Brain, Behavior, and Immunity 123 (2025) 717–724

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

# Acute effects of interferon-alpha on cellular anabolic and catabolic processes are associated with the development of fatigue during Interferon-alpha-based therapy for Hepatitis-C: A preliminary study

Eva Periche-Tomas [a,*], Annamaria Cattaneo [b], Nadia Cattane [c], Claudia Bone [a], Jeremy Tibble [d], Edward T. Bullmore [e], Carmine Pariante [b], Neil A. Harrison [a,f]

[a] CUBRIC, