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Altered Physiological, Affective, and Functional Connectivity Responses to Acute Stress in Patients With Alcohol Use Disorder

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ABSTRACT

BACKGROUND: There is evidence that the processing of acute stress is altered in alcohol use disorder (AUD), but little is known about how this is manifested simultaneously across different stress parameters and which neural processes are involved. The current study examined physiological and affective responses to stress and functional connectivity in AUD.

METHODS: Salivary cortisol samples, pulse rate, and affect ratings were collected on 2 days from 34 individuals with moderate or severe AUD during early abstinence and 34 control participants. On one of the days, stress was induced, and on the other day, a nonstressful control task was performed. Following the intervention, participants underwent functional magnetic resonance imaging to assess functional connectivity, with a focus on cortical and subcortical seed regions previously reported to be involved in AUD and/or stress.

RESULTS: For pulse rate and cortisol, stress responses were blunted in AUD, whereas the affective response was stronger. Neuroimaging analyses revealed stress-related group differences in functional connectivity, involving the connectivity of striatal seeds with the posterior default mode network, cerebellum, and midcingulate cortex and of the posterior default mode network seed with the striatum and thalamus.

CONCLUSIONS: The results suggest a dissociation between subjectively experienced distress and the physiological stress response in AUD as well as stress-related alterations in functional connectivity. These findings highlight the complex interplay between chronic alcohol use and acute stress regulation, offering valuable considerations for the development of therapeutic strategies.

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There is a complex and bidirectional relationship between stress and alcohol use disorder (AUD). On the one hand, stress is a known risk factor for the development of addiction and vulnerability to relapse (1). On the other hand, chronic alcohol consumption is associated with alterations of physiological stress systems, including the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system. For example, basal cortisol levels in blood plasma and saliva, which serve as indicators of HPA axis activity, have been observed to be elevated in patients diagnosed with AUD during intoxication and acute withdrawal compared with healthy control participants [e.g., (2,3)]. During abstinence, cortisol levels generally decline (4,5), and most studies no longer find significant group differences (6). Regarding the autonomic nervous system, lower heart rate variability and higher heart or pulse rate have been reported during early abstinence (7–11).

In addition to these alterations in basal autonomic nervous system and HPA axis tone, phasic responses to stress have also been found to be disturbed. According to recent reviews (6,12), numerous studies have indicated that the cortisol response to acute stress is blunted in AUD during early abstinence, thereby impacting relapse risk (13–15). Furthermore, a weaker heart (or pulse) rate response to acute stress has been observed in several studies, although the results have been more mixed (6). Interestingly, a diminished physiological stress response does not appear to be associated with lower subjective perceived stress. Few studies have examined effects of acute stress on subjective distress or affect, but these have reported equal or stronger negative affect or distress in AUD during abstinence compared with healthy control participants (7,10,16–18). Thus, previous findings suggest a dissociation between physiological and subjective stress responses in AUD during early abstinence; while there is evidence that HPA and autonomic nervous system responses are blunted, experienced distress tends to be higher than in healthy participants.

It is not clear which neural processes are associated with altered stress responses. The investigation of functional connectivity using functional magnetic resonance imaging (fMRI) can provide valuable insights into the organization of brain circuits (19). To our knowledge, there has only been one pilot study of functional connectivity during acute stress, which was

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conducted with 10 long-term abstinent patients with AUD and 11 control participants (20). This study focused on the amygdala as a seed region and found hypoconnectivity with frontal, temporal, parietal, and cerebellar regions in AUD while performing a classic stress task. However, other seed regions may also be of interest such as the thalamus and posterior cingulate cortex—a central node in the default mode network (DMN)—where changes in connectivity have been demonstrated after stress induction in healthy participants [for review, see (21)]. Furthermore, this study did not include a nonstressful control session, so it remains unclear to what extent the changes found are specific to a state of acute stress. Several other studies have also identified differences in functional connectivity between individuals with AUD and healthy control participants in the absence of stress, including hyperconnectivity of the striatum with the anterior insula (22), anterior cingulate cortex (ACC), and superior and inferior frontal gyri (22,23); hypoconnectivity of the thalamus with the striatum (22–24), medial prefrontal cortex (24,25), and ACC (23,24,26); and disrupted connectivity of the DMN (27).

The current study investigated physiological and affective stress responses along with brain connectivity in AUD. Patients with moderate or severe AUD and healthy control participants were examined on 2 separate days with a stress-inducing task on one day and a nonstressful control task on the other (presented in counterbalanced order). Salivary cortisol samples, pulse rate, and questionnaire data on negative affect were collected. Participants underwent fMRI to assess stress-induced changes in functional connectivity, with a focus on cortical and subcortical regions that have been associated with AUD. We expected to find a dissociation between physiological and subjective stress responses in AUD that would be reflected in stronger affective responses but blunted cortisol and pulse rate responses compared with healthy control participants. Furthermore, we explored group differences in stress-associated changes in functional connectivity.

METHODS AND MATERIALS

Sample

Forty-two individuals with a DSM-5 diagnosis of moderate or severe AUD (5–11 criteria fulfilled) and 38 healthy control participants were recruited (see the Supplement for details on recruitment and inclusion/exclusion criteria). All patients were in the phase of early abstinence (10th–40th day) after acute withdrawal symptoms had resolved, and they remained stationary in the clinic throughout the study. A final sample of 34 patients and 34 healthy control participants was included in the study (see the Supplement for details on dropouts and exclusions). The 2 groups were comparable on age and gender. All participants gave informed written consent. The study was approved by the local ethics committee at the University of Lübeck (AZ 17-077).

Procedure

Before participating in the study, participants were informed about the study procedure (but not about the stress induction protocol) and screened to ensure that they met all inclusion and exclusion criteria. After successful inclusion, participants visited the Center of Brain, Behavior and Metabolism in Lübeck on 2 separate days, with a maximum interval between test days of 10 days (mean interval: 3.25 days) for all but one person (see the Supplement for details).

The 2 test days were identical in terms of procedure and differed only in the stress protocol, with the order of the 2 days being counterbalanced across participants (Figure 1A). On one day, participants completed a slightly modified version of the Trier Social Stress Test (TSST) (28), a widely used tool for investigating acute psychosocial stress that includes delivering a free speech and performing mental arithmetic in front of a jury. On the other day, participants performed a control task in which they read written texts out loud and performed easy mental arithmetic in the absence of a jury to mimic the procedure of the TSST without eliciting psychosocial stress (for details on the tasks, see the Supplement). Each test day lasted for approximately 3 hours (8:30 AM–11:30 AM $\pm$ 30 minutes) and started with a check of the inclusion and exclusion criteria and the collection of a first saliva sample (T0)[1]. This was followed by an explanation and practice of the experimental tasks to be performed later during the MRI session. Afterward, a pulse oximeter (PULOX PO-300; Novidion GmbH) was attached to the participant's index finger to record the pulse rate, and the participants watched a calming video showing landscape scenes. Following this rest period, a second saliva sample was collected (T1), and participants completed the state part of the State-Trait Anxiety Inventory (STAI-S) (29) to assess their current affective state (see the Supplement). Then, the TSST or the control task was performed. Another saliva sample (T2) and affective state rating were then collected, and the pulse oximeter was removed. Another saliva sample (T3) was taken right before the start of the 1-hour MRI session. Finally, participants provided a final saliva sample (T4), completed several questionnaires (see the Supplement), and were debriefed on the day of the stress induction. On the second testing day, participants received their remuneration of €60 for their participation in the study.

Analysis of Pulse Rate, Cortisol, and Affective State

For every participant and test day, STAI-S scores were calculated for the 2 time points. Mean pulse rate for the rest, anticipation, and task phase were also computed (see the Supplement). Two control participants had to be excluded from analyses involving pulse rate due to incomplete recording. Cortisol concentrations for saliva samples were measured by chemiluminescence immunoassay with high sensitivity (see the Supplement for details on laboratory analysis).

To assess group differences in basal activity of the sympathetic nervous system, HPA activity, and subjectively experienced distress, averaged baseline scores for cortisol (T0), pulse rate, and STAI-S on the 2 test days were compared between the groups using 2-tailed Welch's 2-sample *t* tests.

To analyze the effects of the experimental stress induction, repeated-measures analyses of variance with group (AUD vs. control) as the between-participant factor and condition (stress

[1]Blood samples were also taken throughout the test day, which are not relevant for this study. See the Supplement for details.

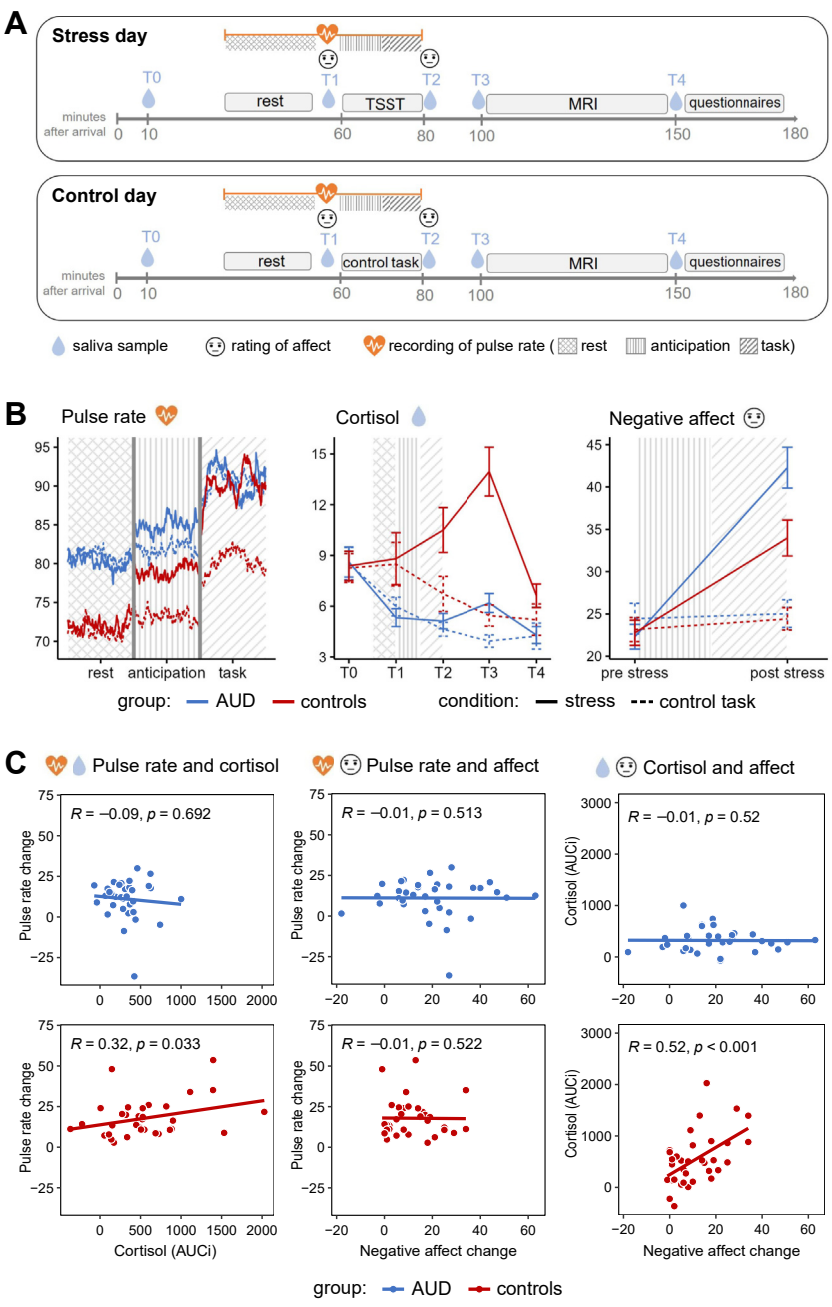

Figure 1. **(A)** Overview of the experimental design. **(B)** Development of pulse rate (beats per minute), cortisol levels (nmol/L), and rated negative affect over the 2 test days in patients with alcohol use disorder (AUD) and control participants. The shaded areas indicate the time of the rest phase as well as the anticipation and task phase of the stress or control task. **(C)** Relationship of stress-induced changes in cortisol, pulse rate, and negative affect. AUCi, area under the curve with respect to increase; MRI, magnetic resonance imaging; TSST, Trier Social Stress Test.

vs. control task) as the within-participant factor were calculated on difference scores of pulse rate (mean pulse rate during task phase − mean pulse rate during rest phase), difference scores of self-reported affective state (STAI-S after stress induction/control task − STAI-S before the task), and changes in cortisol (area under the curve with respect to an increase from T1 to T4) [see (30)].

To analyze whether stress-related increases in pulse rate, cortisol, and negative affect were associated with each other, 1-sided Pearson's correlations were computed for the 3 difference scores described above, and correlations within the 2 groups (AUD and control) were statistically compared using Fisher's *z* transformation (31).

MRI Acquisition and Functional Connectivity Analyses

Participants were examined in the MRI scanner at the Center of Brain, Behavior and Metabolism in Lübeck (3T Siemens MAGNETOM Skyra magnetic resonance tomograph) using a 64-channel head coil. MRI data were preprocessed and analyzed using SPM12 (Wellcome Department of Imaging Neuroscience; http://www.fil.ion.ucl.ac.uk/spm) implemented

in MATLAB (version R2019b; MathWorks Inc). Scan acquisition parameters and preprocessing details are described in the Supplement.

The MRI session comprised 2 experimental tasks and an anatomical scan (see the Supplement for details). The current study was not focused on task-associated brain activity or connectivity (which will be published in the context of other research questions of the project) but on functional connectivity after all task-related activity was removed. As in previous studies (32–34), this was analyzed using a seed-based approach by regressing out hemodynamic responses induced by the task. To be temporally close to the stress induction, the analyses were performed on the first task, which was the Monetary and Social Incentive Delay Task (35,36). This is a well-established reward processing paradigm in which anticipated monetary and social rewards can be won by quickly pressing a button when a target stimulus appears (for details, see the Supplement). Seed regions were the amygdala, ventral and dorsal striatum, thalamus, (dorsal and ventral) insula, ACC, and (anterior and posterior) DMN because alterations in AUD have been reported for these regions in the literature (see above). For details on the seed masks, see the Supplement.

To extract time series for the functional connectivity analysis from the seed regions, a first-level general linear model was created for each participant, including predictor variables for the task (see the Supplement for details), the 6 realignment parameters, and their temporal derivatives as nuisance regressors. Additionally, a scrubbing regressor was added to the general linear model, removing all volumes with a framewise displacement >1 mm [see (37)]. The time series of each seed was then extracted using the first eigenvariate in SPM. Finally, new first-level general linear models were generated for every participant and every seed, including the extracted time series of the respective seed, all previously mentioned regressors, as well as regressors for white matter and cerebrospinal fluid signal. The correlations of each seed's time series with the time series of all other voxels in the brain were analyzed at the second level (group level) using a full factorial model in SPM12. To allow a comparison of group differences with the previous literature that did not perform a stress manipulation, a t test comparing both groups only during the control task was also calculated. We used a false discovery rate–corrected cluster-extent threshold of $p < .05$ based on a $p < .001$ voxel-level threshold as implemented in SPM12. For exploratory analyses, parameter estimates of significant clusters of the group × stress interaction were extracted, and Pearson's correlations with stress-induced changes in cortisol, pulse rate, and affect were calculated (see the Supplement for details).

RESULTS

Sample Characteristics

Descriptive data on demographics, questionnaires, and baseline measures of pulse rate, cortisol, and negative affect can be found in Table 1.

Blunted Physiological but Stronger Affective Stress Responses in AUD

The 3 analyses of variance on changes in cortisol levels (area under the curve with respect to an increase) and difference scores of pulse rate (task phase − rest) and affect (STAI-S after − before the task) all revealed a significant main effect of condition, reflecting a greater increase in cortisol, pulse rate, and negative affect during/after the TSST than the control task across all participants (see Table 2 for statistics and effect sizes). In all 3 analyses, there was no main effect of group, but a significant interaction effect between condition and group (Table 2). For cortisol and pulse rate, stress responses (in contrast to the control condition) were less pronounced in

Table 1. Sample Characteristics

	AUD, n = 34[a]	Control, n = 34[b]	Group Comparisons
Gender	3% female, 97% male	6% female, 94% male	–
Age, Years	42.41 ± 9.15	43.88 ± 9.31	–
Duration of Abstinence, Days	14.76 ± 6.71	–	–
Alcohol Amount, g/Day	301.9 ± 203.3	2.8 ± 3.5	$t_{31.02} = 8.32$, $p < .001$, $d = 2.11$
BDI-FS	3.98 ± 3.10	0.97 ± 1.31	$t_{44.74} = 5.22$, $p < .001$, $d = 1.26$
TICS	21.52 ± 8.41	10.33 ± 5.01	$t_{53.83} = 6.67$, $p < .001$, $d = 1.62$
SRS	56.75 ± 11.05	46.68 ± 7.23	$t_{56.89} = 4.45$, $p < .001$, $d = 1.08$
Baseline Pulse Rate, bpm	79.80 ± 12.38	71.93 ± 12.05	$t_{65.95} = 2.66$, $p = .01$, $d = 0.64$
Baseline Cortisol, nmol/L	8.57 ± 5.08	8.32 ± 4.56	$t_{65.26} = 0.21$, $p = .83$, $d = 0.05$
Baseline STAI-S	23.36 ± 8.32	22.96 ± 7.81	$t_{65.73} = 0.21$, $p = .84$, $d = 0.05$

Values are presented as mean ± SD, if not otherwise specified. For the comparison of groups, Welch's t tests were computed. BDI-FS is a 7-item screening scale that measures the severity of nonsomatic depression symptomatology (59). TICS values represent the 12-item screening scale as a global measure of experienced chronic stress (60). SRS sum value reported as a measure of self-reported general stress reactivity (39).

AUD, alcohol use disorder; BDI-FS, Beck Depression Inventory-Fast Screening; SRS, Stress Reactivity Scale; STAI-S, state part of the State-Trait Anxiety Inventory; TICS, Trier Inventory for Chronic Stress.

[a]For 1 participant with AUD, exact information about the duration of abstinence (between 10 and 40 days) is missing, and for 2 participants with AUD, the exact amount of alcohol before withdrawal is missing, resulting in a sample size of 33 and 32 for these 2 variables, respectively.

[b]Pulse rate could not be recorded completely in 2 control participants, and 1 control participant did not complete the BDI. Therefore, the sample size was reduced to 32 and 33 for these 2 variables, respectively.

Table 2. ANOVA Results on Changes in Pulse Rate, Cortisol, and Affect

	F	η_p^2	p
Pulse Rate Change (Task Phase − Rest)			
Group	$F_{1,64} = 1.47$	0.02	.230
Condition	$F_{1,64} = 13.75$	0.18	<.001
Group × condition	$F_{1,64} = 7.43$	0.10	.008
Change in Cortisol Levels (AUCi)			
Group	$F_{1,66} = 2.79$	0.04	.099
Condition	$F_{1,66} = 30.35$	0.32	<.001
Group × condition	$F_{1,66} = 6.62$	0.09	.012
Change in Negative Affect (After the Task − Before the Task)			
Group	$F_{1,66} = 3.73$	0.05	.058
Condition	$F_{1,66} = 72.12$	0.52	<.001
Group × condition	$F_{1,66} = 7.50$	0.10	.008

ANOVA, analysis of variance; AUCi, area under the curve with respect to increase.

patients with AUD than in control participants, but affective responses were stronger (Figure 1B).

Correlations of Physiological and Affective Stress Responses

Correlational analyses revealed significant positive associations between stress-related changes in cortisol and negative affect and between changes in cortisol and pulse rate in healthy control participants, but no significant correlations in AUD (Figure 1C and Table 3). Statistical comparisons of the correlations in the 2 groups can be found in Table 3.

Weaker Functional Connectivity in AUD

Functional connectivity analyses revealed main effects of group for the connectivity of the dorsal striatum, ACC, and anterior and posterior DMN with several cortical and subcortical regions (Figure 2 and Table 4), reflecting hypoconnectivity in AUD. Additional t tests comparing patients with AUD and healthy control participants on the control task only showed weaker connectivity of the bilateral ventral and dorsal striatum, right dorsal insula, and anterior and posterior DMN with cortical and subcortical regions, specifically the striatum, cerebellum, and precuneus (Table S1).

Interaction Effects of Group and Stress on Functional Connectivity

Functional connectivity analyses did not reveal any main effect of stress in any of the seeds. However, significant interaction effects of group and stress were found for functional connectivity of the left ventral and dorsal striatum with the right precuneus and superior parietal lobule, the left dorsal striatum with the right cerebellum and midcingulate cortex, the right ventral insula with the cerebellum, and the posterior DMN with the left putamen and thalamus (Table 5 and Figure 3). Exploratory correlations of parameter estimates from these clusters with stress-induced changes in cortisol, pulse rate, and affect can be found in the Supplement.

DISCUSSION

In the current study, we investigated physiological and affective responses to acute stress along with functional brain connectivity in AUD. Altered stress responses were found for all stress parameters (cortisol, pulse rate, and negative affect) in patients with AUD compared with control participants. In parallel, stress effects on functional connectivity also differed between groups.

Importantly, the TSST induced increases in cortisol, pulse rate, and negative affect across groups, suggesting successful stress induction. Furthermore, group comparisons of baseline levels of cortisol and pulse rate replicated previous findings in the literature. We found no group difference in basal cortisol, which is consistent with reports that although cortisol levels are elevated during intoxication and acute withdrawal, they decline during abstinence and are similar to those of healthy participants in most studies [see (6) for review]. Baseline pulse rate was elevated in AUD in the current study, consistent with previous findings of elevated basal heart or pulse rate during the first weeks of abstinence (7–10). These alterations are assumed to be a consequence of chronic alcohol consumption (7–10). Acute alcohol intake affects the cardiovascular system via centrally mediated sympathetic activity, and abnormalities of cardiovascular regulation appear to persist into recovery (38).

Responses to acute stress were altered in AUD for all measured parameters, but the effects differed in their direction. Phasic cortisol responses after stress induction were significantly lower in AUD, confirming our hypothesis and replicating previous findings [see (12) for review]. For pulse rate, we also found smaller differences between the stress and control tasks in patients with AUD than in control participants. However, this interaction effect was not due to a blunted response during stress but rather to a stronger response in AUD during the control condition. This finding may explain why many previous studies that have used social stress tasks, but usually did not include a nonstressful control task, have found no group differences [see (6) for review] and suggests that it may be important to include a control condition in future studies to better understand stress-related pulse or heart rate changes in AUD.

In contrast to the findings for cortisol and pulse rate, stress-related negative affect was stronger in patients with AUD than in control participants, replicating previous studies (7,17,18). Consistent with this, Stress Reactivity Scale scores,

Table 3. Correlations of Stress-Related Changes in Pulse Rate, Cortisol, and Negative Affect

	Control	Alcohol Use Disorder	Comparison Between Groups
Cortisol and Pulse Rate	$r = 0.32$, $p = .033$	$r = -0.09$, $p = .692$	$z = -1.66$, $p = .096$
Cortisol and Affect	$r = 0.52$, $p < .001$	$r = -0.01$, $p = .520$	$z = -2.31$, $p = .021$
Pulse Rate and Affect	$r = -0.01$, $p = .522$	$r = -0.01$, $p = .513$	$z = 0.02$, $p = .987$

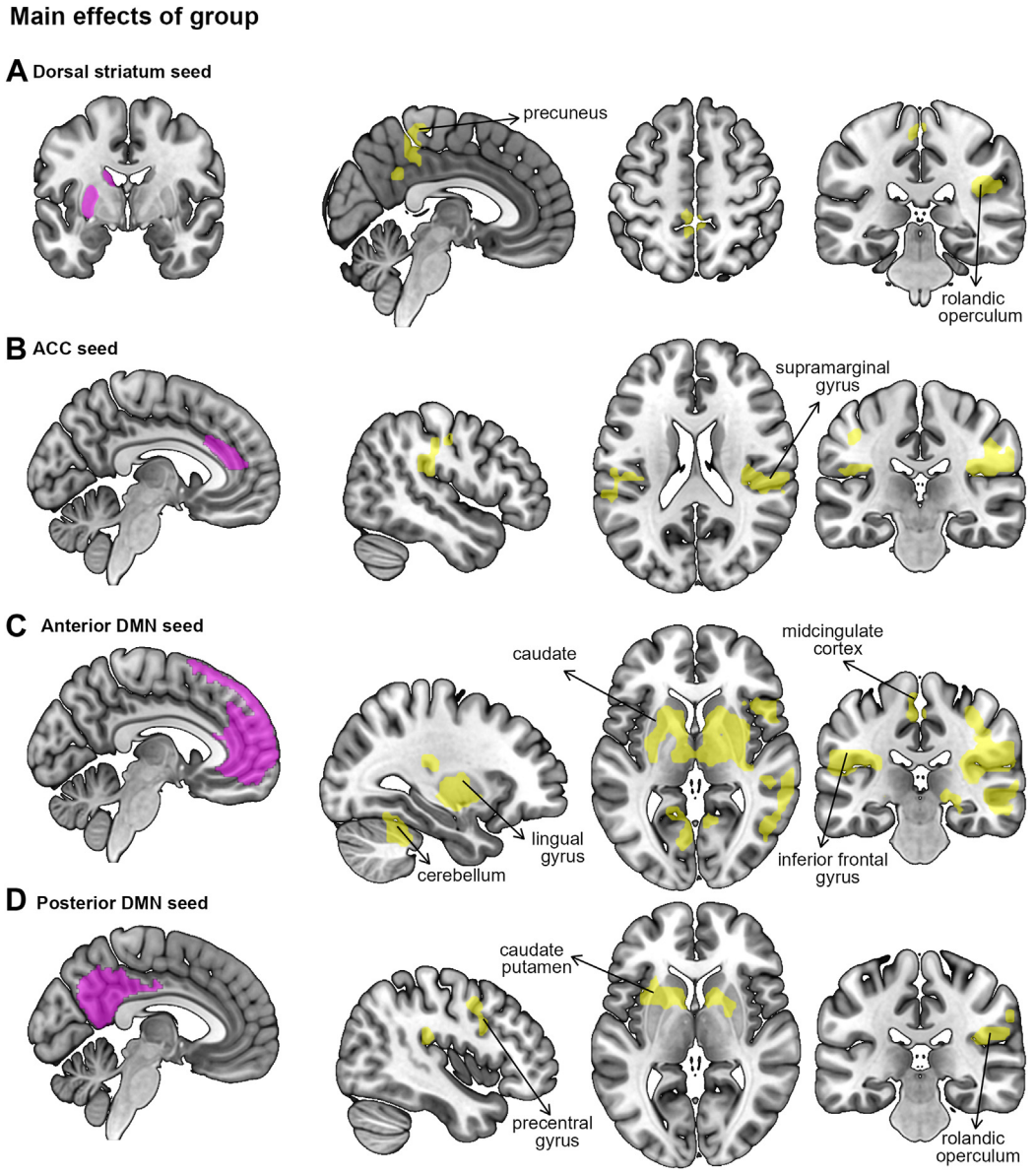

Figure 2. Main effects of group on functional connectivity of **(A)** the dorsal striatum, **(B)** the anterior cingulate cortex (ACC), **(C)** the anterior default mode network (DMN), and **(D)** the posterior DMN. Regions where functional connectivity with the mean time series of the seed (in purple) differed significantly between the groups are shown in yellow. Functional connectivity of all depicted regions was significantly reduced in patients with alcohol use disorder compared with control participants.

which represent affective stress reactivity as a trait (39), were also significantly elevated in AUD. Taken together, these findings suggest a dissociation between subjectively experienced distress (stronger) and the physiological stress response (weaker) in AUD. This dissociation is further corroborated by the finding that stress-related changes in cortisol were significantly correlated with changes in pulse rate and negative affect in healthy participants but not in patients with AUD. We assume that a dysregulation of the physiological stress systems in AUD underlies this finding. Interestingly, postmortem analyses of gene expression in the hippocampus have shown that pathways involved in stress responses are mostly increased in AUD (40), which may indicate that blunted physiological stress responses do not reflect reduced activity of brain stress systems (41). Furthermore, the stronger affective stress response in AUD may also be due to a dysregulation of brain stress systems. In rats, chronic alcohol consumption has been shown to lead to heightened activity of the corticotropin-releasing factor (CRF) system in the central amygdala in response to stress (42), and it has been suggested that alterations in the CRF system underlie enhanced behavioral stress responses (43). These alterations can persist after longer periods of abstinence (41). While these findings suggest that heightened affective stress

