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Highlights

- Mechanisms of brain function and inflammation in IBS-related fatigue are unclear.
- A mesocorticolimbic network relevant to fatigue was established and investigated.
- In IBS, brain connectivity was decreased, when considering fatigue impact.
- High fatigue impact on the lives of IBS patients is related to TNF- α in plasma.
- The vulnerability to fatigue in IBS has both central and inflammatory correlates.

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Fatigue in irritable bowel syndrome is associated with plasma levels of TNF- α and mesocorticolimbic connectivity

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Abstract

Irritable bowel syndrome (IBS) is a symptom-based disorder of gut-brain interactions generating abdominal pain. It is also associated with a vulnerability to develop extraintestinal symptoms, with fatigue often reported as one of the most disturbing. Fatigue is related to brain function and inflammation in several disorders, however, the mechanisms of such relations in IBS remain elusive. This study aimed to elucidate fatigue and its association with a resting state network of mesocorticolimbic regions of known importance in fatigue, and to explore the possible role of circulating TNF- α levels in IBS and healthy controls (HC).

Resting state functional magnetic resonance imaging (fMRI) was conducted in 88 IBS patients and 47 HC of similar age and gender to investigate functional connectivity between mesocorticolimbic regions. Further, fatigue impact on daily life and plasma levels of the proinflammatory cytokine tumor necrosis factor- α (TNF- α), of known relevance to immune activation in IBS, were also measured. The selected mesocorticolimbic regions indeed formed a functionally connected network in all participants. The nucleus accumbens (NAc), in particular, exhibited functional connectivity to all other regions of interest. In IBS, fatigue impact on daily life was negatively correlated with the connectivity between NAc and dorsolateral prefrontal cortex bilaterally (left $p = 0.019$; right $p = 0.038$, corrected for multiple comparisons), while in HC, fatigue impact on daily life was positively correlated to the connectivity between the right NAc and anterior middle insula in both hemispheres (left $p = 0.009$; right $p = 0.011$). We found significantly higher levels of TNF- α in IBS patients compared to HC ($p=0.001$) as well as a positive correlation between TNF- α and fatigue impact on daily life in IBS patients ($\rho=0.25$, $p=0.02$) but not in HC ($\rho=-0.13$, $p=0.37$). There was no association between functional connectivity in the mesocorticolimbic network and plasma levels of TNF- α in either group. In summary, this novel multimodal study provides the first evidence that the vulnerability to fatigue in IBS is associated with connectivity within a mesocorticolimbic network as well as immune activation. These

findings warrant further investigation, both peripherally and potentially with measurements of central immune activation as well.

Key words: Irritable bowel syndrome, vulnerability, fatigue impact, TNF- α , immune activation, resting state fMRI, mesocorticolimbic network, gut-brain interactions, inflammation

1. Introduction

Irritable bowel syndrome (IBS) is a symptom-based disorder of gut-brain interactions, characterized by abdominal pain in combination with altered bowel habits, in the absence of structural or metabolic abnormalities (1-4). It affects about 10% of the general population with a female predominance and has major individual and societal implications (3, 5). Symptoms are difficult to treat and lead to significant reductions in quality of life and high healthcare costs (3, 6, 7). In addition to the defining gastrointestinal symptoms, IBS patients typically report a range of extraintestinal symptoms, of which fatigue is one of the most common and disturbing (8-10). In a recent meta-analysis, 54% of IBS patients experienced fatigue (9), which is in accordance with a previous systematic review that found chronic fatigue syndrome as well as depression and anxiety in a large proportion of IBS patients (11). Higher levels of fatigue in IBS patients are associated with greater stress, depression, and trait anxiety (12). Together, these extraintestinal symptoms contribute to the disease burden and have a negative impact on quality of life (13, 14). Fatigue in IBS may hence increase vulnerability to symptom chronicity and treatment failure as well as contribute to inter-individual variability in this complex biopsychosocial disorder.

As a biopsychosocial disorder, the etiology of IBS is multifactorial. The pathophysiology is heterogeneous and incompletely understood, involving alterations of the gut-brain axis including central nervous system (CNS) processing, visceral hypersensitivity, altered gut microbiota, permeability, and mucosal immune alterations (3, 15, 16). A maladaptive stress response as well as peripheral immune system alterations, including systemic low-grade inflammation, have been proposed as contributing factors in IBS development and symptoms (16-20). This is supported by elevated concentrations of circulating proinflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin (IL)-6, IL-8, IL-1 β in IBS patients (21-23), and consistent with evidence that acute inflammation induces visceral hyperalgesia in healthy individuals (24, 25). Elevated peripheral TNF- α levels were found to correlate with both gastrointestinal IBS symptoms (26), as well as with some extraintestinal comorbidities in IBS, including chronic fatigue syndrome (21). However, existing studies assessing associations between cytokines and symptoms in IBS remain scarce and inconsistent (22, 27), and existing brain imaging studies in IBS have not considered fatigue and its possible connection to peripheral inflammation. In light of current knowledge on the role of peripheral proinflammatory cytokines in hyperalgesia and fatigue (28-30) in other conditions such as cancer, multiple sclerosis, chronic fatigue syndrome, diabetes, and chronic pain (30-35), more research in IBS is called for, especially in the consideration of central mechanisms.

While functional and structural brain alterations in IBS have been well established (36), existing brain imaging studies have not targeted fatigue. Fatigue is primarily a physiological, adaptive response and a somatic signal to rest in response to motor and sensory inputs. This has an important function during, for instance, infection. In chronic fatigue, this adaptive function is lost (28, 37). Within the brain, several regions of the mesocorticolimbic circuit

have been proposed to play a role in different aspects of fatigue. These include the nucleus accumbens (NAc) in the ventral striatum (38), which is reportedly essential for the motivational dimension of fatigue (30), the anterior cingulate cortex (ACC) and the prefrontal cortex, with linkage to the cognitive aspects of fatigue (30, 39, 40), and finally the anterior insular cortex with involvement in the perception of interoceptive signals, adding a physical dimension to fatigue (31, 41-43). During immune activation, proinflammatory cytokines have been demonstrated to inhibit the synthesis, activity, and expression of neurotransmitters such as dopamine and serotonin within the mesocorticolimbic system (44-46). A majority of previous fMRI research on central fatigue focused on functional activity within specific brain regions (30), whereas changes in the connectivity between different regions might also play an important role (47-49). Altered resting state connectivity between specific subregions of the mesocorticolimbic network has recently been demonstrated in multiple sclerosis-related fatigue, proposing the ventral striatum as a key hub (50).

Therefore, we conducted a multimodal study with a focus on fatigue in a comparatively large and well-characterized sample of IBS patients and healthy controls (HC). Using a combination of different analyses, we aimed to elucidate fatigue and its association with a resting state network of mesocorticolimbic regions related to fatigue (30) and to explore the possible role of circulating TNF- α levels in IBS and HC.

2. Methods

2.1. Participants

Eighty-eight IBS patients, fulfilling Rome III criteria (51), were recruited from the Gastroenterology Department, University Hospital in Linköping. None of the participants reported a history of gastroenteritis. Forty-seven HC of similar age and gender without a medical history of gastrointestinal symptoms or complaints were recruited by advertisement. Exclusion criteria for both study groups were any organic gastrointestinal disease including inflammatory bowel disease, coeliac disease, and gastrointestinal malignancies, metabolic or neurological disorders, and severe psychiatric disease (e.g. schizophrenia, bipolar disorder and psychosis). This was determined based on the participants' medical registries. Further exclusion criteria were nicotine intake within the last 2 months and inability to speak Swedish fluently. In addition, IBS patients (n=33) taking a stable and low dose of psychopharmaceuticals, such as antidepressants prescribed for the treatment of IBS symptoms, were not excluded from participation. Prior to Magnetic Resonance Imaging (MRI), a standard screening was further performed to ensure no contraindications for MR scanning (e.g. metallic or electrical implants, history of claustrophobia, pregnancy etc.). All participants gave written informed consent to participate in the study, which was approved by the Regional Ethical Review Board in Linköping, Sweden (Ref. nos. 2013/506-32; 2014/264-32).

Note that data presented here have been collected as part of a larger study on brain-gut interactions. Findings from different subsamples of this study cohort addressing brain glutamate and GABA neurotransmission and their putative role in pain and anxiety (52, 53), gut bacterial passage (54) and its relation to measures of brain connectivity (55), have previously been published. None of these reports included inflammatory markers, fatigue, or the mesocorticolimbic system, which is the focus of the current analysis.

2.2. Procedures

2.2.1. Questionnaires

The following scales were used to assess fatigue impact and to characterize participants with respect to socioeconomic status, IBS symptom severity, anxiety, and depression.

Questionnaires were provided to participants 2-4 weeks before the day of the MRI scan, and were returned to the lab by the time of the blood draw.

2.2.1.1. Modified Fatigue Impact Scale

As a multifaceted symptom, fatigue is challenging to objectively measure and evaluate (30, 56). A frequently applied and reliable method to capture fatigue is to estimate its impact on daily life, as accomplished herein using the modified Fatigue Impact Scale (mFIS) (57-59). In the mFIS questionnaire, fatigue is defined as “a feeling of physical tiredness and lack of energy that many people experience from time to time”. It contains 21 questions addressing the impact of fatigue on physical, cognitive, and psychosocial dimensions of participants’ daily life during the past four weeks. Responses are scored on a five-point scale (0 = never, 1 = rarely, 2 = sometimes, 3 = often, and 4 = almost always), generating a total mFIS score ranging from 0–84. The questionnaire was originally developed for use in multiple sclerosis, but has also been validated for research in gastrointestinal disease (59, 60). For readability, we will refer to the measures of ‘fatigue impact on daily life’ as ‘fatigue impact’ throughout the manuscript.

2.2.1.2. MacArthur scale of subjective social status (SES ladder)

The MacArthur Scale of Subjective Social Status was used to assess the common sense of the individual's social status, which is tightly linked to health status and psychological functioning (61, 62). A summative measure of the subjective social status is provided using a symbolic stepladder image.

2.2.1.3. Hospital Anxiety and Depression Scale

Anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS) (63). Sum scores ranging from 0 to 21 indicate the severity of current symptoms of anxiety and depression, respectively.

2.2.1.4. IBS Severity Scoring System

Gastrointestinal symptom severity was assessed with the IBS Severity Scoring System (IBS-SSS) (64), which evaluates the severity of abdominal pain, distension, stool frequency, consistency, and interference with daily life. The total scores define mild (75–175), moderate (175–300), and severe symptoms of IBS (scores > 300).

2.2.2. Measurements of plasma TNF- α concentrations

Six milliliters of venous blood were collected in EDTA-treated tubes for measurements of TNF- α concentrations in plasma. For reasons of feasibility, this was accomplished sometime between 7:30 a.m and 5 p.m within a mean of 9 days (1-24) after the MR scan for IBS patients and within a mean of 6 days (1-14) for controls. The participants were instructed to refrain from the use of anti-inflammatory drugs within 24 hours prior to blood draw. No systematic differences emerged between groups with respect to the time

of the day when blood was drawn (data not shown). For each ml of blood, a solution of 1.3 mg EDTA and 50 ml Trasylol 10 000 KiE was added. Blood samples were centrifuged (15 min, 3400g) in 4°C, and after centrifugation, plasma was removed and transferred to new tubes that were frozen in -80°C until analysis.

To quantify levels of TNF- α , an ultrasensitive human TNF- α ELISA kit was used according to the manufacturer's instructions (KHC3014 Invitrogen, Thermo Fisher Scientific, Sweden, inter-assay coefficient of variance = 8.5%, intra-assay coefficient of variance = 5.9%, analytical sensitivity <0.09 pg/ml, assay range 0.5-32 pg/ml).

Undiluted plasma samples, controls (positive and negative), and standard point samples were added in duplicates to the plate. Absorbance was measured at 450 nm in a VERSAmax Tunable Microplate Reader (Molecular Devices, CA, USA) using Softmax pro 5 (Molecular Devices).

2.2.3. *Magnetic resonance imaging (MRI)*

All participants were requested to fast for at least four hours (water was acceptable) and to refrain from alcohol consumption and from taking any medications for pain, sleep, or IBS symptoms for at least 24 h prior to scanning.

MRI scanning sessions were accomplished on a 3T Philips Ingenia (Philips Healthcare, Best, The Netherlands) located at the Center for Medical Image Science and Visualization (CMIV) at Linköping University, Sweden, using a 32-channel head coil. Ten minutes of eyes-closed resting state functional MRI (fMRI) data were acquired with a single-shot, gradient-echo, echo planar imaging (EPI) sequence with repetition time and echo time (TR/TE) = 2000/37 ms; voxel

= 3.59x3.59x4 mm³; 28 slices; SENSE factor = 2) that effectively covered the whole brain. In addition, T1-weighted (T1W) images were acquired for all participants to ensure that they were free from any obvious pathologic abnormalities. Due to limited availability of the scanner and logistical/feasibility considerations, the time frame for scheduling MRI scanning sessions ranged from 7:45 am to 8:00 pm. No systematic differences between IBS patients and HC were evident with respect to time of the day of scanning (data not shown).

2.3. Functional connectivity analyses

Resting state fMRI data were preprocessed and analyzed using the CONN functional connectivity toolbox (65) (<http://www.nitrc.org/projects/conn>). Preprocessing was performed using the standard pipeline in CONN with realignment and unwarping for estimation and correction of subject motion; slice timing correction, ART-based outlier detection and scrubbing for the removal of signal intensity and motion artifacts; segmentation of the T1W images into grey matter, white matter, and cerebrospinal fluid as a preparation for normalization of the functional images to Montreal Neurological Institute (MNI) coordinates; smoothing using a Gaussian kernel of 8 mm for spatial convolution to ameliorate inter-subject differences in anatomy. The threshold criterium for motion artifacts was head motion larger than one voxel in any direction.

To denoise the functional images, a band-pass filter of 0.008–0.09 Hz and linear detrending were used. Functional connectivity was calculated as bivariate correlation with hemodynamic response weighting.

Functional connectivity between specific regions of interest (ROIs) according to our hypothesis, were investigated by ROI-to-ROI analysis using the CONN toolbox. Here, we

specifically investigated regions of the mesocorticolimbic network known to be related to motivational, cognitive, and physical aspects of fatigue as suggested by Karshikoff et al. (30). This was done using CONN default masks for bilateral NAc, bilateral middle frontal gyrus (here representing the dorsolateral prefrontal cortex, DLPFC), and the ACC. The CONN default mask for ACC includes only the rostral-dorsal ACC. However, the dorsal part in particular has proposed importance for the cognitive aspects of fatigue (30). Because several previous studies indicate the anterior insula is an important hub for fatigue, without describing anatomical substructures (13, 66), we decided to use manually segmented masks for the anterior-middle insulae (amINS) in this study (66). In total, we investigated the functional connectivity between seven ROIs.

2.4. Statistical analyses

2.4.1. Statistical analyses of TNF- α and questionnaire data

IBM SPSS Statistics for PC version 25 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. As not all measures were consistent with a normal distribution as determined by the Shapiro-Wilk normality test, Mann-Whitney U-tests were used for group comparisons of questionnaire data, plasma TNF- α and the total score of mFIS. Categorical data was compared using the chi-squared-test. The Kruskal-Wallis equality-of-populations rank test was used for subgroup analyses of the three subtypes of IBS. Associations were determined using Spearman's correlation coefficient. Results are presented as a median and interquartile range, unless indicated otherwise. Given the well-established relation between mood disorders and experience of fatigue symptoms (67), HADS as well as age, sex, BMI, and the use of psychotropic medication were controlled for in the association between TNF-

α and mFIS in IBS patients. For that, multiple linear regression analyses were performed in the IBS group, using a multiple regression backward elimination approach. Potential predictive variables were removed one at a time if their p-value was >0.05 . Evaluation of the variance inflation factor (VIF) analyses revealed no multicollinearity problem in the regression models (all VIF values were <3 with <10 as the upper limit of acceptability). The Shapiro-Wilk test did not support the assumption of normally distributed residuals of TNF- α values in IBS ($p<0.001$). For this reason, we used the non-parametric bootstrap (1000 replications) for formal statistical inference in regression models. The unstandardized coefficient (B), the standard error (SE) as well as the standardized coefficient (β), F, and R squared of the final regression model are presented. All tests were two-tailed and statistical significance was set to $p<0.05$.

2.4.2. Statistical analyses of fMRI data

The CONN software was used for all functional connectivity analysis. Firstly, we confirmed that the selected ROIs comprised a functionally connected network in our total sample of IBS patients and HC using one-sample t-tests. We also compared the network's functional connectivity between IBS and HC using two-sample t-tests. Secondly, associations between fatigue impact and functional connectivity within this network were investigated by linear regression analysis using mFIS total scores for each participant as predictor in the model and HADS scores as covariates of non-interest, given that mood disorders could impact connectivity in IBS patients (36). Similarly, associations between TNF- α levels and functional connectivity were also investigated by linear regression analysis using plasma levels of TNF- α as predictor and HADS as well as mFIS scores as covariates of non-interest. The linear regression analyses were performed both within the IBS and HC groups separately and

between groups in order to compare IBS with HC. A Fisher transformation (inverse hyperbolic tangent function) was applied to the correlation measures in order to improve the normality assumption before second level between-subject analyses. To further identify possible relations between fatigue impact or inflammation and functional connectivity, additional seed-to-voxel whole brain analyses were performed with bilateral nuclei accumbens as the seeds and mFIS or TNF- α as regressors.

Results from the functional connectivity analyses are reported with p-values that are false discovery rate (FDR) corrected for multiple comparisons, using a statistical threshold of $p_{\text{FDR}} < 0.05$.

3. Results

3.1. Sample characterization

Eighty-eight IBS patients and 47 HC were included in this study. Data on TNF- α levels were obtained from all participants, whereas other outcome measures were not available from all patients and HC, resulting in missing data as follows: Two IBS patients did not complete the MRI part of the study and fMRI data from 4 IBS was excluded due to excessive motion. Four IBS patients did not fill-in the mFIS questionnaire and HADS scores were missing from one patient. IBS-SSS was not completed by one IBS patient and one HC. The MacArthur Scale of Subjective Social Status was not filled-in by five IBS patients. The body mass index (BMI) was not available for 16 IBS patients. A characterization of the samples is provided in Table 1. Briefly, there were no differences between groups regarding age, gender, or BMI. IBS

patients had a lower common sense of social status than HC. As pointed out in the methods section, 37.5 percent of the IBS patients, but none of the HC used psychotropic medication. IBS-SSS scores were expectedly higher in patients and indicated moderate to severe symptoms in this group. According to stool patterns reported for Rome-criteria, a majority of patients presented with mixed-type IBS. Comparisons of HADS scores indicated significantly more symptoms of anxiety and depression in patients relative to HC. Results from additional analyses regarding fatigue impact and TNF- α in subgroups of IBS patients based on gender, subtypes of IBS, and the usage of psychotropics are presented in Supplementary Table S1. Except for higher TNF- α in women, no statistically significant differences between patient subgroups were seen.

Table 1. Sample characteristics and group comparisons

	IBS	HC	U/ χ^2	p
N	88	47		
Age	30.0 (11.0)	31.0 (19.0)	1962.0	0.624
Women	85% (75)	85% (40)	<0.01	0.985
Men	15% (13)	15% (7)		
mFIS	43.0 (32)	11.0 (18)	525.5	<0.001
HADS Anx	10.0 (7.0)	3.0 (3.0)	506.5	<0.001
HADS Depr	5.0 (6.0)	1.0 (2.0)	551.5	<0.001
Socioeconomic status	6.0 (2)	7.0 (1)	1179.5	<0.001
IBS-SSS	325.0 (119.0)	8.0 (27-0)	13.0	<0.001
IBS-D	28% (n=25)			
IBS-C	18% (n=16)			

IBS-M	53% (n=47)			
BMI	23.4 (5.2)	23.4 (17.1)	1675.0	0.926
Psych med	37.5% (33)	0.0% (0)	23.3	<0.001

Data are given as a median and interquartile range in parentheses or percentage and counts in parentheses. Group comparisons were performed using the Mann-Whitney-U-test or the chi-squared-test. IBS = Irritable bowel syndrome, HC = healthy controls, mFIS = modified fatigue impact scale, , HADS = Hospital Anxiety and Depression Scale, Anx = anxiety, Depr = depression, , IBS-SSS = IBS Severity Scoring System, IBS-D = IBS with predominantly diarrhea, IBS-C = IBS with predominantly constipation, IBS-M = IBS with mixed stool type manifestation, BMI = body mass index, Psych Med= Psychotropic medication.

3.2. *Group comparisons of TNF- α and fatigue impact*

Patients with IBS demonstrated significantly higher plasma levels of TNF- α compared to HC ($U = 1371.5$; $p = 0.001$; Figure 1). Group comparisons further confirmed a substantially higher fatigue impact in patients compared to HC (Table 1).

Figure 1. Plasma levels of TNF- α in IBS patients and HC, $p=0.001$.

3.3. Associations between fatigue impact, TNF- α , psychological symptoms, and IBS severity

Correlational findings are presented in Figure 2 and Table 2. In summary, plasma levels of TNF- α showed a positive correlation with fatigue impact in IBS patients, but not in HC (Figure 2). TNF- α was related to sex in IBS patients. Both, fatigue impact and TNF- α were further related to IBS symptom severity in patients. With respect to psychological symptoms, fatigue impact was positively associated with anxiety as well as depression in both groups, whereas no association in either IBS patients or HC was detected between HADS scores and TNF- α .

Table 2. Within group correlations

	TNF-α		mFIS	
	IBS	HC	IBS	HC
Age	-0.01	-0.20	0.08	-0.13
Male sex	-0.20*	-0.03	-0.20	0.22
BMI	-0.02	0.09	0.05	-0.26
Psych med	0.16	-	0.17	-
HADS Anx	0.09	0.06	0.43**	0.67**
HADS Depr	0.17	-0.06	0.63**	0.42**
IBS-SSS	0.25*	-0.05	0.36**	0.26

Spearman's correlation coefficients indicating associations with fatigue impact and TNF- α of age, sex, BMI, psychotropic medication, psychological symptoms, and IBS symptom severity separately for IBS patients and HC. IBS = Irritable bowel syndrome, HC = healthy controls, mFIS = modified Fatigue Impact Scale, BMI = body mass index, Psych med = Psychotropic medication, HADS = Hospital Anxiety and Depression Scale, Anx = anxiety, Depr = depression, IBS-SSS = IBS Symptom Severity Scale.

*Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Figure 2. Scatterplots of the relationship between TNF- α levels and scores of the modified Fatigue Impact Scale (mFIS) in IBS patients (Spearman's $\rho = 0.28$, $p=0.001$) and healthy controls (HC) (Spearman's $\rho = 0.13$, $p=0.37$)

3.4. Multiple linear regression of the association between fatigue impact and TNF- α in IBS patients

The independent predictive value of fatigue impact in IBS patients for variance in TNF- α levels in plasma was investigated in the patient sample using a multiple regression backward elimination procedure with gender, age, BMI, use of psychotropic medication, and HADS scores of depression and anxiety as potential predictors. Only fatigue impact was a statistically significant predictor ($\beta=0.26$, $B=0.02$, $SE=0.01$, $p=0.009$) in the final model ($R^2=9.31$, $F=5.90$, $p=0.02$).

3.5. Functional connectivity within the mesocorticolimbic network

The selected mesocorticolimbic regions which have previously been related to fatigue (30), formed a functionally connected network in all participants (Supplementary Table S2). The connectome ring in Figure 3 displays how the network ROIs are functionally connected to each other. The regions are further presented in Figure 4. The NAc, in particular, exhibited functional connectivity to all other ROIs. There was no difference in ROI-to-ROI functional connectivity between IBS and HC when not taking fatigue impact into consideration, $p_{FDR} > 0.05$. In other words, comparisons were only significant when total fatigue impact scores were included as a predictor in the model.

Figure 3. Significant functional connectivity between regions of interest comprising the mesocorticolimbic network. Data are presented as a connectome ring with false discovery rate correction ($p_{FDR} < 0.05$). ACC = anterior cingulate cortex; amINS = anterior-middle insula; DLPFC = dorsolateral prefrontal cortex; NAc = nucleus accumbens; ROI = region of interest; L = left; R = right. The color bar represents T-values for the ROI-to-ROI effects.

Figure 4. Visualization of the regions of interest (ROI) used in the functional connectivity analysis. A) Dorsolateral prefrontal cortex (blue), anterior cingulate cortex (green), nucleus accumbens (yellow), left view. B) Dorsolateral prefrontal cortex (left side = purple, right side = blue), anterior cingulate cortex (green), nucleus accumbens (yellow), anterior view. C) Anterior and middle insula, left view.

3.6. Associations between functional connectivity and fatigue impact

In both the IBS and HC groups, fatigue impact was related to functional connectivity between some distinct nodes of the selected network (Table 3). In individuals with IBS, fatigue impact negatively correlated with connectivity between the NAc and the DLPFC in both hemispheres

(Figure 5A), whereas in HC, fatigue impact positively correlated with connectivity between the right NAc and bilateral amINS (Figure 5B).

The between-group analysis showed that IBS patients had less functional connectivity between the right NAc and bilateral amINS than HC in relation to fatigue impact (Table 3). It is important to note that the observed significant difference between groups may be driven by *positive associations* between fatigue impact and the functional connectivity in HC in contrast to the *absence of such association* or trend thereof in IBS (Figures 6A and 6B).

Between-group analysis further revealed IBS patients to exhibit lower fatigue impact-related connectivity between the right NAc and the right DLPFC, but stronger inter-insular connectivity between hemispheres compared with HC (Table 3). Figures 6C and 6D illustrate that the significant group differences were driven by trends of opposite correlations in the two groups. Note that the within-group associations between fatigue impact and connectivity were only significant for the connectivity between the right NAc and the right DLPFC in IBS, see Table 3. See Supplementary Table S3 for all functional connectivity results related to fatigue impact.

Table 3. Relation between fatigue impact and functional connectivity.

Group	Regions	t-value	pFDR
IBS			
	L NAc – L DLPFC	-3.05	0.019
	R NAc – R DLPFC	-2.81	0.038
HC			
	R NAc – L amINS	3.38	0.009
	R NAc – R amINS	3.09	0.011

IBS > HC

R NAc – L amINS	-2.28	0.049
R NAc – R amINS	-2.46	0.049
R NAc – R DLPFC	-2.28	0.049
R amINS – L amINS	2.52	0.045

Significant results from the ROI analyses of the relationship between fatigue impact and connectivity in the selected network. For non-significant results and uncorrected p-values, see Supplementary Table S3. Results are controlled for anxiety and depression and corrected for multiple comparisons. amINS = anterior-middle insula; DLPFC = dorsolateral prefrontal cortex; NAc = nucleus accumbens; L = left; R = right; HC = healthy controls; p_{FDR} = false discovery rate corrected p-value.

Figure 5. Associations between fatigue impact and functional connectivity in IBS (A) and in healthy controls (HC) (B). Blue signifies negative correlations between fatigue impact and connectivity, and red signifies positive correlations. amINS = anterior-middle insula; DLPFC = dorsolateral prefrontal cortex; NAc = nucleus accumbens; L = left; R = right.

Figure 6. Associations between fatigue impact and functional connectivity between regions

of interest in the mesocorticolimbic network. The straight and dotted lines are visual indications of the linear relationships between functional connectivity and fatigue impact in IBS patients and healthy controls (HC), respectively. Note, the regression lines are shown for visualization purpose only. Statistics are presented in Table 3. Spearman correlation coefficients calculated from graph: A) $r_{\text{IBS}} = 0.01$; $r_{\text{HC}} = 0.26$, B) $r_{\text{IBS}} = 0.02$; $r_{\text{HC}} = 0.27$, C) $r_{\text{IBS}} = -0.18$; $r_{\text{HC}} = 0.28$, D) $r_{\text{IBS}} = 0.12$; $r_{\text{HC}} = -0.42$. amINS = anterior-middle insula; DLPFC= dorsolateral prefrontal cortex; NAc = nucleus accumbens; L = left; R = right; mFIS = modified Fatigue Impact Scale.

3.7. Associations between functional connectivity and TNF- α

There were no significant associations between functional connectivity in the selected nodes representing the mesocorticolimbic network and plasma levels of TNF- α in either IBS or HC (Supplementary Table S4).

3.8. Seed-to-voxel whole brain analyses

Seed-to-voxel whole brain analyses with bilateral nuclei accumbens as the seed, and mFIS or TNF- α as regressors, did not reveal any significant relations between functional connectivity with mFIS or TNF- α in all participants nor in IBS only (data not shown).

4. Discussion

We set out to study fatigue and its association with a resting state network of mesocorticolimbic regions of known importance to fatigue, and to explore the possible role of circulating TNF- α levels in this matter, in IBS and HC. In summary, this novel multimodal study provides the first evidence that vulnerability to fatigue in IBS is associated with connectivity within a mesocorticolimbic network, as well as with immune activation.

