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ORIGINAL ARTICLE

ALCOHOLISM CLINICAL & EXPERIMENTAL RESEARCH

Effects of moderate alcohol levels on default mode network connectivity in heavy drinkers

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Abstract

Background: It is well established that even moderate levels of alcohol affect cognitive functions such as memory, self-related information processing, and response inhibition. Nevertheless, the neural mechanisms underlying these alcohol-induced changes are still unclear, especially on the network level. The default mode network (DMN) plays an important role in memory and self-initiated mental activities; hence, studying functional interactions of the DMN may provide new insights into the neural mechanisms underlying alcohol-related changes.

Methods: We investigated resting-state functional connectivity (rsFC) of the DMN in a cohort of 37 heavy drinkers at a breath alcohol concentration of 0.8 g/kg. Alcohol and saline were infused in a single-blind crossover design.

Results: Intranetwork connectivity analyses revealed that participants showed significantly decreased rsFC of the right hippocampus and right middle temporal gyrus during acute alcohol exposure. Moreover, follow-up analyses revealed that these rsFC decreases were more pronounced in participants who reported stronger craving for alcohol. Exploratory internetwork connectivity analyses of the DMN with other resting-state networks showed no significant alcohol-induced changes, but suffered from low statistical power.

Conclusions: Our results indicate that acute alcohol exposure affects rsFC within the DMN. Functionally, this finding may be associated with impairments in memory encoding and self-referential processes commonly observed during alcohol intoxication. Future resting-state functional magnetic resonance imaging studies might therefore also investigate memory function and test whether DMN-related connectivity changes are associated with alcohol-induced impairments or craving.

KEYWORDS
acute alcohol, functional magnetic resonance imaging, resting-state functional connectivity, resting-state networks

Fang and Deza-Araujo contributed equally to this work.

INTRODUCTION

Alcohol (ethanol) directly acts on several brain systems (Koob et al., 1998), and the resulting feelings of relaxation, disinhibition, and euphoria may explain why alcohol is one of the most consumed addictive substances worldwide (Peacock et al., 2018). Heavy alcohol consumption and alcohol use disorder (AUD) impose significant costs for society and devastating consequences for the physical and mental well-being of individuals (Peacock et al., 2018).

Several studies revealed that even moderate levels of alcohol intake impair inhibitory control, memory, self-referential processing and can produce loss of control over alcohol use itself (Bjork & Gilman, 2014; Field et al., 2010; Lyvers & Tobias-Webb, 2010). At a neural level, effects on inhibitory control can be explained by the disruption of conflict-monitoring and top-down control networks (Gan et al., 2014; Marinkovic et al., 2012). In addition, impaired frontal top-down control over subcortical structures such as the ventral striatum and the amygdala may explain why alcohol increases aggressive behavior (Gan et al., 2015; Heinz et al., 2011). Prior research and animal models suggest that acute alcohol exposure can disrupt the formation of new memories, which in turn might lead to amnesic episodes, that is, blackouts (Rose & Grant, 2010). Advancing our understanding of the brain mechanisms by which acute alcohol consumption impairs behavior and cognition could have a major impact on the development of new interventions and public health policies.

One effective way to study alcohol effects on connectivity between brain regions is to analyze resting-state functional magnetic resonance imaging (rs-fMRI) data (Biswal et al., 1995). This technique can measure the interregional synchrony, or functional connectivity, of brain areas in the absence of an overt task (Greicius, 2008). Synchronous brain regions are often referred to as networks. The best studied network is the default mode network (DMN) (Raichle et al., 2001). The DMN mainly includes the ventral medial prefrontal cortex, the precuneus, the inferior parietal lobule, the medial temporal cortex, and parts of the lateral temporal cortex (Andrews-Hanna et al., 2010; Raichle et al., 2001). It has been suggested that the DMN plays an important role in internally focused tasks including autobiographical episodic memory, mnemonic scene construction, self-related prospective thoughts, and other forms of internal mentation (Andrews-Hanna et al., 2010; Buckner et al., 2008). Therefore, this network should be particularly relevant for investigating the effects of acute alcohol consumption on self-referential, self-awareness, and memory-related processes (Hull, 1981).

Functional connectivity studies have disentangled the role of different brain networks in the behavioral manifestations of acute alcohol intake. For instance, the sensations of calm and relaxation produced by moderate levels of alcohol might be the result of a disruption of connectivity between the anterior insula and the dorsolateral anterior cingulate cortex, which might impair the detection and evaluation of emotionally salient stimuli (Gorka et al., 2018). Similarly, alcohol intake affects the connectivity of the visual network and the thalamus, increasing the metabolic demands of these 2 regions, which might explain the impairment of cognitive performance, visual processing, and motor functions observed after acute alcohol exposure (Esposito et al., 2010; Khalili-Mahani et al., 2012; Shokri-Kojori et al., 2017; Spagnolli et al., 2013).

Regarding the DMN, previous studies suggest that acute alcohol exposure alters its function. For instance, a ROI-based connectivity analysis based on 11 healthy participants elucidated that acute alcohol consumption (0.59 g/kg dose of ethanol) increased the resting-state functional connectivity (rsFC) of the hippocampal formation after 60 minutes and decreased the rsFC of the precuneus after 90 minutes, suggesting a sensitivity of the DMN-related rsFC to acute alcohol concentrations (Weber et al., 2014). This notion is supported by Zheng and colleagues (Zheng et al., 2015), revealing that half an hour after acute alcohol administration (a dose of 0.65 g/kg), precuneus-related rsFC was altered compared with baseline (N = 32). However, both studies used a within-subject design with a sober state (instead of placebo) as a control condition, which may bias the responses of participants by increasing the expectancy effects of the intervention (Cyders et al., 2020). At the network level, alcohol intake (0.56 g/kg) induced greater anticorrelation between the anterior DMN and the dorsal attention network in 15 light/moderate drinkers (Lei et al., 2014). On the other hand, 2 studies (Esposito et al., 2010; Khalili-Mahani et al., 2012) with small samples (8 participants having a 0.70 g/kg alcohol dose and 12 participants having a 0.60 g/kg breath alcohol concentration [BrAC]) did not observe DMN connectivity changes after alcohol intake, possibly due to low statistical power. Hence, additional placebo-controlled experiments in larger samples are necessary to establish a clearer picture of alcohol-induced changes in DMN connectivity.

In our study, we therefore investigated the acute effect of alcohol on the intra- and internetwork connectivity of the DMN in heavy drinkers by using a single-blind crossover design with an alcohol and control (saline) condition to maximize statistical power. We employed a well-established alcohol infusion procedure (O'Connor et al., 1998; Plawecki et al., 2007) to obtain similar and constant BrACs of 0.8 g/kg in all individuals. Data for this work were collected during an interventional study with naltrexone (results will be reported elsewhere). Due to the involvement of the DMN in self-related mentation and mnemonic processes, we hypothesized that, compared to saline infusion, alcohol would critically affect the functional architecture of the DMN. Moreover, we investigated whether acute alcohol exposure changed the connectivity between the DMN and other brain networks, which would indicate either compensatory networking or disrupted communication between the DMN and other brain systems.

MATERIALS AND METHODS

Participants

Heavy drinkers were recruited by public advertisement and checked for eligibility during a telephone interview (N = 819) followed by an onsite screening (N = 196). Inclusion criteria were as

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follows: (1) men and women aged between 25 and 55, (2) within the past 45 days as assessed with the Timeline Follow-Back interview (TLFB, Sobell & Sobell, 1992): at least weekly alcohol consumption at medium-risk level (according to "Guideline on the development of medicinal products for the treatment of alcohol dependence" (European Medicines Agency, 2010) and "International guide for monitoring alcohol consumption and related harm" (World Health Organization, 2000)) of on average >40 g/day (men) or >30 g/day (women), ≥6 days with alcohol consumption >100 g/day (men) or >75 g/day (women) and ≥4 nonconsecutive alcohol abstinence days, (3) ≥1 drinking day in each full week between screening and visit 1 and ≤6 alcohol abstinence days in the week before visit 1, and (4) nontreatment-seeking for alcohol consumption. Exclusion criteria were as follows: (1) current or past substance dependence except nicotine according to the Diagnostic and Statistical Manual of Mental Disorders IV, (2) current or past alcohol withdrawal symptoms, (3) history of epileptic seizure or delirium, (4) clinically relevant pancreatitis and liver disease (5) current or past treatment related to alcohol consumption (including counseling and support groups), (6) current mental disorder requiring treatment, (7) history of suicide attempt, (8) current intake of psychotropics, opioid analgesics, or illicit drugs (urine test on visits 1–4; Drug-Screen Multi 10TD Test, nal von minden, Moers, Germany), (9) body weight >130 kg, (10) pregnancy or breastfeeding, (11) contraindications to naltrexone or MRI, (12) participation in clinical trial in the past 4 weeks, and (13) history of hypersensitivity to alcohol or any of the used medicinal products, of their ingredients or medicinal products with similar chemical structures. Forty-six heavy drinkers were randomized, but 9 were excluded from the analyses because MRI data could not be acquired or were incomplete. All analyzed participants were right-handed as assessed with the Edinburgh inventory (Oldfield, 1971) and reported normal or corrected-to-normal vision (participant characteristics are detailed in Table 1). All participants provided written informed consent, were fully debriefed after the experiment, and received payment for participation as approved by the institutional review board of the Technische Universität Dresden. This study was in accordance with the Declaration of Helsinki.

TABLE 1 Demographic information and alcohol consumption of participants (*N* = 37)

Demographics	Mean (SD) or *n* (%)
Age	29.2 (4.7)
Sex (females)	3 (8.1%)
Smokers	24 (64.8%)
School education	
General secondary school (8–10 years)	11 (29.7%)
High school	26 (70.3%)
Professional education	
Currently unemployed/not in training	2 (5.4%)
Apprentice (vocational school)	3 (8.1%)
Student (university/college)	9 (24.3%)
Vocational training completed	13 (35.1%)
Academic degree (university/college)	10 (27.0%)
Drinking history	**Mean (SD) or n (%)**
AUDIT	14.2 (4.1)
Risky consumption (5–14)[a]	21 (56.8%)
Harmful consumption (15–19)[a]	12 (32.4%)
Severe consumption (>20)[a]	4 (10.8%)
TLFB (45 days)	
Drinking days	32.0 (7.5)
Alcohol drinking (g/drinking day)	115.8 (41.9)
Binge days	21.1 (7.8)
Alcohol binge (g/binge day)	152.7 (53.8)
OCDS (total score at visit 4)	9.6 (4.0)
Obsessive	2.1 (2.4)
Compulsive	7.4 (2.5)

AUDIT, Alcohol Use Disorders Identification Test assessed at screening visit.

[a]Number of participants in respective AUDIT category. TLFB: Timeline Follow-Back assessed at screening visit (alcohol consumption during the last 45 days before the study). OCDS: Obsessive–Compulsive Drinking Scale assessed at visit 4.

Experimental procedure

This work is part of the clinical trial "Validation of a test system for development of medications for alcoholism" (EudraCT number: 2015–002831–16, ClinicalTrials.gov identifier: NCT02652585), which aimed to demonstrate that naltrexone would reduce the motivation to work for alcohol in a laboratory experiment, using a mixed design with naltrexone (*n* = 16) and placebo (*n* = 21) as between-subjects variables (double-blind) and alcohol and saline infusions as within-subjects variables (single-blind, counterbalanced). The investigation of alcohol effects on brain function at rest was a secondary objective. Naltrexone (Adepend, Desitin, Hamburg, Germany) and placebo were delivered in indistinguishable opaque, white capsules and taken every morning for 28 days. The whole clinical trial comprised visit 1 (day 0), visit 2 (between days 7 and 10), visit 3 (between days 10 and 26), visit 4 (between days 12 and 28), and visit 5 (between days 31 and 34). Participants who were randomized to naltrexone received 25 mg/day from day 1 (i.e., the day following visit 1) to day 3. From day 4 (i.e., before visit 2) until day 28 (i.e., visit 4 or after), participants received a daily dose of 50 mg naltrexone. It can be assumed that the naltrexone blood level reached a steady state from visit 2 to visit 4 in each individual. Visit 5 took place 3 days after the end of drug intake.

Drinking behavior was assessed with the Alcohol Use Disorders Identification Test (AUDIT, Saunders et al., 1993) when screening for eligibility. The TLFB was used for detailed assessment of the quantity and frequency of drinking when screening. Craving was assessed with the Obsessive Compulsive Drinking Scale (OCDS, Anton et al., 1996) at visit 1 and visit 4.