Table 4. Main Effects of Group on Functional Connectivity

	Side	MNI Coordinates			Cluster Size	F^a	p
		x	y	z			
Seed: Left Dorsal Striatum							
Rolandic operculum, angular gyrus, superior temporal gyrus, supramarginal gyrus	Right	42	−25	20	203	28.31	.010
		45	−49	23		18.40	
		60	−52	20		17.49	
Precuneus, midcingulate cortex	Left/Right	0	−40	56	182	19.13	.010
		−6	−52	38		15.03	
		3	−49	32		14.90	
Seed: ACC							
Rolandic operculum, supramarginal gyrus, postcentral gyrus, precentral gyrus	Right	42	−25	20	294	26.88	.001[b]
		57	−25	23		18.16	
		63	−19	35		17.28	
Supramarginal gyrus, inferior parietal lobule	Left	−57	−40	32	227	18.13	.002[b]
		−45	−52	53		17.17	
		−51	−46	47		17.14	
Seed: Anterior DMN							
Caudate nucleus, rolandic operculum, supramarginal gyrus, putamen	Left/Right	15	8	11	3238	37.33	<.001[b]
		42	−28	20		34.46	
		−54	−25	17		29.44	
Fusiform gyrus, cerebellum, lingual gyrus	Right	30	−40	−22	508	27.84	<.001[b]
		24	−64	−16		25.30	
		30	−43	−34		21.66	
Cerebellum, hippocampus, lingual gyrus	Left	−33	−49	−25	387	24.86	<.001[b]
		−18	−37	−1		21.82	
		−6	−76	−1		19.62	
Precuneus, midcingulate cortex, paracentral lobule	Left/Right	−3	−40	56	190	21.24	.005
		3	−40	44		20.91	
		−3	−25	50		19.03	
Inferior frontal gyrus	Right	54	20	−1	131	27.95	.021
		57	11	8		22.64	
		57	26	14		13.12	
Posterior medial frontal, midcingulate cortex	Left/Right	3	−10	65	103	17.95	.044
		0	−7	47		17.65	
Seed: Posterior DMN							
Caudate nucleus, putamen, insula lobe	Left	−9	8	11	233	23.60	.002[b]
		−24	11	2		20.56	
		−33	5	11		15.65	
Rolandic operculum, inferior parietal lobule, supramarginal gyrus	Right	42	−28	20	138	20.70	.016
		57	−37	47		17.50	
		63	−22	29		14.78	
Precentral gyrus, inferior frontal gyrus	Right	45	2	44	126	18.64	.016
		51	5	35		17.12	
		48	11	26		16.20	
Caudate nucleus, pallidum	Right	9	8	5	95	26.46	.037
		24	2	2		14.95	

A false discovery rate–corrected cluster-extent threshold based on $p < .001$ voxel-level threshold was used.

ACC, anterior cingulate cortex; DMN, default mode network; MNI, Montreal Neurological Institute.

[a] $df = 1,131$.

[b] Results that survived Bonferroni correction to correct for multiple testing of the 15 seed regions ($p < .05/15 = .0033$).

responses are a result of chronic alcohol consumption, there may also be trait factors, such as negative emotionality, that precede the onset of substance abuse (44). Longitudinal studies are needed to clarify whether the stronger affective stress responses are a consequence of alcohol consumption or a trait and risk factor.

Neuroimaging analyses revealed weaker functional connectivity of the left dorsal striatum, ACC, and anterior and

Table 5. Interaction Effects of Group and Stress on Functional Connectivity

	Side	MNI Coordinates x	y	z	Cluster Size	F^a	p
Seed: Left Ventral Striatum							
Precuneus, superior parietal lobule	Right	15	−52	50	246	18.75	.001[b]
		21	−64	59		17.90	
		24	−52	50		16.58	
Seed: Left Dorsal Striatum							
Cerebellum	Right	15	−82	−25	132	24.21	.032
		36	−76	−28		23.33	
		9	−79	−40		17.53	
Precuneus, superior parietal lobule, midcingulate cortex	Right	15	−52	50	124	21.54	.032
		18	−64	56		14.68	
		6	−40	44		14.26	
Seed: Right Ventral Insula							
Cerebellum	Right	27	−55	−28	136	18.54	.007
		39	−67	−34		18.42	
		33	−49	−37		15.05	
Seed: Posterior DMN							
Putamen, thalamus	Left	−30	−16	−4	128	22.73	.011
		−15	−19	−7		16.84	
		−9	−13	−1		13.79	

A false discovery rate–corrected cluster-extent threshold based on $p < .001$ voxel-level threshold was used.

DMN, default mode network; MNI, Montreal Neurological Institute.

[a]$df = 1,131$.

[b]Results that survived Bonferroni correction to correct for multiple testing of the 15 seed regions ($p < .05/15 = .0033$).

posterior DMN with several cortical and subcortical regions in AUD on both test days. A separate analysis of the control day revealed hypoconnectivity of the bilateral ventral striatum, right dorsal striatum, and right dorsal insula with various regions (mainly the striatum, cerebellum, and precuneus) in AUD. The largest alterations of connectivity were found for the anterior and posterior DMN seeds, which supports the notion that this network plays an important role in substance use disorders (27,45). Several of our findings replicate previous results of resting-state fMRI research in AUD, including weaker connectivity between the anterior and posterior DMN (27), the anterior DMN and the putamen (25), the ventral striatum and angular gyrus (26), the caudate and superior frontal gyrus as well as posterior DMN (26), and the insula and cerebellum (46). However, there have also been contradictory findings in the literature such as hyperconnectivity between the striatum and superior frontal gyrus, inferior parietal gyrus, or cerebellum (22,23). This may be related to the analytical approach of the current study. We assessed functional connectivity during the processing of a reward task. Although the effects of the task were adjusted for [see (34)], it may have affected the connectivity of the striatum, a central structure of the reward system. However, there was also great heterogeneity in the results of previous resting-state fMRI research, possibly related to variations in the abstinence duration and other sample variables.

Previous research has reported altered connectivity of the amygdala, thalamus, and DMN (posterior cingulate cortex) after stress induction in a healthy population (21). In the current study comprising healthy individuals and patients with AUD, we did not observe any significant effect of stress for any of these seeds across all participants. However, interaction effects of stress and group were found for the left ventral and dorsal striatum, right ventral insula, and posterior DMN seed, reflecting hypoconnectivity on the control day but normal connectivity after stress in patients with AUD.