To the best of our knowledge, central correlates of fatigue have not been studied in IBS. We confirmed a functionally connected network of mesocorticolimbic regions known to be related to cognitive, physical, and motivational aspects of fatigue in both IBS patients and healthy volunteers. When taking fatigue impact into consideration, IBS patients had a general tendency toward reduced functional connectivity within this network in comparison to controls. The NAc, the main hub in the mesolimbic dopamine circuit involved in the cognitive processing of motivation aversion and reward (38), seems to have a central role in the network since, unlike the other regions, it was found to be functionally connected to all other regions. In IBS, fatigue impact was negatively correlated with the connectivity between NAc and DLPFC bilaterally, while in controls it was positively correlated to the connectivity between the right NAc and amINS in both hemispheres. These results fit well with findings in other patient groups with fatigue and other overlapping symptoms. Specifically, reduced functional connectivity has previously also been demonstrated in chronic fatigue syndrome (68), PTSD (69) and depression (70). In a study of MS-related fatigue, reduced functional connectivity of the NAc to sensorimotor and reward networks was also observed (50). To further understand the motivational aspects of fatigue, investigations of dopaminergic input regions of the striatum could be of interest. For example previous research has also suggested toxic effects of proinflammatory cytokines on dopaminergic neurons (71), though we could not find evidence supporting an association between the functionally connected

mesocorticolimbic network and levels of the proinflammatory cytokine TNF- α . In addition other research indicates an association between inflammation and central alterations in fatigue (30, 31). Potential immune-to-brain pathways for this association between inflammatory mechanisms and the CNS include both neural (peripheral activation of the vagus nerve) and humoral pathways (28, 29). The humoral pathways are suggested to be mediated by peripheral cytokines activating microglia alongside brain vessels where the blood-brain barrier is weaker (29). It is also known that activated microglia produce cytokines locally in the brain which additionally have the potential to modify monoaminergic neurotransmission (45). Increased plasma IL-6, IL-1 β and IL-1, inflammatory receptor antagonists, have also been associated with decreased functional connectivity within a corticostriatal reward circuitry in relation to depression (70). TNF- α was also measured in that study, but like in this present study, no association with connectivity of striatal ROIs was found. Hence, while our multimodal analyses supported a role for altered mesocorticolimbic network connectivity in fatigue in IBS, correlational analyses of the connectivity data in this study do not support elevated peripheral TNF- α as a key mechanism. One potential explanation for the absence of this association between functional connectivity and inflammation as measured by TNF- α could be the specific ROI selection, as our initial hypotheses for the association between fatigue impact and functional connectivity in IBS was based on a general theory of fatigue as presented by Karshikoff *et al.* (30),. However, exploratory whole brain analyses did not detect further significant relations between functional connectivity and fatigue impact or TNF- α , supporting the relevance of the network investigated.

IBS patients in this study experienced significantly more fatigue as well as elevated levels of the proinflammatory cytokine TNF- α , when compared to HC. Both results support previous findings documenting fatigue as a key extraintestinal symptom (9, 72), as well as peripheral

inflammation as a putative mechanism in IBS (16, 21, 23, 26). Interestingly, our data provide novel, initial evidence for the specific association between fatigue and peripheral inflammatory alterations in IBS patients. This complements existing knowledge, which has thus far been rather scarce and inconsistent. For example, in one study of female IBS patients, elevated TNF- α was associated with comorbid chronic fatigue syndrome (22), while another group found no association between fatigue impact and levels of proinflammatory cytokines including TNF- α (23). The present study supports a possible pathophysiological link between fatigue and inflammatory mechanisms in IBS, in keeping with knowledge on the role of fatigue and inflammatory mediators as key components of sickness behavior in healthy individuals and across multiple patient groups (28, 30, 31). It is noteworthy that unlike one previous study (23), our data revealed no correlations between TNF- α levels and anxiety or depression when assessed with HADS scores, suggesting a possible specificity for fatigue rather than broader psychiatric comorbidities. However, in light of the extensive literature on inflammatory mechanisms in mood disorders, especially depression, (73, 74) the putative role of immune system mediators in relation to the broad range of extraintestinal symptoms in IBS, clearly deserves further study.

4.1. Limitations and strengths

This study has several limitations. Firstly, the cross-sectional and correlational approaches applied can give some guidance, but do not lend themselves to establish causal relationships. Prospective or longitudinal studies are needed to further elucidate the putative directionality of associations between fatigue, inflammation, and brain connectivity.

Secondly, for this study the focus on TNF- α was based on existing literature. However, other pro- and anti-inflammatory cytokines are likely relevant, and more inflammatory markers could have shed further light on the putative role of altered neuro-immune communication in contributing to the likelihood of experiencing fatigue in IBS. Considering the known interaction between stress and immune responses in IBS (18, 19) specific measures of, for instance, corticotropin-releasing factor could also be of importance. In support of the need to study several cytokines in the context of sickness behavior and resting state network analyses, a previous study showed that acute systemic inflammation during experimental endotoxemia affected multiple resting state networks (75), supporting a role for IL-6 but not TNF- α .

A third limitation to this study is that for practical reasons, TNF- α , behavioral scales, and connectivity analyses were conducted at different time points and times of the day. That could possibly lead to false negative results. However, no systematic differences emerged between groups. Also, the extraintestinal symptom of fatigue in IBS is not an acute condition. Hence corresponding physiological changes as well as symptom measures could be expected to be stable over the weeks, during which the study was conducted.

Finally, regarding limitations - comorbid sleep disturbances were not assessed. In MS, sleep disorders are strongly linked to fatigue and the treatment of sleep disorders was able to improve symptoms of fatigue (76). Disruptions in functional connectivity of the insular cortices have also been reported to correlate with several sleep-related, cognitive, and psychological parameters (77). We also know that sleep complaints are common in IBS (78, 79) and that sleeping disturbances are associated with immune activation (80). However, previous research has also established that actual objective sleep alterations are not present in IBS (81), leading to the question of whether sleep complaints in this matter actually could be consistent with the experience of fatigue.

Strengths of this study are the multimodal approach and a relatively large sample-size. Furthermore, anxiety and depression were taken into consideration and controlled for in both connectivity and TNF- α analyses. Our findings actually indicate that the association between TNF- α levels and fatigue impact is specific to the fatigue component and not linked to a general association between low-grade inflammation and severity of psychological complaints. Similarly, the association between the investigated mesocorticolimbic network and fatigue was independent of depression and anxiety.

4.2. Future directions

Besides more thorough and prospective studies of peripheral and central neuroendocrine-immune mechanisms of fatigue in IBS, the findings of this study give rise to a broad range of future research questions. We chose to study fatigue impact on daily lives but future studies of the different dimensions of the fatigue experience in relation to central alterations are of interest. Also, while supplementary analyses did not reveal differences in either fatigue impact or TNF- α levels between IBS patients with different stool patterns, sample sizes, particularly of IBS-C were small. Putative differences between patients with different predominant stool patterns, including possible connectivity differences, call for further research in larger subsamples. Correspondingly, initial evidence of higher TNF- α in female IBS patients arose from our supplementary analyses, yet the sample of male patients was small. These results warrant further investigation into putative gender differences in IBS patients regarding both mesocorticolimbic connectivity, the experience of fatigue, and potential associations with peripheral inflammation.

Of great importance for the patients suffering from IBS and fatigue is the treatment of this burdening symptom. This study suggests that treatment-oriented studies should consider both local and peripheral inflammatory mechanisms together with brain measures. Our findings further indicate that treatments altering the motivational drive seem to be of importance. In MS and HC, a functional neuroimaging study showed that stimulation of the frontostriatal network through goal attainment led to decreased fatigue in both groups (82).

It has also previously been demonstrated that exercise of moderate intensity has an anti-inflammatory effect (83-85). In studies of patients with fibromyalgia, exercise has been associated with both improved physical fatigue (86) as well as a partial normalization of aberrant resting-state functional connectivity of regions involved in pain perception, including the insula (87). Finally, a long-term follow-up of an interventional study of increased physical activity in IBS interestingly showed both improved IBS-related and psychological symptoms, including fatigue impact (88).