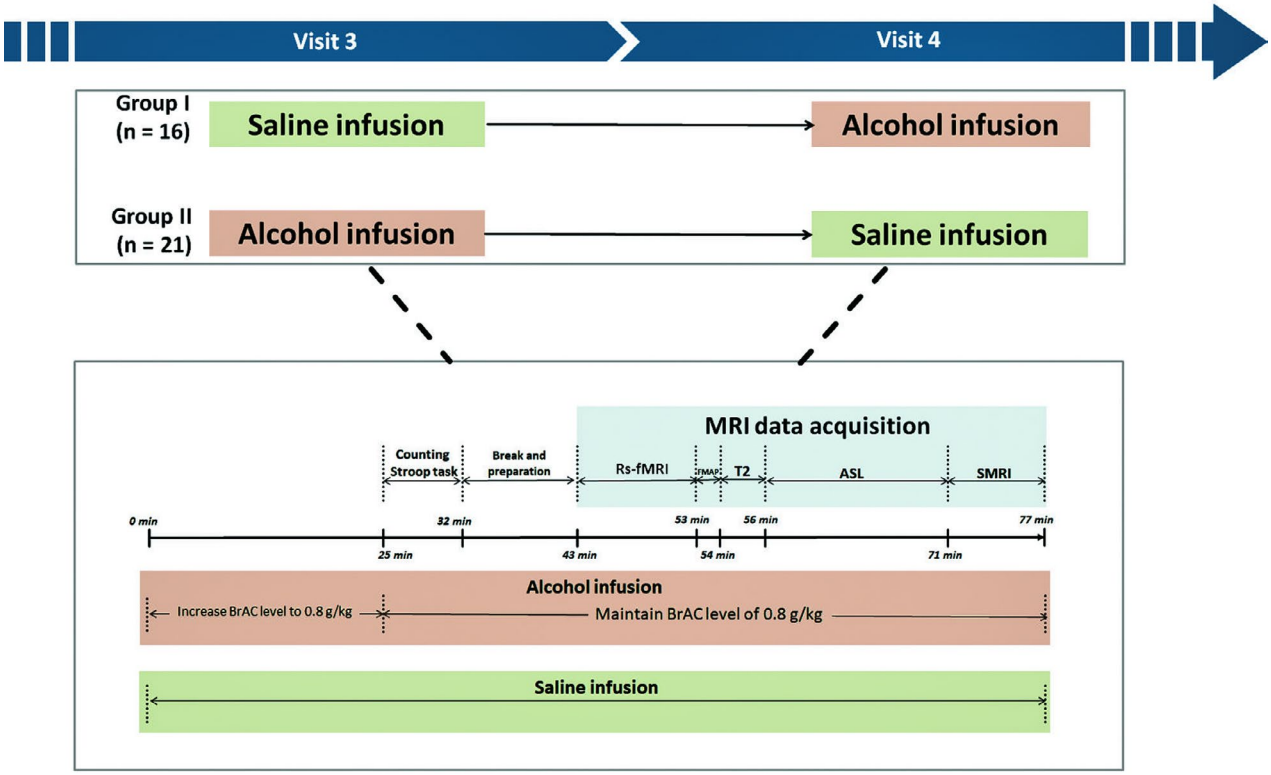

FIGURE 1 Schematic presentation of data collection. Participants received alcohol and saline infusions in a randomized single-blind crossover design. ASL: arterial spin labeling; BrAC: breath alcohol concentration; FMAP: field map; Rs-fMRI: resting-state functional magnetic resonance imaging; SMRI: structural magnetic resonance imaging; T2: T2-weighted image

MRI data (details regarding MRI sessions are provided in Figure 1) were acquired at visits 3 and 4. The interval between the 2 MRI scans was 2–12 days. At the first MRI scan, participants were for 10–20 days on naltrexone (mean = 14.3 days). At the second MRI scan, participants had received medication for 13–28 days (mean = 20.8 days). To minimize alcohol expectancy effects (Cyders et al., 2020), participants were told that alcohol would be administrated on both days (in a random order that they would not know), but in different amounts up to a BrAC of 0.8 g/kg. Participants were sober when arriving for MRI visits (BrAC of 0.0 g/kg as measured with Alcotest 6810 breathalyzer [Dräger, Lübeck, Germany]) and randomly received an intravenous infusion of alcohol (6% v/v, mixture of normal saline with 95% ethanol [Braun, Melsungen, Germany]) or normal saline in a single-blind crossover design, using a computer-assisted infusion system (O'Connor et al., 1998) as described previously (Jünger et al., 2017). Briefly, the infusion rate was adjusted based on age, sex, weight, height, and repeated BrAC measurements, to reach a BrAC of 0.8 g/kg within 25 minutes, before performing MRI, and to maintain this alcohol level until the end of the scan.

Visual Analog Scales (VASs) ranging from 0 ("no, not at all") to 10 ("yes, extremely strong") were administrated at the beginning of the session (T1), during the session (T2, after the BrAC reached 0.8 g/kg), and at the end of the session (T3), to obtain subjective ratings of stimulation ("I feel exhilarated at the moment"), sedation ("I feel subdued at the moment"), negative feelings ("I feel unwell at the moment"), craving ("I now feel like drinking more alcohol"), well-being ("I feel good right now"), subjective drinking amount ("I feel as if I just had… drinks (0–30)"), feeling drunk ("At the moment I feel drunk"), and thirst ("I am thirsty right now"). To investigate the effects of alcohol on these ratings across the experimental session, ratings were entered as a dependent variable in a repeated-measures ANOVA with time points (T1, T2, and T3) and intervention (alcohol vs. saline) as within-subject factors. Effects of alcohol were tested via the time point-by-intervention interaction and via post hoc paired t-tests at time points. Significance was assumed at $p < 0.05$ without correction for multiple comparisons.