This effect was found for the connectivity of the left ventral and dorsal striatum seed with the right superior parietal lobule and precuneus and similarly for the posterior DMN seed (encompassing the precuneus) with the putamen. It has been previously reported that the striatum is functionally connected to the precuneus/posterior DMN and superior parietal regions in healthy participants (47) and that resting-state connectivity between the posterior DMN and striatum is weaker in individuals with drug addiction (27). Our results now suggest that in AUD, this connection is more strongly involved after acute stress (increase in connectivity after stress while control participants show a decrease). It is unclear what mechanisms underlie this effect. It has been suggested that altered connectivity between the DMN and subcortical areas enhances negative emotions in substance use disorders, but the role of specific connectivity tracts has not been investigated (27). Alterations in the dopamine system in AUD may play a role here. Chronic drug administration alters striatal dopamine signaling, and studies in healthy human subjects and animals suggest that changes in dopamine modulation may affect (de) activation of the DMN (27). The dopaminergic system is also involved in stress: Acute stress induces CRF release, which leads to dopamine release in the striatum (48). However, if the stress axes are dysregulated due to chronic substance consumption, the ability of CRF to modulate dopamine levels may be eliminated, as has already been postulated for chronic stress (48). These changes in dopamine modulation may be a potential mechanism by which alterations in connectivity between the striatum and posterior DMN after acute stress may occur in AUD. They may also underlie the interaction effect found for the connectivity between the posterior DMN and the thalamus, because nuclei within the thalamus are innervated by dopamine and CRF, among other neurotransmitters, and are closely connected to the striatum (49–51), which has been proposed to be linked to stress-associated changes in dopamine tone (52).

Interaction effects of stress and group in the same direction were also found for the connectivity of both the dorsal striatum seed and the ventral insula seed with the cerebellum. A recent study also reported hypoconnectivity between the insula and the cerebellum in AUD (53). In addition, several studies have implicated the cerebellum in the stress response [see (54) for review]. It has been suggested that the cerebellum may play an important role in stress-associated neurobehavioral effects because it expresses the cellular machinery necessary to process neurochemical mediators such as glucocorticoids and CRF, among others (54). Thus, acute stress may have an altered effect on cerebellar connectivity in AUD via these systems.

Several limitations of the current study should be considered when interpreting the study results. First, as part of a broader project, blood samples were collected, which already caused stress in some individuals, as evidenced by increasing cortisol levels from T0 to T1 in a subsample. Although there

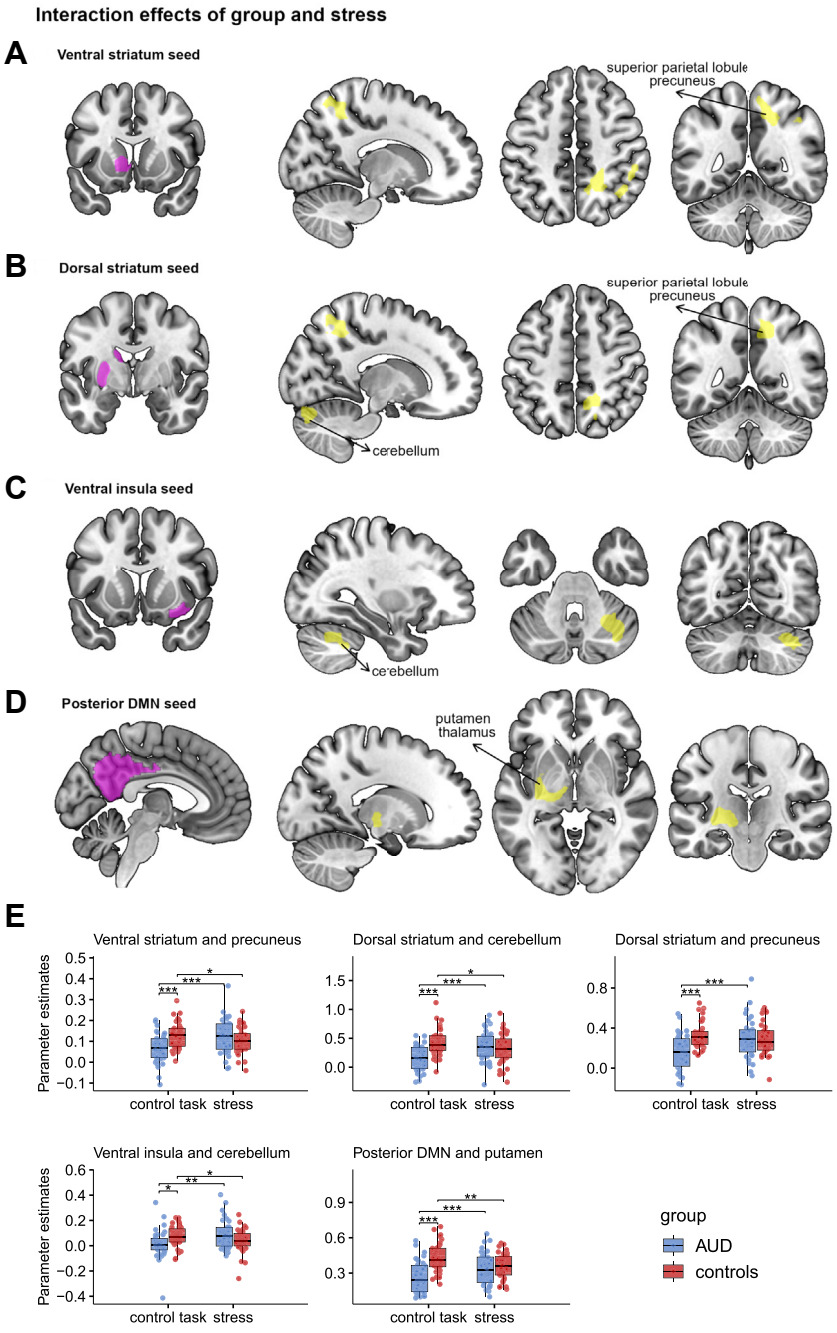

Figure 3. Group × stress interaction effects on functional connectivity of **(A)** the ventral striatum seed, **(B)** the dorsal striatum seed, **(C)** the ventral insula seed, and **(D)** the posterior default mode network (DMN) seed. Seed regions are shown in purple. Clusters in which functional connectivity with the mean time series of the seed showed significant group × stress interaction effects are displayed in yellow. **(E)** From these clusters, parameter estimates of functional connectivity (reflecting the average of all voxels within the cluster) were extracted for patients with alcohol use disorder (AUD) and healthy control participants for both days. These show that functional connectivity was weaker in patients with AUD on the control day. After stress, functional connectivity increases in patients with AUD and decreases in control participants, leading to comparable results. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

was still a rest period between the blood draw and the time of the prestress data collection, this may have influenced the results for cortisol in particular, because there is a time lag between the rise and fall of salivary cortisol levels. Second, our sample consisted mainly of male participants. The results are consistent if the few female participants are excluded (see the Supplement), but it is not clear to what extent the results can be generalized to female patients with AUD. Third, the 2 groups differed on several variables that could potentially confound the results, such as recent stress, past drug consumption, depressive symptoms, and smoking. For example, chronic and acute nicotine have been shown to affect functional connectivity (55) and the physiological stress response (56). Due to the high prevalence of smoking among patients with AUD, it is difficult to clearly separate the effects of nicotine use and AUD. Furthermore, it is not clear to what extent the

results are specific to AUD. Previous research has also reported blunted cortisol and cardiovascular responses to stress in other disorders such as depression, eating disorders, and posttraumatic stress disorder (57,58). It has been proposed that all of these disorders may reflect frontolimbic dysregulation predisposed by genes and childhood experiences (58). Fourth, the current study examined patients in the early abstinence phase, i.e., at a time when withdrawal symptoms were no longer present but when the last consumption was only 10 to 40 days before. It is not possible to say to what extent the results can be generalized to other phases. Fifth, the functional connectivity analyses should be considered exploratory because there were no clear a priori hypotheses, and so future studies are needed for confirmation. In addition, it is not clear to what extent the results are comparable to those from resting-state measurements. Previous research that contrasted resting-state data with event-related residuals showed that correlation profiles were qualitatively similar but also that differences existed (34).