5. Conclusion

IBS patients are particularly vulnerable to experiencing fatigue. In this novel multimodal study, we established both a negative association between fatigue impact and functional connectivity within regions of the mesocorticolimbic network and a positive association between fatigue impact and the proinflammatory cytokine TNF- α , in IBS. The possible relationship between immune activation and fatigue in IBS patients calls for further investigation, both regarding peripheral and central measurements of immune activation.

Based on the results of this study, future therapeutic trials in IBS targeting fatigue could involve both immune and psychological or motivational factors.

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7. Conflict of interest statement

All authors declare no conflict of interest.

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Supplementary Table S1. Subgroup analyses of fatigue impact and TNF- α in IBS

	mFIS			TNF- α		
	Median (IQR)	X ² /U	p	Median (IQR)	X ² /U	p
IBS-D	35.5 (29.0)	1.3	0.253	5.5 (1.1)	2.8	0.516
IBS-C	43.0 (38.0)			5.1 (1.2)		
IBS-M	44.0 (31.0)			5.1 (1.7)		
Psych. med.	48.0 (33.0)	668.0	0.131	5.3 (1.9)	737.5	0.143
No psych. med.	42.5 (28.0)			5.1 (1.3)		
Women	44.0 (31.0)	311.5	0.063	5.1 (1.4)	310.0	0.037
Men	27.0 (25.0)			4.7 (1.0)		

patients.

Differences were evaluated using the Kruskal-Wallis equality-of-populations rank test with 2 d.f. for the three subtypes of IBS and the Mann-Whitney-U-test for IBS patients with or without low-dose psychotropic medication as well as for gender. IBS = Irritable bowel syndrome, IBS-D = IBS with predominantly diarrhea, IBS-C = IBS with predominantly constipation, IBS-M = IBS with mixed stool type manifestation. Psych. med.= Psychotropic medication.

Supplementary Table S2. Functional connectivity in the selected network.

Seed region	Connected region	T-value	P _{unc}	P _{FDR}	Intensity	Size	F-value
L amINS			<0.001	<0.001	114.55	5	696.32

	R amINS	62.03	<0.001	<0.001			
	ACC	28.32	<0.001	<0.001			
	L NAc	13.02	<0.001	<0.001			
	R NAc	8.91	<0.001	<0.001			
	R DLPFC	-2.27	0.025	0.030			
R amINS			<0.001	<0.001	125.80	5	661.63
	L amINS	62.03	<0.001	<0.001			
	ACC	31.88	<0.001	<0.001			
	L NAc	14.51	<0.001	<0.001			
	L DLPFC	-10.18	<0.001	<0.001			
	R NAc	7.19	<0.001	<0.001			
L DFLPC					60.33	5	280.27
	R DLPFC	31.2	<0.001	<0.001			
	R amINS	-10.18	<0.001	<0.001			
	R NAc	9.30	<0.001	<0.001			
	ACC	-5.20	<0.001	<0.001			
	L NAc	4.45	<0.001	<0.001			
L NAc					93.58	6	238.45
	R NAc	32.68	<0.001	<0.001			
	ACC	21.18	<0.001	<0.001			
	R amINS	14.51	<0.001	<0.001			
	L amINS	13.02	<0.001	<0.001			
	R DLPFC	7.74	<0.001	<0.001			

	L DLPFC	4.45	<0.001	<0.001			
R DLPFC					44.32	4	229.35
	L DFLPC	31.20	<0.001	<0.001			
	L NAc	7.74	<0.001	<0.001			
	R NAc	3.11	0.002	0.005			
	L amINS	-2.27	0.025	0.037			
R NAc					76.56	6	200.80
	L NAc	32.68	<0.001	<0.001			
	ACC	15.38	<0.001	<0.001			
	L DLPFC	9.30	<0.001	<0.001			
	L amINS	8.91	<0.001	<0.001			
	R amINS	7.19	<0.001	<0.001			
	R DLPFC	3.11	0.002	0.002			
ACC					101.94	5	192.80
	R amINS	31.88	<0.001	<0.001			
	L amINS	28.32	<0.001	<0.001			
	L NAc	21.18	<0.001	<0.001			
	R NAc	15.38	<0.001	<0.001			
	L DLPFC	-5.20	<0.001	<0.001			

Results from the region of interest, ROI-to-ROI, functional connectivity analysis. ACC = anterior cingulate cortex; amINS = anterior-middle insula; DLPFC = dorsolateral prefrontal cortex; NA = nucleus accumbens; L = left; R = right; p_{unc} = uncorrected p-value; p_{FDR} = false discovery rate corrected p-value.

Supplementary Table S3. Relation between fatigue impact and functional connectivity.

Group	Regions	t-value	p_{UNC}	p_{FDR}
IBS				
	Seed L NAc			
	L NAc – R NAc	-1.63	0.1070	0.2715
	L NAc – L DLPFC	-3.05	0.0032	0.0190
	L NAc – R DLPFC	-1.51	0.1358	0.2715
	L NAc – L amINS	0.86	0.3923	0.4707
	L NAc – R amINS	0.91	0.3646	0.4707
	L NAc – ACC	-0.03	0.9794	0.9794
	Seed R NAc			
	R NAc – L NAc	-1.63	0.1070	0.2673
	R NAc – L DLPFC	-1.52	0.1337	0.2673
	R NAc – R DLPFC	-2.81	0.0063	0.0378
	R NAc – L amINS	0.26	0.7929	0.7929
	R NAc – R amINS	0.49	0.6240	0.7929
	R NAc – ACC	-0.39	0.6975	0.7929
	Seed L DLPFC			
	L DLPFC – L NAc	-3.05	0.0032	0.0190
	L DLPFC – R NAc	-1.52	0.1337	0.4010
	L DLPFC – R DLPFC	-1.03	0.3069	0.4025
	L DLPFC – L amINS	-1.07	0.2875	0.4025
	L DLPFC – R amINS	-0.84	0.4025	0.4025
	L DLPFC – ACC	-0.88	0.3815	0.4025

Seed R DLPFC

R DLPFC – L NAc	-1.51	0.1358	0.4073
R DLPFC – R NAc	-2.81	0.0063	0.0378
R DLPFC – L DLPFC	-1.03	0.3069	0.5414
R DLPFC – L amINS	-0.75	0.4578	0.5493
R DLPFC – R amINS	-0.15	0.8840	0.8840
R DLPFC – ACC	-0.92	0.3609	0.5414

Seed L amINS

L amINS – L NAc	0.86	0.3923	0.6012
L amINS – R NAc	0.26	0.7929	0.7929
L amINS – L DLPFC	-1.07	0.2875	0.6012
L amINS – R DLPFC	-0.75	0.4578	0.6012
L amINS – R amINS	1.38	0.1724	0.6012
L amINS – ACC	0.68	0.5010	0.6012

Seed R amINS

R amINS – L NAc	0.91	0.3646	0.6037
R amINS – R NAc	0.49	0.6240	0.7488
R amINS – L DLPFC	-0.84	0.4025	0.6037
R amINS – R DLPFC	-0.15	0.8840	0.8840
R amINS – L amINS	1.38	0.1724	0.6037
R amINS – ACC	0.90	0.3725	0.6037

Seed ACC

ACC – L NAc	-0.03	0.9794	0.9794
ACC – R NAc	-0.39	0.6975	0.8370
ACC – L DLPFC	-0.88	0.3815	0.7515

ACC – R DLPFC	-0.92	0.3609	0.7515
ACC – L amINS	0.68	0.5010	0.7515
ACC – R amINS	0.90	0.3725	0.7515

HC

Seed L NAc

L NAc – R NAc	-1.49	0.1438	0.5274
L NAc – L DLPFC	-0.23	0.8183	0.8183
L NAc – R DLPFC	0.68	0.5027	0.6032
L NAc – L amINS	1.18	0.2440	0.5274
L NAc – R amINS	1.13	0.2637	0.5274
L NAc – ACC	-0.49	0.6243	0.7492