MRI data acquisition

Imaging data were acquired by using single-shot echo-planar imaging (EPI) on a 3 T Magnetom Trio Tim scanner (Siemens, Erlangen, Germany) equipped with a 12-channel head coil. Following brief localizer scans (~1 min), rs-fMRI data were acquired with an axial 2D EPI sequence with voxel size = 3.0 mm × 3.0 mm × 2.0 mm, slice gap = 1.0 mm, repetition time (TR) = 2410 ms, echo time (TE) = 25 ms, field of view (FOV) = 192 mm ×192 mm, flip angle = 81°, matrix = 64 × 64, bandwidth (BW) = 2112 Hz/Px, 42 slices, 243 volume. Subsequently, a B0 field map was acquired with voxel size = 3.0 mm × 3.0 mm × 2.5 mm, gap = 0.5 mm, TR = 482 ms, TE 1 = 5.19 ms, TE 2 = 7.65 ms, FOV = 192 mm × 192 mm, flip angle = 46°, matrix = 64 × 64, BW = 260 Hz/Px, 44 slices. Each subject underwent 2 rs-fMRI scans lasting approximately 10 minutes each, 1 at visit 3

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and the other at visit 4. Structural T1-weighted data were acquired at visit 3 using a sagittal 3D, magnetization-prepared rapid gradient echo (MPRAGE) sequence with voxel size = 1.0 mm × 1.0 mm × 1.0 mm, TR = 1900 ms, TE = 2.26 ms, inversion time = 900 ms, FOV = 256 mm × 224 mm, flip angle = 9°, matrix = 256 × 256, thickness = 1.0 mm, slices = 176, BW = 200 Hz/Px. Foam padding, headphones, and earplugs were used to reduce head movement and protect hearing. Participants were instructed to relax, to look at a fixation cross, to think about nothing in particular, and to not fall asleep.

Image preprocessing

The rs-fMRI data were preprocessed by using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL version 5.0.9, www.fmrib.ox.ac.uk/fsl) (Jenkinson et al., 2012) and the Data Processing Assistant for Resting-State fMRI (version 4.3, rfmri.org/DPARSF). The latter is based on Statistical Parametric Mapping (SPM12, www.fil.ion.ucl.ac.uk/spm) and the toolbox for Data Processing and Analysis of Brain Imaging (DPABI version 2.3, rfmri.org/DPABI) (Yan et al., 2016).

Preprocessing steps included motion correction with FSL-MCFLIRT, distortion correction based on the field map images, brain extraction of the EPI data with Brain Extraction Tool (BET version 2.1, fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET), spatial smoothing with a Gaussian kernel of full width at half maximum of 6 mm, and global 4D mean intensity normalization. Independent component analysis (ICA)-based nonaggressive denoising was then applied on the individual resting-state data with the Automatic Removal of Motion Artifacts (ICA-AROMA version 0.3; Pruim et al., 2015). Next, nuisance regression was conducted to remove signals associated with white matter and cerebrospinal fluid. The T1-weighted image was coregistered with the denoised EPI images, and T1-based normalization to MNI space was done based on a unified segmentation approach (Ashburner & Friston, 2005) using SPM12. Functional images were resampled to a resolution of 2 mm ×2 mm ×2 mm. Finally, high-pass temporal filtering (>0.01 Hz) using DPABI was applied to remove low-frequency noise.

For each individual, frame-wise displacement (FD) (Power et al., 2011) was calculated. Scrubbing was not performed given that ICA-AROMA was applied (see above). The mean FD for each participant was <0.5 mm. No participant was excluded from further analyses. There was no significant difference in mean FD between the alcohol and saline infusion conditions (mean FD saline condition = 0.15, mean FD alcohol condition = 0.16, p = 0.37, paired t-test, 2-tailed), suggesting that head motion did not confound our analyses.

Intranetwork connectivity of the default mode network

We obtained participant-specific spatial maps of the DMN by employing a dual regression analysis (Beckmann et al., 2009), which involved 3 steps. First, we used Incremental Group-principal Component Analysis (MIGP) as implemented in Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC version 3.14, fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC) (Beckmann et al., 2005) to perform probabilistic ICA. This procedure generated a set of spatially independent components at the group level (i.e., group ICA; d = 20). Then, 10 major brain networks were identified among these 20 independent components by spatially matching the maps with existing templates (Figure 2) (Beckmann et al., 2009). After this identification, only the map representing the DMN was used in the following steps. Second, for each participant, the group-level DMN map was used as an independent variable and the preprocessed rs-fMRI data as dependent variables in a regression analysis to obtain individual time series related to his/her DMN signal (i.e., spatial regression). Third, to obtain participant-specific spatial maps, we variance-normalized the time series and used them as a set of temporal regressors in a second regression analysis, whose dependent variables were also the preprocessed rs-fMRI data (i.e., temporal regression). Finally, voxel-wise paired t-tests (two-tailed) of the resulting spatial maps were used to reveal the differences in the intraconnectivity of the DMN between the 2 infusion conditions averaging over the drug effect. Following, we did a Gaussian random field correction (Friston et al., 1994; Open Science, 2015) as implemented in the DPABI toolbox: First a threshold of p_{uncorr} = 0.001 was applied at the voxel-level; then, a cluster-level threshold of p_{corr} < 0.05 was used in a whole-brain mask with a resolution of 2 × 2 × 2 mm^3. This correction controls a false discovery rate (FDR) at a 0.05 level (Kessler et al., 2017).

Internetwork connectivity of the default mode network

We explored alcohol-induced changes in the coupling between the DMN and the other 9 resting-state networks (Beckmann et al., 2009) by using FSLNets (version 0.6.3, fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets). Based on the variance-normalized, subject-, and session-specific time courses of the 10 networks, we calculated Pearson's correlations between the DMN time series and the other 9 networks for each participant and infusion condition. After converting correlation scores into z scores with Fisher's transformation, differences between the 2 infusion conditions were tested by using nonparametric tests as implemented in FSL Randomise (version 2.9, fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise), with 10,000 permutations. An FDR-corrected p < 0.05 (for 9 tests, 2-tailed) was considered significant.

