scientific reports

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Functional resting state connectivity is differentially associated with IL-6 and TNF-α in depression and in healthy controls

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Inflammatory processes have been implicated in the pathophysiology of depression. In human studies, inflammation has been shown to act as a critical disease modifier, promoting susceptibility to depression and modulating specific endophenotypes of depression. However, there is scant documentation of how inflammatory processes are associated with neural activity in patients with depression. We therefore tested the hypothesis that the peripheral inflammation markers IL-6 and TNF-α correlate with neural resting state network functional connectivity in depression using functional magnetic resonance imaging (fMRI) and compared it with healthy controls. We used fMRI to investigate the functional connectivity (FC) of the resting state Default Mode Network (DMN) and Salience/Ventral Attention Network (SAL) and their association with the peripheral inflammation markers IL-6 and TNF-α in 25 patients with depression and compared it to 24 healthy subjects. Results of this imaging study revealed that both DMN and SAL resting state networks are differentially associated with distinct immunological pathways depending on whether a person has a depressive phenotype or is healthy. While the DMN FC correlated with the concentration of the cytokine IL-6 in healthy subjects, SAL FC's connectivity correlated with the cytokine TNF-α's concentration. This study highlights the importance of peripheral inflammatory processes in depression and suggests a modulatory effect on neural resting state networks depending on the state of depression.

Depression is one of the most frequent psychiatric conditions, with a worldwide estimated prevalence of 4.4% in adults[1], leading to a high disease burden and high costs for health systems[2]. A subgroup of patients with depression shows low-grade inflammation as a relevant disease mechanism of mood disorders[3,4]. These inflammatory processes appear to be associated with, among others, interleukin-6 (IL-6)[5] and tumor necrosis factor-alpha (TNF-α)[6].

Various meta-analyses have consistently reported increased IL-6 and TNF-α concentrations in depressed patients compared to healthy controls[7–9]. IL-6 and TNF-α are associated with specific symptoms and behaviors that co-occur with depression[10–13]. Both translational and human studies have shown that the dysfunctional release of IL-6 and TNF-α leads to depression-like phenotypes, may be linked to diminished psychomotor activity[11], and, in the context of depression, may predict cognitive impairment[14,15].

In the general context of inflammation, IL-6 is a multifunctional pleiotropic cytokine produced by various immune cells, which is involved in systemic inflammatory processes and acute-phase responses[16]. In chronic

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inflammation, such as chronic stress, IL-6 stimulates the release of glucocorticoids[17,18], increases the production of immunoglobulins and B-cell differentiation[19], and enhances the recruitment of monocytes[20]. Further, TNF-α is a Th1 cytokine primarily produced by macrophages and T-cells, which acts as a proinflammatory cytokine [16]. In the context of chronic stress[21], prolonged exposure to TNF-α, reported also in depression[7,22,23], may contribute to an inadequate response of the HPA axis, leading to dysregulated cortisol production and disrupted neuroendocrine responses to stress[6,24–27].

Previous studies have shown that inflammatory processes are also associated with aberrant functional connectivity (FC) within resting state brain networks of patients with depression[28], predominantly the default mode network (DMN) and the salience/ventral attention network (SAL)[29,30]. Meta-analyses have consistently shown that depression is associated with a hyperconnectivity of the DMN[31], and multiple studies demonstrated that aberrant DMN FC is associated with depressive symptoms, such as self-referential thoughts and rumination[32]. What is more, resting state brain networks appear to be associated with changes in peripheral inflammatory activity, as reviewed by Swartz and colleagues[33]. Low-grade inflammation corresponds with high levels of peripheral IL-6 and TNF-α and can influence FC in the DMN and SAL, as suggested by previous research[34,35]. In healthy subjects, for instance, IL-6 levels modulate DMN connectivity[36], and various inflammatory cytokines (e.g., IL-6 and TNF-α) are associated with the functional connectivity of regions involved in the salience network[37]. Given that neural resting state networks show aberrant FC in depression, there is scant documentation of how inflammatory processes are associated with the functional connectivity of resting state networks in people with depression, such as the study of Aruldass and colleagues[28]. Finally, there is a lack of evidence regarding how the peripheral markers of inflammation IL-6 and TNF-α are associated with aberrant network FC of DMN or SAL in depression.

Given this background, we used fMRI to study the association of IL-6 and TNF-α and the functional connectivity of DMN and SAL networks in twenty-four participants with depression and twenty-five community healthy controls. As primary hypothesis, we aimed to compare the association of DMN FC with IL-6 both in people experiencing depressive episodes and healthy participants. As a secondary analysis, we compared the association of DMN FC with TNF-α as well as SAL FC with both IL-6 and TNF-α in both populations.

Based on prior studies, we expect that peripheral inflammation, measured using peripheral IL-6 and TNF-α concentrations, will be associated with decreased FC in both resting state networks DMN and SAL. In addition, we hypothesize that peripheral inflammation will be higher in participants with depression than in the healthy control group, as confirmed in various meta-analyses. Following previous studies[38,39], we expect that DMN and SAL FC will differ between people with depression and healthy controls (H_A: depression ≠ healthy controls).

Results

Sample characteristics

A total of 24 outpatients with a depressive episode (15 females and 9 males) and 25 community healthy control participants (15 females and 10 males) were initially included in the study (Table 1). No statistical differences were observed between patients with depression (DE) and healthy controls (HC) concerning the variables age ($t = -1.62$, $p = 0.113$), BMI ($t = 0.56$, $p = 0.581$), sex ($\chi 2 = 0.03$, $df = 1$, $p = 0.858$), or smoking behavior ($\chi 2 = 0.27$, $df = 1$, $p = 0.667$) as reported in a previous study[40]. Concerning BDI-FS values, as reported previously[40], we observed significant differences between outpatients with depression and community healthy controls ($t = -4.78$, $p < 0.001$). Using the conversion formula of Poole and colleagues[41], participants with depression had shown a converted BDI-II scores of 29.22 ± 14.32 points, and healthy controls a value of 14.13 ± 5.98 points. Study groups differed significantly regarding the level of IL-6, with the outpatient group having significantly higher concentrations than the healthy control subjects ($U = 205.00$, $p = 0.029$; Fig. 1a). In the group of depressed patients, IL-6 concentrations did not correlate with the duration of the disease (Spearman's rho = 0.339, $p = 0.106$), and there was no statistically significant difference in IL-6 levels in patients under medication compared to patients without pharmacological treatment ($U = 25.50$, $p = 0.126$). In contrast to the group differences reported on IL-6, TNF-α concentrations were not significantly different between patients and healthy controls ($U = 247.50$, $p = 0.856$; Fig. 1b). Additionally, TNF-α concentrations did not correlate with the duration of the

	DE	HC	p-Values
Age (in years)	31.88 (12.76)	26.80 (8.72)	0.133
BMI (in kg/m2)	24.05 (4.16)	24.86 (5.86)	0.581
Sex (female/male)	15/9	15/10	0.858
Psychiatric medication (yes/no)	19/5	0/25	< 0.001
Smoking behavior (yes/no)	3/21	2/23	0.667
Disease duration (in years)	9.16 (7.84)		
BDI-FS	7.25 (5.17)	1.80 (2.16)	< 0.001
IL-6 (in pg/mL)	2.25 (2.23)	1.38 (0.78)	0.029
TNF-α (in pg/mL)	8.59 (1.42)	8.95 (1.61)	0.856

Table 1. Sample characteristics (data extracted from[40]). Age and BMI values are expressed as mean (standard deviation). Due to the non-parametric distributions, cytokine concentrations are expressed as median (interquartile range). As described in the methods' section, U-tests for cytokines are expressed with a one-tail p-value.

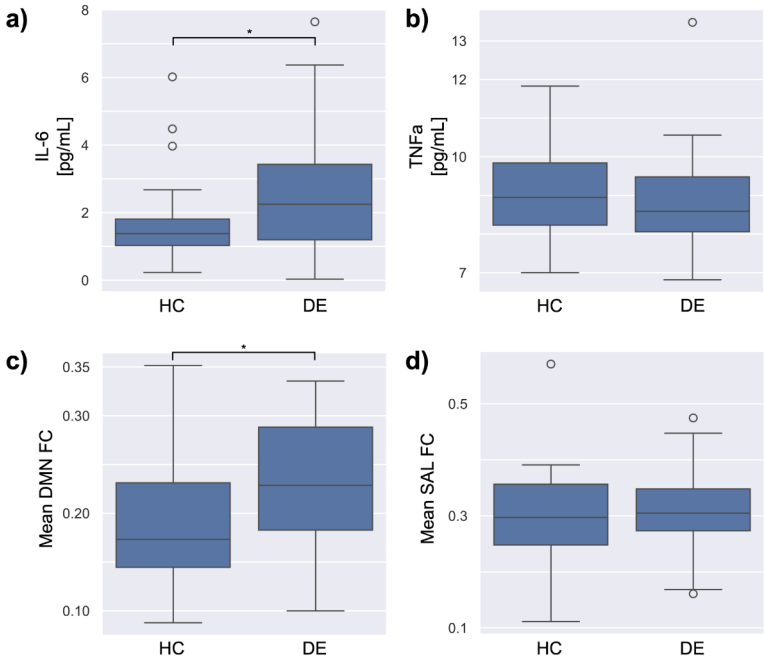

Fig. 1. DE patients show significantly higher IL-6 and mean DMN FC compared to HCs. (**a**) DE and HC showed a significant difference of IL-6 levels (U = 205.0, p = 0.029) with higher IL-6 levels in DEs. (**b**) DE and HC showed similar TNF-α levels (U = 352.5, p = 0.86). (**c**) DEs and HCs showed a significant difference of mean DMN FC (t = -2.37, p = 0.022) with a higher DMN FC in DEs. (**d**) DEs and HCs showed similar mean SAL FC (t = -0.39, p = 0.70). *p < 0.05.

disease (Spearman's rho = 0.133, p = 0.535), and there was no statistically significant difference in TNF-α levels in patients under medication compared to patients without pharmacological treatment (U = 39.50, p = 0.593).

DE patients show significantly higher IL-6 and mean DMN connectivity compared to HCs

In this study, the DMN definition was based on previous work by Schaefer and colleagues[42], parcellating the cerebral cortex into 100 ROIs and attributing them to resting state networks. Corresponding with prior studies, the Student's t-test showed significantly higher DMN connectivity in DE patients compared to HCs (t = -2.37, p = 0.022; Fig. 1c). In contrast to the group differences of DMN FC, DE patients and HCs did not have significant differences of SAL FC (t = -0.39, p = 0.70; Fig. 1d).

IL-6 correlates with DMN connectivity in healthy subjects, but not in patients with depression

Recent studies demonstrated that resting state FC might be associated with peripheral inflammatory markers in humans with depression[28,43]. In contrast to these prior studies, we could not show a significant negative correlation between mean DMN FC and IL-6 level (r = -0.21, p = 0.33; Fig. 2a). This finding starkly contrasts the HCs that did show a significant correlation between mean DMN FC and IL-6 level (r = 0.46, p = 0.02; Fig. 2a). Thereby, HC and DE groups showed significantly different correlations of mean DMN FC and IL-6 levels (t = 2.32, p = 0.02; Fig. 2a).

Within the scope of our secondary analysis, we wanted to investigate how TNF-α, a pro-inflammatory cytokine, is associated with DMN FC. In this analysis, neither DEs nor HCs showed a correlation of mean DMN FC and TNF-α level (DE: r = 0.02, p = 0.91; HC: r = 0.25, p = 0.23; Fig. 2b). Thus, IL-6 but not TNF-α is associated with DMN FC in healthy subjects. However, neither IL-6 nor TNF-α is associated with DMN FC in depressed patients.

TNF-α correlates with SAL FC in patients with depression but not in healthy subjects

A previous similar study has shown that systemic inflammatory exogenous responses, reflected as increases in peripheral IL-6 and TNF-α after LPS-induced inflammation, did not show an association with SAL FC in healthy humans[35]. In our study, mean SAL FC did not show a significant association with IL-6 levels in either group (DE: r = 0.20, p = 0.34; HC: r = 0.12, p = 0.58; Fig. 2c). Instead, the SAL network was negatively correlated with TNF-α levels in DE group (r = -0.43, p = 0.034; Fig. 2d) but not in HCs (r = 0.13, p = 0.53; Fig. 2d). Thereby, HCs and DEs showed significantly different correlations of mean SAL FC and TNF-α levels (t = 1.96, p = 0.049; Fig. 2d). Thus, whereas DMN mean FC is correlated with IL-6 in HCs, the SAL FC is correlated with TNF-α in DE group.

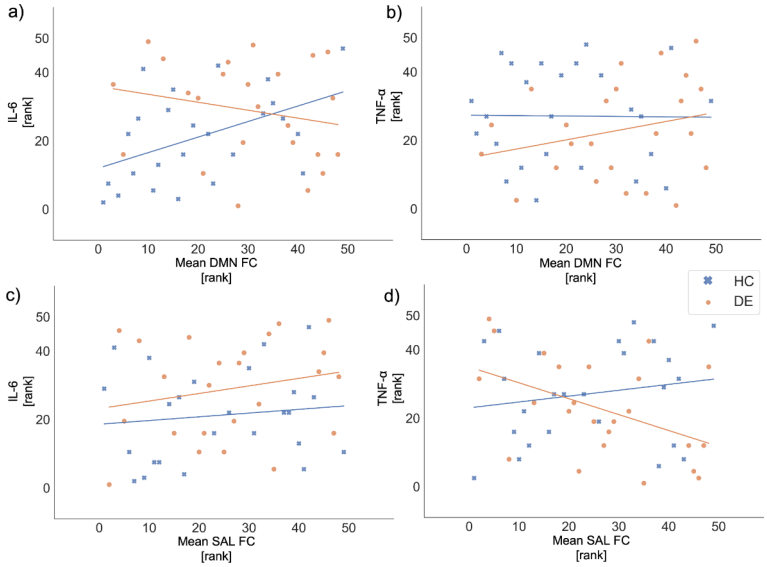

Fig. 2. DMN FC is significantly correlated with IL-6 in HCs, but not in DEs, SAL FC is significantly modulated by TNF-α in DEs, but not in HCs. (**a**) Healthy controls (HCs) showed a significant correlation of mean DMN FC and IL-6 level ($r = 0.46$, $p = 0.02$). Depressed patients (DEs) did not show a significant correlation of mean DMN FC and IL-6 level ($r = -0.21$, $p = 0.33$). HCs and DEs show significantly different correlations of mean DMN FC and IL-6 levels ($t = 2.32$, $p = 0.02$). (**b**) Neither DEs nor HCs showed a correlation of mean DMN FC and TNF-α level (DE: $r = 0.02$, $p = 0.90$; HC: $r = 0.25$, $p = 0.23$). (**c**) Neither DEs nor HCs showed a correlation of mean SAL FC and IL-6 level (DE: $r = 0.20$, $p = 0.34$; HC: $r = 0.12$, $p = 0.58$). (**d**) DEs showed a significant correlation of mean SAL FC and TNF-α level ($r = -0.43$, $p = 0.034$). HCs did not show a significant correlation of mean SAL FC and TNF-α level ($r = 0.13$, $p = 0.53$). HCs and DEs show significantly different correlations of mean SAL FC and TNF-α levels ($t = 1.96$, $p = 0.049$).

Discussion

This study investigated the association between peripheral inflammatory markers and neural resting state connectivity. Firstly, people with depression showed higher IL-6 concentrations compared to healthy subjects, while TNF-α levels did not differ between both groups. Secondly, we could show that depressed patients experience elevated DMN FC in comparison to healthy subjects, while SAL FC did not differ between both groups. We further investigated the link between peripheral inflammation and resting state FC in both depressed patients and healthy controls. Here, resting state networks DMN and SAL were associated with different inflammatory markers depending on whether a person has a depressive phenotype or is healthy. Firstly, IL-6 levels correlated with DMN FC in healthy subjects, while this association is absent in the SAL network. In patients with depression, IL-6 did neither correlate with DMN nor SAL FC. Secondly, TNF-α levels correlated with SAL FC in patients with depression, but not DMN FC. Conversely, in healthy subjects, TNF-α was not associated with the FC in either of these networks.

As previously reviewed by various meta-analyses, people with depression show increased peripheral proinflammatory cytokines, especially IL-6 and TNF-α[7,23,44]. In accordance with these previous reports, we were able to demonstrate higher IL-6 concentrations in people with depression in comparison with healthy controls. However, results of the current study suggest that TNF-α concentration in participants with depression was not higher than the healthy controls. The latter finding contrasts meta-analytic evidence suggesting that both TNF-α and IL-6 are among the typically increased peripheral proinflammatory cytokines in depression[7,23,44]. As prior studies described significant differences in TNF-α concentrations in patients with severe forms of depression, i.e., inpatients[45,46], or patients with suicidal behavior[47] compared to healthy controls, these findings may be attributed to the outpatient collective.

A well-established functional neuroimaging finding in humans is that patients with depression show altered DMN connectivity[48,49]. In our study, patients with depression had increased DMN FC in comparison to healthy subjects, consistent with previous research[50,51]. We could further show that depressed patients and healthy controls have similar SAL FC. In contrast to the reported results in our study, Huang and colleagues showed that depressed patients have reduced SAL FC compared to healthy individuals[39]. These differing results might be attributed to sample differences between both studies: for instance, while we evaluated exclusively outpatients, Huang and colleagues included both inpatients and outpatients in their study.

We could further show that resting state networks DMN and SAL were associated with inflammatory markers depending on whether a person has a depressive phenotype or is healthy.

In healthy subjects, DMN FC correlated with IL-6 levels. Even though few studies have investigated the association of resting state brain networks and inflammation in healthy subjects, this finding corresponds with earlier similar studies on DMN FC in inflammation[36,52–54]. In depressed patients, we did not find significant associations between IL-6 and DMN FC. To our knowledge, there are only two studies that have investigated

the relationship between depressive symptoms, IL-6, and DMN FC[36,43] that show contradictory results. On the one hand, Park and colleagues demonstrated that IL-6 had mediating effects between DMN FC and levels of depressive symptoms[43]. On the other hand, Marsland and colleagues did not find any association between DMN FC, depressive symptoms, and IL-6 concentrations[36]. Our results align with the latter study, as we cannot report a significant association between DMN FC and IL-6 levels in depression.

In people with depression, SAL FC correlated negatively with TNF-α levels. To our knowledge, this is the first study that reported the association of TNF-α on the SAL FC in patients with depression. Related studies in healthy subjects have focused on the association between peripheral inflammation and connectivity of salience networks, with inconclusive results regarding the interplay of TNF-α levels and SAL FC. For example, in a study assessing anxiety in healthy subjects, resting state FC in salience networks did not show any association with the inflammatory markers TNF-α[35]. This finding corroborates our results that TNF-α is not correlating with SAL FC in healthy subjects.

Furthermore, we could not show an association of peripheral IL-6 levels and SAL FC in both groups. Also, DMN FC did not show significant associations with TNF-α concentrations in both groups. This is in line with related previous studies of healthy subjects that could not report associations between peripheral IL-6 concentrations and the SAL network. For example, mean resting state connectivity of the anterior SAL network did not correlate with IL-6 levels after a surgical procedure in mentally healthy subjects[55]. IL-6 levels were also not correlated with SAL FC in healthy adults and patients with mild cognitive impairment[56].

Our results suggest that the peripheral inflammatory markers IL-6 and TNF-α might exert a modulatory effect on neural resting state networks depending on the status (i.e., depression diagnosis or healthy controls). The different effects on neural resting state networks might be attributed to the distinct biological functions of both cytokines.

Neuropsychiatric disorders, such as depression, reveal peripheral inflammatory processes that challenge traditional understanding of brain-immune boundaries. In general, both IL-6 and TNF-α are proinflammatory cytokines and peripherally could cross the blood brain barrier[57] and stimulate central cytokine production[58,59], which in turn generates microglial activation[60]. Critically, these inflammatory dynamics manifest without widespread blood–brain barrier disruption, challenging previous assumptions about immune system penetration[61]. Instead, translational studies reveal nuanced, region-specific permeability changes, particularly in areas like the nucleus accumbens[62], but not prefrontal cortex or hippocampus[62]. This sophisticated mechanism suggests that immune interactions with neural tissue are highly localized and targeted, rather than systemic. These targeted inflammatory processes may explain the differential effects observed on various resting state brain networks. By modulating neural connectivity through precise, region-specific mechanisms, inflammatory markers potentially contribute to the pathophysiological complexity of neuropsychiatric disorders.

Moreover, the specific immune signaling pathways and receptors involved within the CNS may differ between IL-6 and TNF-α. While TNF-α primarily acts through TNF receptors in order to activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)[6,63], IL-6 signals through the IL-6 receptor complex, which includes gp130[64–66]. These differences in receptor pathways may lead to distinct downstream effects and interactions within the CNS. Overall, this might have an implication in the different activation patterns in the CNS, although both cytokines could stimulate centrally the cytokine production[58] and increase through signaling the amount of glutamate in the CNS[67,68]. Based on these mechanisms, we interpret the different correlation patterns found in our data as a possible indicator of the biological differences that exist between the signaling pathways of IL-6 and TNF-α in the CNS, and these differences might be influenced by the varying effects of these cytokines on individuals diagnosed with depression compared to those without psychiatric disorders. In any case, larger studies are needed to confirm the different biological effects of both proinflammatory cytokines on the FC networks as well its differences in depression.

In addition, IL-6 and TNF-α might have different effects on the HPA axis under chronic stress conditions. IL-6 leads to increased cortisol production under chronic stress conditions[69,70], while prolonged exposure to TNF-α might suppresses the HPA axis activity[71] and, in some cases, TNF-α is negatively associated with cortisol production[6,72], although this finding is being widely discussed in the case of depression[59,72]. As previous studies have reported that resting state FC is associated with the activity of the HPA axis[73,74] and IL-6 as well as TNF-α show in some cases opposing biological effects on the HPA axis[25,75,76], IL-6 and TNF-α might exert distinct effects on DMN and SAL FC potentially via the HPA axis. Although the differential effects of both proinflammatory cytokines on the HPA axis could explain the correlation patterns, more studies are needed to confirm and understand the different biological effects of both cytokines under chronic stress conditions, such as depression.

Our findings highlight the importance of systemic inflammatory processes and suggest the modulation of neural resting state networks, a potential interface for influencing cognitive processes and symptoms occurring during depression[77]. Different immunological processes might differentially affect neural resting state networks in depressive patients and healthy controls. Specifically, TNF-α showed significant association with SAL FC in depressed patients, while IL-6 was associated with DNM FC in healthy controls.

However, it is important to acknowledge several limitations of this study when interpreting our results. Firstly, although significant associations were observed between TNF-α and SAL FC in depression, causal inferences cannot be made due to the cross-sectional nature of the study. Longitudinal studies with appropriate control groups are needed to establish causality. Secondly, factors such as medication intake, diet, genetic factors, socioeconomic stressors, and disease duration within the patient group may have influenced the expression of peripheral cytokines and the resting state FC. Further studies on immunological markers with a therapy-naive group are necessary to validate these findings. Additionally, while differences in DMN and IL-6 were observed between patients and healthy controls, these clinical sample characteristics corresponded to the collective of outpatients. Therefore, studies of patients with severe depressive episodes might allow further conclusions

regarding the association of peripheral inflammation and neural resting state FC in depression. Finally, it is worth noting that the sample included more females than males for both groups. However, as previously mentioned[40], these proportions are in line with statistical and epidemiological reports on depression.

In light of our results, we can conclude that peripheral IL-6 is associated with DMN FC in healthy subjects, but not in depression. Additionally, we found that peripheral TNF-α is correlated with SAL FC exclusively in people with depression. The different correlation patterns between groups and connectivity networks may partially depend on the distinct signaling pathways of these proinflammatory cytokines in the central nervous system and their differential effects on the HPA axis in chronic stress conditions, such as depression. However, additional longitudinal studies with larger sample sizes and the inclusion of diverse patient subgroups (e.g., therapy-naïve participants) are required to enhance the generalizability and confirm the robustness of these findings. Future studies should consider models that include health-related variables, such as lifestyle habits, nutrition, and sleep quality, which may interact with the relationship between depression diagnosis, peripheral inflammation, and imaging findings. Additionally, these studies should incorporate mediation analyses to examine the proportion of explained variance in the associations between inflammation and connectivity in depression.

Materials and methods

Study design

This study is incorporated into a larger project investigating brain endophenotypes related to depression and perceived fatigue[40,78]. The following neuroimaging case–control study was conducted at the University Hospital of Giessen, Germany, from June to September 2019. This investigation was performed according to the principles of the Helsinki Declaration and its most recent revisions, as well as the ethical standards of the American Psychology Association. It was also approved by the ethics committee of the medical faculty of Justus-Liebig University in Giessen (AZ 81/18). Before participation, each participant or its legal authorized representative was fully informed of this study and gave their written consent to participate. Declarations of consent in the original language (German) are available on request.

Participants

Fifty-one participants were initially recruited for this study (19 males and 32 females) with a mean (SD) age of 29.45 (11.16) years (range 18–64 years), of which two revoked their consent due to their refusal to have blood sampled in the experimental study (a 21-year-old female healthy control participant and a 44-year-old female participant with depression). Finally, twenty-four participants with depressive episodes (DE group; 9 males and 15 females) with a mean (SD) age of 31.88 (12.76) years (range 20–63 years) and twenty-five community health controls (HC group; 10 males and 15 females) with a mean (SD) age of 26.80 (8.73) years (range 18–64 years) completed the experimental investigation as previously described[40].

Patients were eligible for inclusion if they met the clinical criteria for depressive episodes (F33.x and F32.x) as defined by the International Classification of Diseases, 10th edition (ICD-10). Assessments were conducted by clinical experts from the Department of Psychiatry at the University Hospital of Giessen. Patients with psychotic disorders, individuals with any medical condition that might interfere with the investigation (especially infection-related diseases or autoimmune diseases), and those with MRI contraindications were not eligible to partake in this study. Comorbid personality or anxiety disorders were tolerated in this study.

Evaluation of burden of depression symptoms: Beck depression inventory—fast screening

As previously reported in our DTI publication[79], we used the German-validated version of the Beck Depression Inventory—Fast Screening (BDI-FS) to assess depressive symptoms and their impact on each participant[80]. The BDI-FS is a validated short version of the BDI-II, which evaluates self-criticism, self-aversion, past failure, pessimism, anhedonia, sadness, and suicidal behavior. The BDI-FS consists of 7 items, with scores ranging from 0 to 21 points. These scores can be categorized into four levels of severity: minimal (0–3 points), mild (4–8 points), moderate (9–12 points), and severe (13–15 points)[78]. According to Poole and colleagues[41], the formula to convert BDI-FS scores into BDI-II scores is: BDI-II = [2.77*(BDI-FS)] + 9.14 points.

Laboratory measures

Based on prior studies[5,23,81,82] and in accordance with our previous findings on systemic inflammation and fatigue in depression[82], we aimed to study the association of neural resting state networks with IL-6 and TNF-α levels.

For the measurement of IL-6, the blood sample extraction and processing protocols were established based on previous studies conducted by our working group[40,78]. Participants underwent peripheral venous sampling, with approximately 5 mL of fasting samples drawn into EDTA tubes (K-EDTA, SARSTEDT AG & Co. KG, Nümbrecht, Germany). Immediately after collection, the blood samples were centrifuged at 1100xg at 4 °C for 15 min. Subsequently, plasma was extracted from the two sample tubes, and the remaining sample was discarded. The plasma was initially preserved at -20 °C and then transported at -80 °C to a university center specialized in clinical immunology before further processing and measurement.

For IL-6 and TNF-α measurements, a Quantikine ELISA kit (R&D Systems Inc., Minneapolis, Minnesota, United States of America) was deployed, following the protocols outlined in our previous studies[40,78]. Intra- and inter-precision values were maintained below 10%. Any values below the minimum detectable dose (n = 1 for IL-6) were considered zero and included in the analysis. Cytokine concentrations were estimated using a Tecan Reader with the Magellan Reader Software (Tecan Group Ltd., Männedorf, Switzerland), with parameters calculated using Marquardt's 4-parameter estimation method, as specified in our previous protocols[40,78].

MRI protocol

Images were acquired on a high-resolution Siemens Prisma 3.0 MRI scanner (Siemens Healthineers, Germany) with a 64-channel head/neck coil at the Bender Institute for Neuroimaging, Faculty of Psychology, University of Giessen[40].

The MRI protocol included a structural T1 weighted image (MPRAGE; sMRI) and blood-oxygen-level dependent (BOLD) functional MR imaging (fMRI) sequences. T1-weighted structural images were obtained with a repetition time (TR) of 1.58 s, echo time (TE) = 2.3 ms, flip angle = 8°, 176 slices, slice thickness = 0.94 mm, voxel dimensions = 0.94 × 0.94 × 0.94 mm, FoV = 240 × 240 mm.

An echo planar imaging (EPI) sequence (TR = 1500 ms, TE = 30 ms, flip angle = 71°, FOV = 214 × 214 mm, voxel dimensions = 3.0 × 3.0 × 3.0 mm, gap 0.8 mm, 40 slices, with iPAT, accel. factor slice 2, multiband acceleration factor 2, bandwidth = 1876 Hz/px) was used for BOLD fMRI. Resting state fMRI was obtained with eyes open for 5.5 min looking at a projected fixation cross.

Field maps (TR = 1000 ms, TE1 = 10.00 ms, TE2 = 12.46 ms, voxel dimensions = 2.0 × 2.0 × 2.5 mm) were acquired using a gradient echo planar imaging sequence to correct field distortions in the functional images.

Preprocessing

Imaging data were minimally preprocessed using the fMRIPrep pipeline, version 23.2.0[83]. Specifically, the following preprocessing stages were performed: (a) brain extraction of the sMRI scan; (b) normalization of the sMRI scan to the standard T1 template of the Montreal Neurological Institute (MNI template: MNI152NLin2009cAsym, 2 mm resolution); (c) segmentation and (d) surface reconstruction of the sMRI scan; (e) head-motion estimation and (f) slice-time correction of the fMRI scan; (g) coregistration of the fMRI to the sMRI scan; (h) fieldmap estimation and susceptibility distortion correction of the fMRI scan.

In accordance with the guidelines for functional connectivity (FC) processing[84], 36 parameter nuisance regression (six degrees of motion, global signal, white matter and CSF, with temporal, quadratic, and quadratic temporal derivatives) were used for the denoising step deploying the function clean_img of the toolbox nilearn along with a 0.009 to 0.1 Hz band-pass filtering[85]. Spatial smoothing was applied with a 6 mm FWHM kernel and the first five acquired volumes were discarded. All fMRI scans contained less than 25% of volumes with a framewise displacement > 0.5 mm[86]. Both groups contained comparable numbers of motion outliers with mean (standard deviation) of 4.2 (9.3) volumes in healthy subjects having a framewise displacement of > 0.5 mm and 1.4 (2.7) volumes being affected by motion outliers in depressed patients. According to Shapiro's test, outlier values for either group followed a normal distribution. No statistical differences of motion outlier data were observed between patients and healthy controls using a two-sample t-test (t = -1.45, p = 0.15).

Functional connectivity (FC)

Following the preprocessing, functional connectivity (FC) between each pair of ROIs was calculated based on the pairwise Pearson correlation coefficient of the signal "time series" of each ROI. The resulting N x N FC matrix showed the pairwise connectivity strength between ROIs. This procedure was repeated independently for each scan of patients and healthy controls.

Converging evidence suggests that the connectivity within the DMN is a key feature of the neurobiology of depression[30–32]. Therefore, we specifically evaluated ROI-to-ROI FC within the DMN as defined by Schaefer and colleagues[42], consisting of 24 bilateral regions including temporal lobe (left/right Temp_1, left/right Temp_2 and right Temp_3), parietal lobe (left/right Par_1 and left Par_2), left prefrontal cortex (PFC_1 to PFC_7), right ventral prefrontal cortex (PFCv_1 and PFCv_2), left and right precuneus posterior cingulate cortex (pCunPCC_1 and pCunPCC_2) and right dorsal as well as medial prefrontal cortex (PFCdPFCm_1 to PFCdPFCm_3). More precisely, whole-brain connectivity matrices of 100 ROIs were reduced to these 24 DMN regions. Next, we calculated the mean connectivity within the DMN. For the association of the mean DMN-connectivity with the immunological parameters IL-6 and, as a secondary hypothesis, TNF-α we used Spearman's correlation and compared the group of patients with depression and healthy controls with Fisher's algorithm for unpaired correlations (see Statistics).

With the Salience/Ventral Attention network (SAL) being another major network affected by depression[87], we tested this network and its association to the immunological markers IL-6 and TNF-α as an exploratory hypothesis. We specifically evaluated ROI-to-ROI FC within the SAL as defined by Schaefer and colleagues[42], consisting of 12 bilateral regions including the parietal operculum (left ParOper_1), left and right frontal operculum and insula (left/right FrOperIns_1 and FrOperIns_2), left lateral prefrontal cortex (left PFCl_1), left and right frontal and parietal medial cortex (left/right Med_1, left/right Med_2, left Med_3) and right temporal occipital and temporal parietal cortex (right TempOccPar_1 and right TempOccPar_1). Further analysis was performed according to the procedure described above.

