central autonomic network that form the VmPFC-MOb feedback circuit governing ANS modulation (Thayer, et al., 2012; Thayer and Lane, 2000). The MOb is a major region of ANS regulation (Mitchell and Victor, 1996), modulating ANS activities including cardiovascular functions (Gordan, et al., 2015; Pyatigorskaya et al., 2016). Impairments in the MOb have been linked to disruptions of cardiovascular autonomic activity (Colombari, et al., 2001). The MOb closely interacts with the VmPFC (Pace et al., 2024; Zagon et al., 1994) in autonomic regulation, as the VmPFC governs the autonomic networks, exerting regulatory control over ANS activity including MOb function (Owens, et al., 1999; Thayer et al., 2012). Accordingly, impairment in the VmPFC has been associated with compromised autonomic regulation of the cardiovascular system (Buchanan et al., 2010), suggesting a close interaction between the VmPFC and ANS system.

Consistent with this, an inverse relationship was observed between the VmPFC and MOb in our study, such that decreased VmPFC activity was associated with increased MOb activity during stress exposure. This suggests that VmPFC hypoactivity may contribute to difficulties in controlling ANS arousal especially under emotionally challenging situations (e.g., stress), which could potentially increase vulnerability to risky alcohol use. It is also likely that altered functional connectivity within this circuit could be a potential mechanism underlying dysfunctional interactions, impacting the regulation of stress-related adaptive behaviors.

4.3. Stress, altered functional connectivity, and alcohol misuse

In support of this, we examined functional connectivity with the MOb as a seed region during stress and showed that the HD group exhibited reduced connectivity between the VmPFC/ACC and MOb, whereas the LD group displayed increased connectivity between the ACC and MOb. Furthermore, the VmPFC-MOb connectivity during stress was found to be associated with increased alcohol intake and motivation. Individuals with more decreased connectivity between the VmPFC and MOb during stress consumed greater amount of alcohol beverage during a laboratory alcohol taste test, reported higher weekly alcohol consumption, and exhibited greater alcohol-related problems. These findings highlight the significance of the integrity of the VmPFC/ACC connectivity with the MOb in regulating ANS function and alcohol misuse in the context of stress.

The VmPFC plays a crucial role in regulating stress-related adaptive behaviors via its control over autonomic functions (McKlveen, et al., 2015; Thayer et al., 2012). Altered VmPFC function during stress has been associated with ANS dysregulation, impaired coping ability, and increased alcohol consumption in healthy individuals (Sinha, et al., 2016). The critical role of the VmPFC has also been indicated in AUD. In studies with AUD, VmPFC dysfunction has been associated with high alcohol craving (Myrick, et al., 2004), alcohol consumption (Grusser, et al., 2004), and a shorter time to relapse (Rando, et al., 2011), and stress-related relapse vulnerability (Seo, et al., 2013), emphasizing the critical role of the VmPFC function in regulating stress- and alcohol-related adaptive behaviors. In addition, altered functional connectivity in the VmPFC/ACC area has been observed in individuals with AUD. Specifically, reduced intrinsic connectivity in the cingulate cortex has been linked to a shorter time to relapse in AUD patients (Zakiniaeiz, et al., 2017). Further, compromised connectivity in the VmPFC and ACC regions during stress was associated with stress-related impulse control difficulties in AUD patients (Seo, et al., 2016).

Using a sample of non-dependent social drinkers, our study contributes to the existing literature by showing that the VMPFC-MOb connectivity during stress plays a crucial role in ANS modulation and the development of hazardous drinking. A dysfunctional interaction between the two regions may result in compromised VmPFC control over MOb leading to heightened ANS arousal under stress, thereby increasing vulnerability to excessive alcohol use. This suggests that the weakened connectivity in the VmPFC-ANS circuit during stress may serve as a potential neural marker indicative of impaired control over ANS regulation and risky alcohol use. Despite the significance of these findings, several limitations should be considered in interpreting the results. Firstly, the cross-sectional nature of the study limits the ability to

fully understand the neural mechanisms underlying stress, ANS dysregulation, and alcohol misuse. Longitudinal studies will help provide a more comprehensive understanding of these relationships. For example, sophisticated real-world measures such as ecological momentary assessment could improve the prediction of alcohol use data. Secondly, this study primarily focused on basal HR due to the significance of basal ANS disruptions in AUD pathology reported in previous studies (e.g. (Hwang, et al., 2022),). While this provides insights into ANS dysregulation in risky drinking, exploring various ANS measures could help understand the nature of ANS functions associated with risky alcohol use, such as heart rate variability, respiration, and blood pressure. In addition, the sample size was modest with a very low number of women included. Therefore, future research is needed to explore sex differences and validate the current findings in larger samples of women.

5. Conclusion

In conclusion, the current study provides important insights into the neurobiological mechanisms of stress and ANS dysregulation associated with alcohol misuse. Findings revealed that a dysfunctional interaction between the VmPFC and ANS regions contribute to stress-related ANS disruption, potentially leading to hazardous alcohol consumption. This study also emphasizes the continuity of autonomic dysregulation in non-dependent high-risk drinkers. Similar to AUD patients, elevated basal heart rate and compromised VmPFC function during stress were found in high-risk social drinkers, which may indicate prodromal patterns of AUD pathology. In addition, a critical aspect of the study focused on the stress-related alterations in the VmPFC-medulla feedback circuit, highlighting impaired regulatory control exerted by the VmPFC over the MOb that may lead to compromised autonomic regulation and maladaptive coping behaviors. The examination of functional connectivity further revealed decreased connectivity between the VmPFC/ACC and MOb in risky drinkers during stress, highlighting the integral role of the VmPFC-MOb circuit in regulating autonomic regulation and alcohol consumption. Taken together, this study highlights the importance of stress, ANS regulation, and the integrity of the VmPFC-MOb circuit and provides insights into the neurobiological mechanisms that may contribute to alcohol misuse. Future studies should investigate this association in a larger sample using more sophisticated methods that integrate neuroimaging, ANS, and longitudinal clinical outcome approaches to further elucidate this link. The utilization of brain-ANS markers will advance early identification and the development of effective preventive intervention strategies for individuals vulnerable to risky drinking behaviors.

CRediT authorship contribution statement

Dongju Seo: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jorge S. Martins:** Writing – review & editing, Methodology, Formal analysis. **Rajita Sinha:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

All authors declare that they do not have any financial and personal relationships with other people or organizations that could inappropriately influence this work. All authors made a substantial contribution to the analysis and/or interpretation of data and contributed to the writing and intellectual content of the article.

Data availability

Data will be made available on request.

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