Associations of connectivity changes with alcohol use and craving

In a follow-up analysis, we examined correlations between the alcohol-induced intranetwork rsFC changes in the DMN and measures of risky drinking (AUDIT), alcohol craving (OCDS, assessed at the beginning of visit 4 = second MRI scan), and alcohol-related feelings (rated with VAS at both MRI scans). First, we extracted individual mean rsFC from a

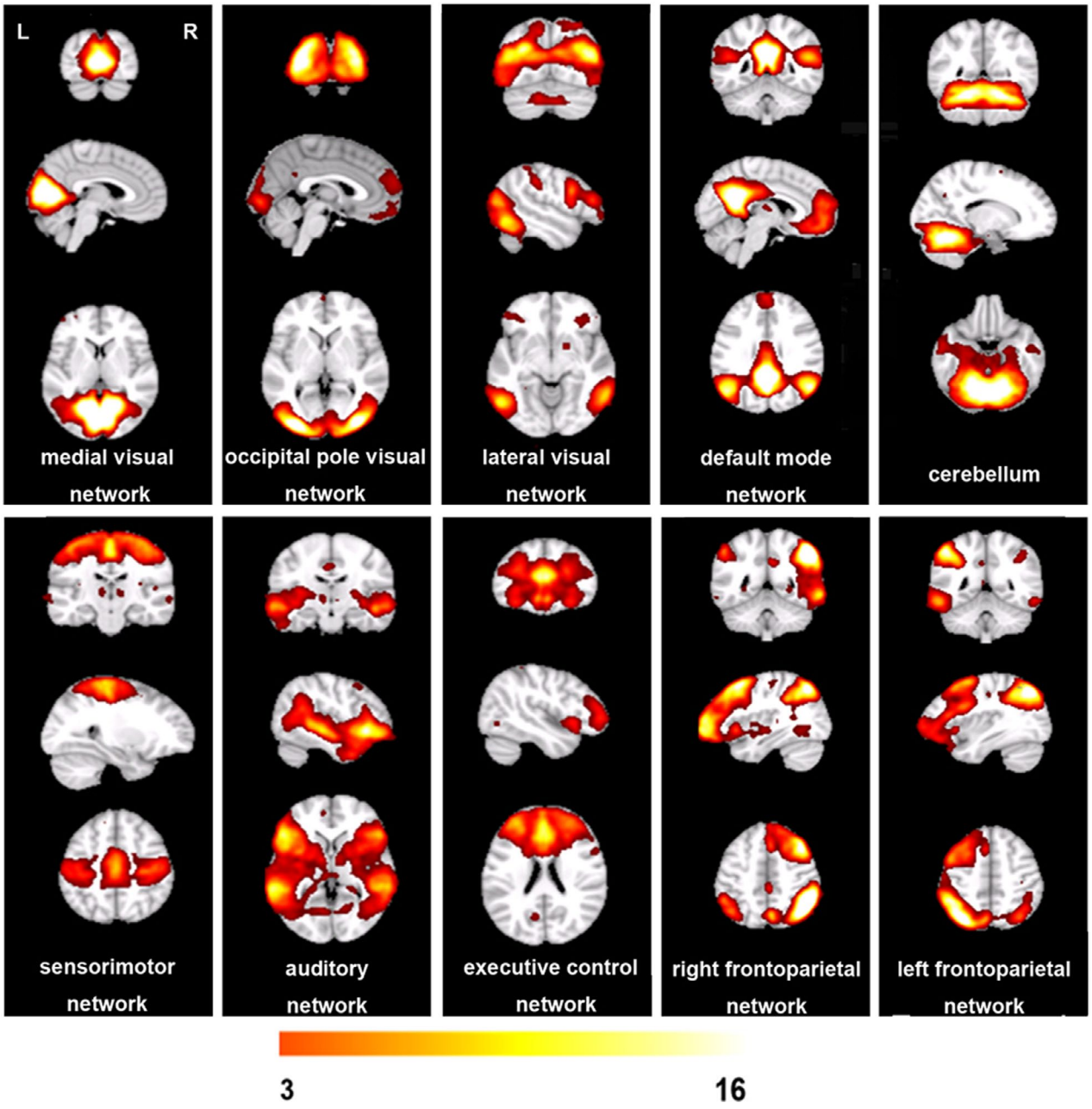

FIGURE 2 Ten resting-state networks identified with group ICA across all participants. All the spatial maps shown were converted to *z* statistic images via a normalized mixture–model fit and then thresholded at $z = 3$. The results are overlaid on the average MNI152 brain. The color bar indicates *z*-values

6-mm-radius sphere centered on the coordinates of peak activity in each significant cluster (Table 3) from each of the 2 conditions. For correlation analyses of the rsFC and AUDIT and OCDS, we obtained the differences (normalized) in the rsFC of each significant cluster between the 2 conditions (i.e., the rsFC strength under saline subtracted from the strength under alcohol for each subject) and performed Pearson's correlations between these differences and the AUDIT and OCDS scores. To investigate associations of alcohol-induced rsFC changes (normalized) and changes in VAS scores (the sum of T2 and T3 scores under saline subtracted from the sum under alcohol for each subject, i.e., T2(alcohol)+T3(alcohol)-T2(saline)-T3(saline)), we performed Pearson's correlations between the differences in each measure. Because these follow-up analyses were not intended to be confirmatory, but rather to provide avenues for future research, we did not correct for multiple comparisons (Streiner & Norman, 2011).

RESULTS

Subjective ratings of alcohol-related feelings

Ratings revealed that alcohol significantly affected (time point-by-intervention interactions) craving, $F(1.68, 60.54) = 7.11$; $p = 0.003$, $\eta^2_P = 0.16$, subjective drinking amount, $F(1.26, 46.44) = 47.55$;

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TABLE 2 Alcohol-related feelings (N = 37)

	Condition	Score Mean (SD) T1	T2	T3	Interaction p value
Stimulation "I feel exhilarated at the moment"	alc	5.4 (2.4)	6.4 (1.9)	5.2 (2.3)	0.11
	p value	0.87	<0.01*	0.55	
	Sal	5.5 (2.0)	5.3 (2.1)	5.0 (2.2)	
Sedation "I feel subdued at the moment"	alc	3.9 (2.6)	3.5 (2.1)	4.9 (2.8)	0.33
	p value	0.46	0.33	0.46	
	sal	3.53 (2.4)	3.95 (2.5)	5.24 (2.6)	
Negative feelings "I feel unwell at the moment"	alc	0.4 (1.1)	1.0 (1.5)	1.0 (1.5)	0.70
	p value	0.31	0.85	0.62	
	Sal	0.50 (1.2)	0.95 (1.9)	0.91 (1.4)	
Well-being "I feel good right now"	alc	6.9 (1.7)	7.1 (1.6)	6.5 (1.8)	0.36
	p value	0.93	0.23	0.88	
	Sal	6.9 (1.5)	6.7 (1.6)	6.6 (1.5)	
Feeling drunk "At the moment I feel drunk"	alc	0.0 (0.0)	4.8 (2.1)	4.8 (2.3)	<0.001*
	p value	0.06	<0.001*	<0.001*	
	Sal	0.1 (0.1)	1.1 (1.4)	1.7 (1.9)	
Subjective drinking amount "I feel as if I had just had… drinks. (0–30)"	alc	0 (0)	4.3 (2.2)	5.3 (3.2)	<0.001*
	p value	-	<0.001*	<0.001*	
	Sal	0 (0)	1.4 (1.5)	2.06 (2.4)	
Craving "I now feel like drinking more alcohol"	alc	2.2 (2.3)	4.9 (2.5)	4.3 (2.6)	<0.001*
	p value	0.61	<0.001*	<0.001*	
	Sal	2.1 (2.1)	3.3 (2.3)	3.8 (2.4)	
Thirst "I am thirsty right now"	alc	3.7 (2.7)	4.3 (2.6)	5.4 (2.9)	0.32
	p value	0.60	0.80	0.16	
	Sal	3.9 (2.5)	4.2 (2.7)	4.9 (2.5)	