Seed R NAc

R NAc – L NAc	-1.49	0.1438	0.2158
R NAc – L DLPFC	0.52	0.6032	0.6032
R NAc – R DLPFC	1.13	0.2650	0.3180
R NAc – L amINS	3.38	0.0016	0.0094
R NAc – R amINS	3.09	0.0035	0.0106
R NAc – ACC	1.62	0.1130	0.2158

Seed L DLPFC

L DLPFC – L NAc	-0.23	0.8183	0.9169
L DLPFC – R NAc	0.52	0.6032	0.9169
L DLPFC – R DLPFC	-1.08	0.2884	0.9169
L DLPFC – L amINS	-0.11	0.9169	0.9169
L DLPFC – R amINS	-0.33	0.7453	0.9169
L DLPFC – ACC	-0.68	0.5029	0.9169

Seed R DLPFC

R DLPFC – L NAc	0.68	0.5027	0.6032
R DLPFC – R NAc	1.13	0.2650	0.4325
R DLPFC – L DLPFC	-1.08	0.2884	0.5325
R DLPFC – L amINS	-1.32	0.1948	0.4325
R DLPFC – R amINS	-1.34	0.1874	0.4325
R DLPFC – ACC	-0.38	0.7093	0.7093

Seed L amINS

L amINS – L NAc	1.18	0.2440	0.3659
L amINS – R NAc	3.38	0.0016	0.0094
L amINS – L DLPFC	-0.11	0.9169	0.9169
L amINS – R DLPFC	-1.32	0.1948	0.3659
L amINS – R amINS	-1.98	0.0543	0.1629
L amINS – ACC	-0.49	0.6243	0.7492

Seed R amINS

R amINS – L NAc	1.13	0.2637	0.3956
R amINS – R NAc	3.09	0.0035	0.0211
R amINS – L DLPFC	-0.33	0.7453	0.7453
R amINS – R DLPFC	-1.34	0.1874	0.3748
R amINS – L amINS	-1.98	0.0543	0.1629
R amINS – ACC	0.40	0.6877	0.7453

Seed ACC

ACC – L NAc	-0.75	0.4602	0.7093
ACC – R NAc	1.62	0.1130	0.6780
ACC – L DLPFC	-0.68	0.5029	0.7093

ACC – R DLPFC	-0.38	0.7093	0.7093
ACC – L amINS	-0.49	0.6243	0.7093
ACC – R amINS	0.40	0.6877	0.7093

IBS > HC**Seed L NAc**

L NAc – R NAc	0.46	0.6492	0.6492
L NAc – L DLPFC	-1.26	0.2113	0.6339
L NAc – R DLPFC	-1.32	0.1878	0.6339
L NAc – L amINS	-0.57	0.5695	0.6492
L NAc – R amINS	-0.58	0.5660	0.6492
L NAc – ACC	0.55	0.5818	0.6492

Seed R NAc

R NAc – L NAc	0.46	0.6492	0.6492
R NAc – L DLPFC	-1.19	0.2377	0.2852
R NAc – R DLPFC	-2.36	0.0200	0.0491
R NAc – L amINS	-2.28	0.0245	0.0491
R NAc – R amINS	-2.46	0.0151	0.0491
R NAc – ACC	-1.71	0.0907	0.1360

Seed L DLPFC

L DLPFC – L NAc	-1.26	0.2113	0.7130
L DLPFC – R NAc	-1.19	0.2377	0.7130
L DLPFC – R DLPFC	0.41	0.6862	0.8856
L DLPFC – L amINS	-0.43	0.6685	0.8856
L DLPFC – R amINS	-0.15	0.8810	0.8865
L DLPFC – ACC	0.14	0.8856	0.8856

Seed R DLPFC

R DLPFC – L NAc	-1.32	0.1878	0.5635
R DLPFC – R NAc	-2.36	0.0200	0.1199
R DLPFC – L DLPFC	0.41	0.6862	0.8234
R DLPFC – L amINS	0.71	0.4768	0.7151
R DLPFC – R amINS	1.03	0.3055	0.6110
R DLPFC – ACC	-0.09	0.9321	0.9321

Seed L amINS

L amINS – L NAc	-0.57	0.5695	0.6685
L amINS – R NAc	-2.28	0.0245	0.0736
L amINS – L DLPFC	-0.43	0.6685	0.6685
L amINS – R DLPFC	0.71	0.4768	0.6685
L amINS – R amINS	2.52	0.0129	0.0736
L amINS – ACC	0.75	0.4547	0.6685

Seed R amINS

R amINS – L NAc	-0.58	0.5660	0.8491
R amINS – R NAc	-2.46	0.0151	0.0453
R amINS – L DLPFC	-0.15	0.8810	0.9231
R amINS – R DLPFC	1.03	0.3055	0.6110
R amINS – L amINS	2.52	0.0129	0.0453
R amINS – ACC	0.10	0.9231	0.9231

Seed ACC

ACC – L NAc	0.55	0.5818	0.9321
ACC – R NAc	-1.71	0.0907	0.5439
ACC – L DLPFC	0.14	0.8856	0.9321

ACC – R DLPFC	-0.09	0.9321	0.9321
ACC – L amINS	0.75	0.4547	0.9321
ACC – R amINS	0.10	0.9231	0.9321

All results from the region of interest analyses of the relation between fatigue impact and connectivity in the selected network. Results are controlled for anxiety and depression. The table shows T-statistics, uncorrected p-values, p_{UNC} , and false discovery rate corrected p-values, p_{FDR} . ACC = anterior cingulate cortex; amINS = anterior-middle insula; DLPFC = dorsolateral prefrontal cortex; NAc = nucleus accumbens; L = left; R = right; HC = healthy controls.

Supplementary Table S4. Relation between TNF- α and functional connectivity.

Group	Regions	t-value	p_{UNC}	p_{FDR}
IBS				
	Seed L NAc			
	L NAc – R NAc	-0.18	0.8538	0.8538
	L NAc – L DLPFC	-1.61	0.1120	0.6415
	L NAc – R DLPFC	-1.35	0.1805	0.5415
	L NAc – L amINS	-0.42	0.6764	0.8538
	L NAc – R amINS	-0.33	0.7390	0.8538
	L NAc – ACC	0.39	0.6980	0.8538
	Seed R NAc			
	R NAc – L NAc	-0.18	0.8538	0.9509
	R NAc – L DLPFC	-0.68	0.4968	0.9509
	R NAc – R DLPFC	-0.06	0.9509	0.9509
	R NAc – L amINS	-0.83	0.4093	0.9509
	R NAc – R amINS	-0.17	0.8628	0.9509
	R NAc – ACC	-0.24	0.8125	0.9509
	Seed L DLPFC			
	L DLPFC – L NAc	-1.61	0.1120	0.1953
	L DLPFC – R NAc	-0.68	0.4968	0.5962
	L DLPFC – R DLPFC	-0.24	0.8106	0.8106
	L DLPFC – L amINS	-2.19	0.0319	0.1911
	L DLPFC – R amINS	-1.81	0.0744	0.1953
	L DLPFC – ACC	-1.53	0.1302	0.1953
	Seed R DLPFC			