Statistical analysis

According to Shapiro's test, IL-6 and TNF-α values did not show a normal distribution. Thus, we used Spearman's correlation coefficient to assess the association of the mean DMN-connectivity with the immunological parameter IL-6 of each group as the primary hypothesis. The correlation coefficient data of both groups were compared using a two-sample t-test after Fisher's z-transform method (book 'Statistical Methods for Psychology')[88]. Significant differences in the FC of resting state networks between groups were defined with a two-tailed p-value of $p \leq 0.05$. Significant differences for the non-parametric parameters IL-6 and TNF-α were tested with a Mann–Whitney-U-Test, using a one-tailed p-value of $p \leq 0.05$ based on reports of prior meta-analyses that indicate a high IL-6 and TNF-α concentrations in depression[7,44]. As secondary hypotheses, we used Spearman's correlation coefficient to assess the association of the mean SAL-connectivity with the immunological parameter IL-6 of each group and the mean DMN- and SAL-connectivity with the immunological parameter TNF-α of each

group. Significant associations of FC of resting state networks and immunological markers were defined with a two-tailed p-value of $p \leq 0.05$.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to the Data protection law of the European Union, but are available from the corresponding author on reasonable request.

Received: 19 September 2024; Accepted: 3 January 2025
Published online: 13 January 2025

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Acknowledgements

The authors thank Bernd Hanewald, M.D., and his team for supporting patient recruitment. Additionally, we acknowledge the Department of Internal Medicine and Rheumatology at the JLU for their assistance in analyzing blood samples. We sincerely thank Ms. Carina Scheiyäck and Ms. Mona Arnold from this department for their support in blood sample analysis. This study was made possible with the support of DAAD (German Academic Exchange Service) and the Foundation "Immunität und Seele."

Author contributions

CNS: Conceptualization, formal analysis, methods, writing—original draft, visualization GS: Idea, conceptualization, supervision, funding, data processing, writing—review. EN: data curation, reaction times processing laboratory methods, supervision, writing—review. CB: writing—review, methods, data curation GG: writing—review, visualization HA: writing—review, visualization EKL: writing—review, visualization BPP: Idea, conceptualization, data curation, formal analysis, writing—original draft, visualization.

Funding

This study is part of a project entitled: "Correlations between systemic inflammation, brain endophenotypes and fatigue in participants with depression". This project has received a financial support from the German Exchange Academic Program (DAAD). This program included also the financial support of the project, that involved mainly material expenses and participants' expenses for participation. Additionally, the authors of this project contacted the owner of the "*Immunität und Seele*" foundation (Prof. Dr. Norbert Müller), that awarded this project and supported financially. Both supporters have no role in the design of the study, data collection, analysis and interpretation of results.

Employment: The authors of this study were not hired by any organization that may gain or lose financially through this publication.

Personal financial interests: With exception of point "Funding", for this study the authors did not receive any compensation (monetary, shares, etc.) from companies, consultation fees or other forms of remuneration from any organizations.

Declarations

Competing interests

Gerhard Gründer has served as a consultant for Boehringer Ingelheim, Institute for Quality and Efficiency in Health Care (IQWiG), Janssen-Cilag, Lundbeck, MindMed, Otsuka, Recordati, Roche, ROVI, Sage, and Takeda. He has served on the speakers' bureau of Gedeon Richter, Janssen Cilag, Lundbeck, Otsuka, Recordati, and ROVI. He has received grant support from Beckley Psytech and Boehringer Ingelheim. He is co-founder and/or shareholder of Mind and Brain Institute GmbH, OVID Health Systems GmbH and MIND Foundation gGmbH. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interest. Funding: This study is part of a project entitled: "Correlations between systemic inflammation, brain endophenotypes and fatigue in participants with depression". This project has received a financial support from the German Exchange Academic Program (DAAD). This program included also the financial support of the project, that involved mainly material expenses and participants' expenses for participation. Additionally, the authors of this project contacted the owner of the "Immunität und Seele" foundation (Prof. Dr. Norbert Müller), that awarded this project and supported financially. Both supporters have no role in the design of the study, data collection, analysis and interpretation of results.

Ethics approval and consent to participate

Ethical approval and consent to participate: This study was approved by the ethic committee of Faculty of Medicine of the Justus Liebig University. Additionally, this study is part of a big project to investigate inflammatory factors and fatigue in patients with depression and multiple sclerosis. The code of this project in

the ethic committee is AZ 81/18. Ethical approval and the informed consents in its original language (German) are by request.

Consent for publication

Not applicable.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-85514-0.

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