Alcohol-related feelings were self-rated on Visual Analog Scales during the alcohol (ALC) and saline (SAL) sessions, at the beginning of the session (T1), after reaching a breath alcohol concentration (BrAC) of 0.8 g/kg (T2, before the MRI session) and at the end of the session (T3).

*p < 0.01 uncorrected for multiple comparisons.

p < 0.001, η^2_p = 0.56, and feeling drunk, F(1.61, 58.10) = 71.82; p < 0.001, η^2_p = 0.66. Post hoc tests showed that participants in the alcohol session compared with the saline session reported more craving for alcohol at T2, t(36) = 5.22, p < 0.001, and T3, t(36) = 4.84, p < 0.001, feeling of drinking a greater amount at T2, t(36) = 9.40, p < 0.001, and T3, t(36) = 6.78, p < 0.001, and feeling more intoxicated at T2, t(36) = 10.12, p < 0.001, and T3, t(36) = 7.90, p < 0.001.

We did not observe any interaction effect on the ratings of stimulation, sedation, negative feelings, well-being, and thirst (all p values > 0.05). Descriptive statistics and comparisons between sessions for each time point are presented in Table 2.

Intranetwork connectivity of the default mode network

By comparing the participant-specific DMN maps between conditions, we quantified individual changes in rsFC related to DMN activity at a voxel-wise level (Rytty et al., 2013). Compared to saline, alcohol produced significant decreases in rsFC of the DMN in clusters located in the right hippocampus ($p_{corrected}$ = 0.037) and in the right middle temporal gyrus (MTG) ($p_{corrected}$ = 0.038). Details are presented in Table 3 and Figure 3. A power analysis based on G*Power (version 3.1, www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower.html) revealed that the achieved power of these tests is 0.980 for both regions (two-tailed paired t-test; Cohen's d > 0.723, α = 0.05, N = 37; Faul et al., 2009). Compared to saline, no increased rsFC was found under alcohol.

Of note, participants took either naltrexone or placebo during neuroimaging, but alcohol-induced rsFC changes within the DMN did not differ between medication groups, indicating that naltrexone was not a confounding variable.

Internetwork connectivity of the default mode network

There was a stronger anticorrelated connectivity between the DMN and the medial visual network under saline compared with

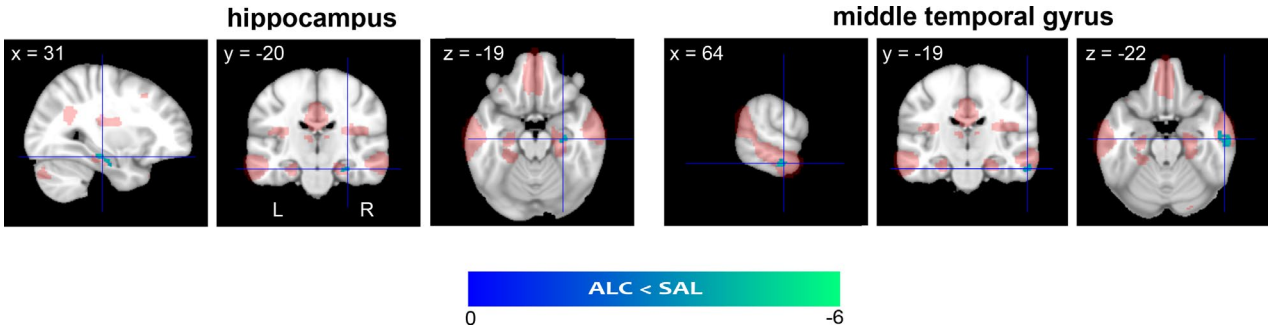

FIGURE 3 Main effect of alcohol (ALC) on connectivity within the DMN. Compared to saline (SAL), no increased rsFC was found under alcohol. The panel indicates brain regions with significant alcohol-induced differences (two-tailed) in the connectivity within the DMN ($p_{corrected}$ < 0.05). The results are overlaid on the DMN template (represented in pale red) and the average MNI152 brain. The color bar indicates *t* values

TABLE 3 Decreased rsFC within the default mode network (DMN) after alcohol infusion (Gaussian random field correction with single voxel level p = 0.001, k = 129 voxels)

Brain regions in the DMN	Brodmann area	MNI coordinates (x, y, z)	Peak *t* value	Cluster size (voxels)	$p_{cluster}$ value (corrected)
Right hippocampus (extending to inferior temporal gyrus)	20, 36	32, −24, −16	−4.91	139	0.037
Right MTG	21	62, −16, −20	−4.87	138	0.038

Abbreviations: MNI, Montreal neurological institute; MTG, middle temporal gyrus.