Conclusions

In conclusion, our findings provide evidence for a dissociation between physiological and affective stress responses in AUD, as well as stress-related alterations in functional connectivity of striatal, insular, cerebellar, and default mode regions. These results suggest a complex interplay between chronic alcohol use and acute stress regulation at physiological, affective, and neural levels, which may be important to consider for therapeutic approaches.

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Funding was acquired by LR. LR, JV, SK, and FMP designed the study. YS, JV, AS, OV, SD, and KJ recruited the participants and acquired the data. LR, YS, and FMP analyzed data. YS and LR drafted the manuscript. All authors critically reviewed the manuscript and approved the final version.

YS has previously presented data from this study at Deutscher Psychotherapie Kongress, May 2023, Berlin, Germany and at the Psychologie und Gehirn Conference, June 2023, Tübingen, Germany. A previous version of this article was published as a preprint on bioRxiv: https://doi.org/10.1101/2024.01.18.576207.

Data and code of this study are openly available at https://osf.io/cm7kb/.

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REFERENCES

1. Milivojevic V, Sinha R (2018): Central and peripheral biomarkers of stress response for addiction risk and relapse vulnerability. Trends Mol Med 24:173–186.
2. Adinoff B, Ruether K, Krebaum S, Iranmanesh A, Williams MJ (2003): Increased salivary cortisol concentrations during chronic alcohol intoxication in a naturalistic clinical sample of men. Alcohol Clin Exp Res 27:1420–1427.
3. Kutscher S, Heise DJ, Banger M, Saller B, Michel MC, Gastpar M, *et al.* (2002): Concomitant endocrine and immune alterations during alcohol intoxication and acute withdrawal in alcohol-dependent subjects. Neuropsychobiology 45:144–149.
4. Price JL, Nixon SJ (2021): Retrospective hair cortisol concentrations from pretreatment to early recovery in alcohol use disorder. Alcohol Alcohol 56:181–184.
5. Stephens MAC, Wand G (2012): Stress and the HPA axis: Role of glucocorticoids in alcohol dependence. Alcohol Res 34:468–483.
6. Chen K, Hollunder B, Garbusow M, Sebold M, Heinz A (2020): The physiological responses to acute stress in alcohol-dependent patients: A systematic review. Eur Neuropsychopharmacol 41:1–15.
7. Sinha R, Fox HC, Hong KA, Bergquist K, Bhagwagar Z, Siedlarz KM (2009): Enhanced negative emotion and alcohol craving, and altered physiological responses following stress and cue exposure in alcohol dependent individuals. Neuropsychopharmacology 34:1198–1208.
8. Tolic I, Soyka M (2018): Stress response in persons with alcohol addiction in the context of abstinence duration and disease severity. Fortschr Neurol Psychiatr 86:356–367.
9. Demmel R, Rist F, Olbrich R (2000): Autonomic reactivity to mental stressors after single administration of lorazepam in male alcoholics and healthy controls. Alcohol Alcohol 35:617–624.
10. Seo D, Lacadie CM, Tuit K, Hong K-I, Constable RT, Sinha R (2013): Disrupted ventromedial prefrontal function, alcohol craving, and subsequent relapse risk. JAMA Psychiatry 70:727–739.
11. Ralevski E, Petrakis I, Altemus M (2019): Heart rate variability in alcohol use: A review. Pharmacol Biochem Behav 176:83–92.
12. Dunne N, Ivers J-H (2023): HPA axis function in alcohol use disorder: A systematic review and meta-analysis. Addict Neurosci 8:100114.
13. Junghanns K, Tietz U, Dibbelt L, Kuether M, Jurth R, Ehrenthal D, *et al.* (2005): Attenuated salivary cortisol secretion under cue exposure is associated with early relapse. Alcohol Alcohol 40:80–85.
14. Adinoff B, Junghanns K, Kiefer F, Krishnan-Sarin S (2005): Suppression of the HPA axis stress-response: Implications for relapse. Alcohol Clin Exp Res 29:1351–1355.
15. Junghanns K, Backhaus J, Tietz U, Lange W, Bernzen J, Wetterling T, *et al.* (2003): Impaired serum cortisol stress response is a predictor of early relapse. Alcohol Alcohol 38:189–193.
16. Starcke K, van Holst RJ, van den Brink W, Veltman DJ, Goudriaan AE (2013): Physiological and endocrine reactions to psychosocial stress in alcohol use disorders: Duration of abstinence matters. Alcohol Clin Exp Res 37:1343–1350.
17. Romero-Martínez Á, Vitoria-Estruch S, Moya-Albiol L (2019): Emotional and autonomic dysregulation in abstinent alcoholic men: An idiosyncratic profile? Alcohol 77:155–162.
18. Bernardy NC, King AC, Lovallo WR (2003): Cardiovascular responses to physical and psychological stress in female alcoholics with transitory hypertension after early abstinence. Alcohol Clin Exp Res 27:1489–1498.
19. van den Heuvel MP, Hulshoff Pol HE (2010): Exploring the brain network: A review on resting-state fMRI functional connectivity. Eur Neuropsychopharmacol 20:519–534.
20. Wade NE, Padula CB, Anthenelli RM, Nelson E, Eliassen J, Lisdahl KM (2017): Blunted amygdala functional connectivity during a stress task in alcohol dependent individuals: A pilot study. Neurobiol Stress 7:74–79.
21. van Oort J, Tendolkar I, Hermans EJ, Mulders PC, Beckmann CF, Schene AH, *et al.* (2017): How the brain connects in response to acute stress: A review at the human brain systems level. Neurosci Biobehav Rev 83:281–297.
22. Kohno M, Dennis LE, McCready H, Hoffman WF (2017): Executive control and striatal resting-state network interact with risk factors to