R DLPFC – L NAc	-1.35	0.1805	0.9509
R DLPFC – R NAc	-0.06	0.9509	0.9509
R DLPFC – L DLPFC	-0.24	0.8106	0.9509
R DLPFC – L amINS	-0.27	0.7915	0.9509
R DLPFC – R amINS	-0.37	0.7109	0.9509
R DLPFC – ACC	-0.53	0.6004	0.9509
Seed L amINS			
L amINS – L NAc	-0.42	0.6764	0.7915
L amINS – R NAc	-0.83	0.4093	0.7271
L amINS – L DLPFC	-2.19	0.0319	0.1911
L amINS – R DLPFC	-0.27	0.7915	0.7915
L amINS – R amINS	1.24	0.2194	0.6583
L amINS – ACC	0.70	0.4847	0.7271
Seed R amINS			
R amINS – L NAc	-0.33	0.7390	0.8628
R amINS – R NAc	-0.17	0.8628	0.8628
R amINS – L DLPFC	-1.81	0.0744	0.4466
R amINS – R DLPFC	-0.37	0.7109	0.8628
R amINS – L amINS	1.24	0.2194	0.6583
R amINS – ACC	0.77	0.4409	0.8628
Seed ACC			
ACC – L NAc	0.39	0.6980	0.8125
ACC – R NAc	-0.24	0.8125	0.8125
ACC – L DLPFC	-1.53	0.1302	0.7810
ACC – R DLPFC	-0.53	0.6004	0.8125

ACC – L amINS	0.70	0.4847	0.8125
ACC – R amINS	0.77	0.4409	0.8125

HC**Seed L NAc**

L NAc – R NAc	1.10	0.2796	0.4194
L NAc – L DLPFC	0.38	0.7045	0.7045
L NAc – R DLPFC	0.71	0.4837	0.5805
L NAc – L amINS	-1.51	0.1391	0.2783
L NAc – R amINS	-1.69	0.980	0.2783
L NAc – ACC	-1.77	0.0845	0.2783

Seed R NAc

R NAc – L NAc	1.10	0.2796	0.4194
R NAc – L DLPFC	0.74	0.4622	0.5546
R NAc – R DLPFC	-1.33	0.1901	0.4194
R NAc – L amINS	-0.04	0.9680	0.9680
R NAc – R amINS	-1.13	0.2642	0.4194
R NAc – ACC	-1.55	0.1291	0.4194

Seed L DLPFC

L DLPFC – L NAc	0.38	0.7045	0.7045
L DLPFC – R NAc	0.74	0.4622	0.6439
L DLPFC – R DLPFC	-0.66	0.5107	0.6439
L DLPFC – L amINS	-0.72	0.4778	0.6439
L DLPFC – R amINS	-0.96	0.3405	0.6439
L DLPFC – ACC	-0.62	0.5366	0.6439

Seed R DLPFC

R DLPFC – L NAc	0.71	0.4837	0.6131
R DLPFC – R NAc	-1.33	0.1901	0.5703
R DLPFC – L DLPFC	-0.66	0.5107	0.6131
R DLPFC – L amINS	-0.30	0.7681	0.7681
R DLPFC – R amINS	-1.45	0.1539	0.5703
R DLPFC – ACC	-0.66	0.5109	0.6131
Seed L amINS			
L amINS – L NAc	-1.51	0.1391	0.8348
L amINS – R NAc	-0.04	0.9680	0.9680
L amINS – L DLPFC	-0.72	0.1391	0.8348
L amINS – R DLPFC	-0.30	0.7681	0.9217
L amINS – R amINS	-0.46	0.6458	0.9217
L amINS – ACC	-0.63	0.5341	0.9217
Seed R amINS			
R amINS – L NAc	-1.69	0.0980	0.4086
R amINS – R NAc	-1.13	0.2642	0.4086
R amINS – L DLPFC	-0.96	0.3405	0.4086
R amINS – R DLPFC	-1.45	0.1539	0.4086
R amINS – L amINS	-0.46	0.6458	0.6458
R amINS – ACC	-1.06	0.2940	0.4086
Seed ACC			
ACC – L NAc	-1.77	0.0845	0.3874
ACC – R NAc	-1.55	0.1291	0.3874
ACC – L DLPFC	-0.62	0.5366	0.5366
ACC – R DLPFC	-0.66	0.5109	0.5366

ACC – L amINS	-0.63	0.5341	0.5366
ACC – R amINS	-1.06	0.2940	0.5366

IBS > HC**Seed L NAc**

L NAc – R NAc	-0.95	0.3437	0.3801
L NAc – L DLPFC	-1.27	0.2080	0.3778
L NAc – R DLPFC	-1.38	0.1698	0.3778
L NAc – L amINS	0.88	0.3801	0.3801
L NAc – R amINS	1.15	0.2519	0.3778
L NAc – ACC	1.45	0.1505	0.3778

Seed R NAc

R NAc – L NAc	-0.95	0.3437	0.5153
R NAc – L DLPFC	-1.01	0.3164	0.5153
R NAc – R DLPFC	1.14	0.2552	0.5153
R NAc – L amINS	-0.51	0.6086	0.6086
R NAc – R amINS	0.79	0.4294	0.5153
R NAc – ACC	1.18	0.2413	0.5153

Seed L DLPFC

L DLPFC – L NAc	-1.27	0.2080	0.7163
L DLPFC – R NAc	-1.01	0.3164	0.7163
L DLPFC – R DLPFC	0.36	0.7163	0.7163
L DLPFC – L amINS	-0.74	0.4610	0.7163
L DLPFC – R amINS	-0.39	0.6983	0.7163
L DLPFC – ACC	-0.44	0.6605	0.7163

Seed R DLPFC

R DLPFC – L NAc	-1.38	0.1698	0.7556
R DLPFC – R NAc	1.14	0.2552	0.7556
R DLPFC – L DLPFC	0.36	0.7163	0.9527
R DLPFC – L amINS	0.06	0.9527	0.9527
R DLPFC – R amINS	0.84	0.4006	0.8012
R DLPFC – ACC	0.25	0.8044	0.9527
Seed L amINS			
L amINS – L NAc	0.88	0.3801	0.6914
L amINS – R NAc	-0.51	0.6086	0.7303
L amINS – L DLPFC	-0.74	0.4610	0.6914
L amINS – R DLPFC	0.06	0.9527	0.9527
L amINS – R amINS	1.11	0.2711	0.6914
L amINS – ACC	0.92	0.3617	0.6914
Seed R amINS			
R amINS – L NAc	1.15	0.2519	0.5153
R amINS – R NAc	0.79	0.4294	0.5133
R amINS – L DLPFC	-0.39	0.6983	0.6983
R amINS – R DLPFC	0.84	0.4006	0.5153
R amINS – L amINS	1.11	0.2711	0.5153
R amINS – ACC	1.29	0.1993	0.5153
Seed ACC			
ACC – L NAc	1.45	0.1595	0.4825
ACC – R NAc	1.18	0.2413	0.4825
ACC – L DLPFC	-0.44	0.6605	0.7926
ACC – R DLPFC	0.25	0.8044	0.8044

ACC – L amINS	0.92	0.3617	0.5425
ACC – R amINS	1.29	0.1993	0.4825

All results from the region of interest analyses of the relation between TNF- α and connectivity in the selected network. Results are controlled for anxiety, depression and fatigue impact. The table shows T-statistics, uncorrected p-values, p_{UNC} , and false discovery rate corrected p-values, p_{FDR} . ACC = anterior cingulate cortex; amINS = anterior-middle insula; DLPFC = dorsolateral prefrontal cortex; NAc = nucleus accumbens; L = left; R = right; HC = healthy controls.