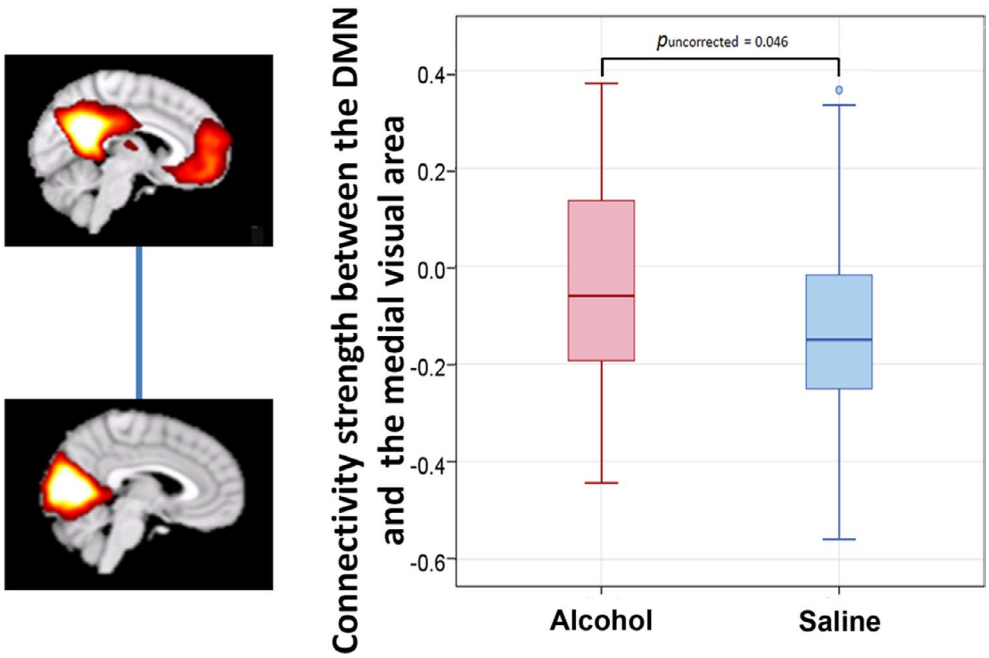

FIGURE 4 Main effect of alcohol for significant network coupling between the DMN and medial visual network ($p_{uncorrected}$ < 0.05)

alcohol infusion ($p_{uncorrected}$ = 0.046; Figure 4). This effect of acute alcohol on internetwork connectivity was small to medium (Cohen's d = 0.285, α = 0.05, N = 37). Computation of post hoc power analysis based on G*Power 3.1 indicated that such a small-to-medium effect size could have been detected in our study with a power of 0.394 (Faul et al., 2009). Nonetheless, the result was no longer significant after FDR correction for nine 2-tailed tests (i.e., the number of correlations between the DMN and all other rsFC networks examined).

Alcohol-induced changes in internetwork connectivity between the DMN and all other networks were not significant (even without FDR correction) and showed a small effect size (range of Cohen's d: 0.022–0.285). Our study only had a low power (range of power estimates: 0.052–0.394) to detect such small effect sizes.

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TABLE 4 Associations between alcohol-induced resting-state functional connectivity (rsFC) changes and Alcohol Use Disorders Identification Test (AUDIT), Obsessive–Compulsive Drinking Scale (OCDS), and Visual Analog Scale (VAS) measures

	Alcohol-induced rsFC changes	
Measures	Right hippocampus (*r* value; *p* value)	Right MTG (*r* value; *p* value)
AUDIT	$r = 0.104$; $p = 0.542$	$r = 0.028$; $p = 0.869$
OCDS	$r = 0.336$; $p = 0.042$	$r = 0.336$; $p = 0.042$
Alcohol-induced VAS changes		
Craving	$r = 0.145$; $p = 0.390$	$r = 0.076$; $p = 0.656$
Feeling drunk	$r = 0.166$; $p = 0.325$	$r = -0.208$; $p = 0.218$
Subjective drinking amount	$r = -0.121$; $p = 0.476$	$r = -0.282$; $p = 0.091$

Similar to the connectivity within the DMN, alcohol-induced changes in internetwork connectivity were also not affected by medication group, indicating that results are not confounded by this factor.

Associations between rsFC changes, drinking, and craving measures

The AUDIT scores showed neither a significant correlation with the alcohol-induced rsFC changes (compared to saline) observed in the right hippocampus ($r = 0.104$, $p = 0.542$) nor the right MTG ($r = 0.028$, $p = 0.869$). Interestingly, craving (assessed with the OCDS) was correlated with the alcohol-induced rsFC changes in both regions (right hippocampus: $r = 0.336$, $p = 0.042$; MTG: $r = 0.336$, $p = 0.042$). Nevertheless, we did not see significant associations of alcohol-induced craving (assessed with VAS) with rsFC changes in these 2 regions (Table 4).

DISCUSSION

We examined the effects of acute alcohol administration (BrAC of 0.8 g/kg) on the DMN in heavy drinkers by employing intra- and internetwork analyses. The intranetwork analysis revealed decreased connectivity within the DMN under alcohol compared with saline infusion in right temporal regions. We found no significant changes in the network coupling between the DMN and 9 other major networks under alcohol infusion. To our knowledge, this is the largest study that investigated the effects of moderate exposures to ethanol (BrAC of 0.8 g/kg) on rsFC of the DMN in individuals at risk for developing alcohol-related disorders.

One of the 2 main observed effects was an alcohol-related reduction in the rsFC in the hippocampus, a key region of memory-related processes. Seminal work on the DMN identified the hippocampus as part of the so-called "medial temporal lobe" subsystem of the DMN (Andrews-Hanna et al., 2010), which is responsible for encoding and consolidating new episodic memory, such as self-experienced events, into long-term memory (i.e., long-term potentiation) (Andrews-Hanna et al., 2010; Burgess et al., 2002). The impairment of this function is evident as intoxicated individuals can perform complex actions without later recollection (i.e., alcohol-induced blackout) (Lee et al., 2009). Studies in animal models suggest that the mechanism of action of acute alcohol exposure on memory-related systems is the alcohol-induced suppression of the firing in hippocampal brain cells (White, 2003; White et al., 2000), and the modification of the responses in memory circuits (Petruccelli et al., 2018). In this light, the reduced rsFC of the hippocampus observed in our study might reflect alcohol-induced changes in the hippocampus at a cellular level, but this interpretation must be considered with caution since a direct connection between rsFC and neuronal interactions has not been established.

The current study also revealed reduced rsFC of the MTG under alcohol compared with saline infusion. Previous studies have consistently described structural (Luciana et al., 2013; Squeglia et al., 2014; Wilson et al., 2015) and functional (Tu et al., 2018) changes in the MTG of young alcohol-dependent individuals. Additionally, chronic alcohol intake decreased rsFC of the MTG in heavy drinkers, compared with healthy controls under sober conditions (Shokri-Kojori et al., 2017). Functional changes related to acute alcohol exposure, as observed in our study, may, therefore, indicate changes in the early stages of alcohol abuse, which may be potentiated by acute alcohol intake.