influence treatment outcomes in alcohol-use disorder. Front Psychiatry 8:182.
23. Camchong J, Stenger A, Fein G (2013): Resting-state synchrony in long-term abstinent alcoholics. Alcohol Clin Exp Res 37:75–85.
24. Liu J, Cai W, Zhao M, Cai W, Sui F, Hou W, *et al.* (2019): Reduced resting-state functional connectivity and sleep impairment in abstinent male alcohol-dependent patients. Hum Brain Mapp 40:4941–4951.
25. Wang J, Fan Y, Dong Y, Ma M, Ma Y, Dong Y, *et al.* (2016): Alterations in brain structure and functional connectivity in alcohol dependent patients and possible association with impulsivity. PLoS One 11: e0161956.
26. Müller-Oehring EM, Jung Y-C, Pfefferbaum A, Sullivan EV, Schulte T (2015): The resting brain of alcoholics. Cereb Cortex 25:4155–4168.
27. Zhang R, Volkow ND (2019): Brain default-mode network dysfunction in addiction. Neuroimage 200:313–331.
28. Kirschbaum C, Pirke KM, Hellhammer DH (1993): The "Trier Social Stress Test"—A tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28:76–81.
29. Grimm J (2009): State-Trait-Anxiety Inventory Nach Spielberger. Deutsche Lang-Und Kurzversion. Methodenforum Der Universität Wien. MF-Working Paper 2009/02. Available at: https://empcom.univie.ac.at/fileadmin/user_upload/p_empcom/pdfs/Grimm2009_StateTraitAngst_MFWorkPaper2009-02.pdf. Accessed February 15, 2023.
30. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH (2003): Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28:916–931.
31. Diedenhofen B, Musch J (2015): cocor: A comprehensive solution for the statistical comparison of correlations. PLoS One 10:e0121945.
32. Esslinger C, Walter H, Kirsch P, Erk S, Schnell K, Arnold C, *et al.* (2009): Neural mechanisms of a genome-wide supported psychosis variant. Science 324:605.
33. Paulus FM, Krach S, Bedenbender J, Pyka M, Sommer J, Krug A, *et al.* (2013): Partial support for ZNF804A genotype-dependent alterations in prefrontal connectivity. Hum Brain Mapp 34:304–313.
34. Fair DA, Schlaggar BL, Cohen AL, Miezin FM, Dosenbach NUF, Wenger KK, *et al.* (2007): A method for using blocked and event-related fMRI data to study "resting state" functional connectivity. Neuroimage 35:396–405.
35. Kirsch P, Schienle A, Stark R, Sammer G, Blecker C, Walter B, *et al.* (2003): Anticipation of reward in a nonaversive differential conditioning paradigm and the brain reward system: An event-related fMRI study. Neuroimage 20:1086–1095.
36. Rademacher L, Krach S, Kohls G, Irmak A, Gründer G, Spreckelmeyer KN (2010): Dissociation of neural networks for anticipation and consumption of monetary and social rewards. Neuroimage 49:3276–3285.
37. Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE (2014): Methods to detect, characterize, and remove motion artifact in resting state fMRI. Neuroimage 84:320–341.
38. Irwin MR, Ziegler M (2005): Sleep deprivation potentiates activation of cardiovascular and catecholamine responses in abstinent alcoholics. Hypertension 45:252–257.
39. Schulz P, Jansen LJ, Schlotz W (2005): Stressreaktivität: Theoretisches Konzept und Messung. Diagnostica 51:124–133.
40. McClintick JN, Xuei X, Tischfield JA, Goate A, Foroud T, Wetherill L, *et al.* (2013): Stress-response pathways are altered in the hippocampus of chronic alcoholics. Alcohol 47:505–515.
41. Fosnocht AQ, Briand LA (2016): Substance use modulates stress reactivity: Behavioral and physiological outcomes. Physiol Behav 166:32–42.
42. Retson TA, Reyes BA, Van Bockstaele EJ (2015): Chronic alcohol exposure differentially affects activation of female locus coeruleus neurons and the subcellular distribution of corticotropin releasing factor receptors. Prog Neuropsychopharmacol Biol Psychiatry 56:66–74.
43. Valdez GR, Zorrilla EP, Roberts AJ, Koob GF (2003): Antagonism of corticotropin-releasing factor attenuates the enhanced responsiveness to stress observed during protracted ethanol abstinence. Alcohol 29:55–60.
44. Measelle JR, Stice E, Springer DW (2006): A prospective test of the negative affect model of substance abuse: Moderating effects of social support. Psychol Addict Behav 20:225–233.
45. Taebi A, Becker B, Klugah-Brown B, Roecher E, Biswal B, Zweerings J, Mathiak K (2022): Shared network-level functional alterations across substance use disorders: A multi-level kernel density meta-analysis of resting-state functional connectivity studies. Addict Biol 27:e13200.
46. Halcomb ME, Chumin EJ, Goñi J, Dzemidzic M, Yoder KK (2019): Aberrations of anterior insular cortex functional connectivity in nontreatment-seeking alcoholics. Psychiatry Res Neuroimaging 284:21–28.
47. Di Martino A, Scheres A, Margulies DS, Kelly AMC, Uddin LQ, Shehzad Z, *et al.* (2008): Functional connectivity of human striatum: A resting state FMRI study. Cereb Cortex 18:2735–2747.
48. Kumar P, Berghorst LH, Nickerson LD, Dutra SJ, Goer FK, Greve DN, Pizzagalli DA (2014): Differential effects of acute stress on anticipatory and consummatory phases of reward processing. Neuroscience 266:1–12.
49. García-Cabezas MA, Rico B, Sánchez-González MA, Cavada C (2007): Distribution of the dopamine innervation in the macaque and human thalamus. Neuroimage 34:965–984.
50. Hsu DT, Kirouac GJ, Zubieta J-K, Bhatnagar S (2014): Contributions of the paraventricular thalamic nucleus in the regulation of stress, motivation, and mood. Front Behav Neurosci 8:73.
51. Huang AS, Mitchell JA, Haber SN, Alia-Klein N, Goldstein RZ (2018): The thalamus in drug addiction: From rodents to humans. Philos Trans R Soc Lond B Biol Sci 373:20170028.
52. Parsons MP, Li S, Kirouac GJ (2007): Functional and anatomical connection between the paraventricular nucleus of the thalamus and dopamine fibers of the nucleus accumbens. J Comp Neurol 500:1050–1063.
53. Manuweera T, Kisner MA, Almira E, Momenan R (2022): Alcohol use disorder-associated structural and functional characteristics of the insula. J Neurosci Res 100:2077–2089.
54. Moreno-Rius J (2019): The cerebellum under stress. Front Neuroendocrinol 54:100774.
55. Fedota JR, Stein EA (2015): Resting-state functional connectivity and nicotine addiction: Prospects for biomarker development. Ann N Y Acad Sci 1349:64–82.
56. Rohleder N, Kirschbaum C (2006): The hypothalamic-pituitary-adrenal (HPA) axis in habitual smokers. Int J Psychophysiol 59:236–243.
57. Metz S, Duesenberg M, Hellmann-Regen J, Wolf OT, Roepke S, Otte C, Wingenfeld K (2020): Blunted salivary cortisol response to psychosocial stress in women with posttraumatic stress disorder. J Psychiatr Res 130:112–119.
58. Carroll D, Ginty AT, Whittaker AC, Lovallo WR, de Rooij SR (2017): The behavioural, cognitive, and neural corollaries of blunted cardiovascular and cortisol reactions to acute psychological stress. Neurosci Biobehav Rev 77:74–86.
59. Beck AT, Brown GK, Kliem S, Steer RA: Beck-Depressions-Inventar-FS, 2013, deutsche Bearbeitung; Manual, Pearson Available at: https://www.pearsonclinical.de/bdi-fs.html. Accessed July 24, 2024.
60. Schulz P, Schlotz W, Becker P, Schulz P, Schlotz W, Becker P (2004): Trierer Inventar zum Chronischen Stress (TICS) [Trier inventory for chronic stress (TICS)]. Available at: https://eprints.soton.ac.uk/50017/. Accessed February 15, 2023.