Follow-up analyses showed a positive association between craving measured at the second MRI scan (with the OCDS) and rsFC changes in clusters with significant effects of alcohol, indicating that larger decreases between the alcohol and saline conditions were present in individuals who reported stronger alcohol craving. Measures of alcohol craving are associated with memory bias for alcohol cues (Franken et al., 2003) and have been used to assess the risk of relapse in alcohol use disorders (Flannery et al., 2003; Stohs et al., 2019). Similarly, recent studies have shown that the intranetwork connectivity of the DMN is a potential biomarker to predict alcohol use severity in heavy drinkers (Fede et al., 2019; Zhang & Volkow, 2019). Because the association between rsFC changes and OCDS was modest and revealed in a follow-up analysis, we suggest that future studies further explore a combination of rsFC measures with behavioral variables of craving before considering the promising joint use of these instruments as prognostic variables in the field of addiction research.

Our internetwork connectivity analysis did not yield any significant results after correction for multiple testing. Thus, we could not replicate the observation of a stronger correlation between the anterior DMN and the dorsal attention network in 15 light/moderate drinkers after acute alcohol administration (Lei et al., 2014). This discrepancy might be due to differences in sample characteristics since our participants were heavy drinkers with a binge drinking

history. A higher alcohol tolerance may have reduced our participants' response to acute alcohol exposure, characterized by a lower functional integration (i.e., nonsignificant interactions between the networks) in their brains compared with those of lighter drinkers. Another explanation might be the low statistical power of the internetwork analyses, which suggested that, in the current work, there was a low probability of discovering alcohol effects on the interactions between the networks if the effects are true (Button et al., 2013). If that is the case, the power analysis indicates that our sample size is not sufficient for the internetwork analyses, albeit it is larger than in previous studies (Esposito et al., 2010; Khalili-Mahani et al., 2012; Lei et al., 2014; Weber et al., 2014; Zheng et al., 2015). Moreover, the relatively low temporal resolution due to a TR of 2410 ms in our fMRI data may also limit the detection of alcohol-induced changes on internetwork synchrony (i.e., coupling between large-scale networks).

The literature on alcohol-induced changes in DMN-related rsFC is inconsistent (Zhang et al., 2019). While some studies showed increases in DMN-related rsFC under acute alcohol exposure (Zheng et al., 2015; Zhu et al., 2015), more studies demonstrated reductions in rsFC of the DMN under similar conditions (Muller-Oehring et al., 2015; Shokri-Kojori et al., 2017; Vergara et al., 2017; Weber et al., 2014). It is possible that the differences in populations under study contributed to these inconsistencies (Zhang et al., 2019). Our study focused on heavy drinkers, which differ from healthy people (Zheng et al., 2015) and patients with alcohol use disorder (Zhu et al., 2015), and thus, differences in the impact of acute alcohol are not surprising. Moreover, a recent study demonstrated similar reductions in rsFC of the DMN under acute alcohol intake in heavy drinkers (Shokri-Kojori et al., 2017). However, since there are only a few studies in this high-risk population, more research is needed to verify our findings.

Several limitations of the present study should be considered. First, since the experimenter needs to carefully control the infusion protocol, we used a single-blind design (i.e., only participants were blind), which might increase bias and preconceived notions about the effects of the interventions (Friedman et al., 1998). Moreover, due to the mere pharmacological effects of the ethanol exposures we used, a certain degree of unblinding on the side of the participants was inevitable. Following Testa and colleagues (Testa et al., 2006), we tried to control expectancy effects by instructing participants that alcohol would be administered in both sessions, albeit different amounts. This instruction proved effective as participants reported that they felt as if they had consumed 1 to 2 drinks on average when receiving saline. Our results showed that changes in alcohol-related feelings were not correlated with alcohol-induced changes in rsFC, and confounding of our findings due to partial unblinding seems improbable. Thus, although a perfect blinding was not possible, we made all efforts to reduce bias due to unblinding. Second, we did not collect behavioral measures that may have confirmed the direct implication of the observed connectivity changes in memory and self-referential processes. Therefore, our interpretations in this regard are speculative and merit further investigation. Third, this study only included heavy drinkers who were predominantly male. Thus, we cannot generalize our findings of alcohol-induced connectivity changes to other groups with different drinking patterns and females. Forth, since the DMN is the most prominent network at rest, the present study focused solely on identifying DMN-related functional connectivity changes. However, targeted investigations of other regions, such as subcortical areas, are needed in future studies to present a whole picture of the acute effects of alcohol on rsFC. Finally, since the internetwork connectivity analyses were underpowered in retrospect (Cohen's $d < 0.285$, alpha = 0.05, $N = 37$), the current study may not have captured effects of alcohol on internetwork connectivity. So far as we know, effect sizes for DMN internetwork connectivity changes at rest were not reported in previous studies using alcohol interventions (Lei et al., 2014; Zhang et al., 2019; Zheng et al., 2015). Therefore, we had to base post hoc power analyses on the effect sizes in our current data to provide a rough estimation. Further studies should address these issues in larger and representative cohorts to fully characterize the neural and behavioral effects of drinking behavior.

In conclusion, studying heavy drinkers with a well-controlled BrAC of 0.8 g/kg revealed that acute alcohol intake induced rsFC reductions in DMN regions involved in self-referential processes. Specifically, reduced rsFC of the right hippocampus and the right MTG was observed during alcohol infusion compared with saline infusion. Furthermore, these reductions were more pronounced in participants who reported stronger alcohol craving (assessed with the OCDS), suggesting a potential marker that can be used to assess problematic drinking patterns. Our results support the notion of disrupted neural bases of mnemonic processes and impaired self-referential thought during acute alcohol consumption and provide a potential mechanism that may lead to chronic impairments. Future research is needed to confirm potential brain-behavior associations and to better understand how the observed connectivity changes may promote and maintain excessive alcohol consumption.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

MNS, USZ, and MS were responsible for the study concept and design. MS, PR, and JP contributed to data acquisition. XF and YIDA analyzed the data. XF, YIDA, MNS, MM, JP, PR, and SJO interpreted

the findings. YIDA, XF, and JP drafted the manuscript. MNS, PR, MM, and SJO provided critical revision of the manuscript. All authors reviewed and approved the final version for publication.

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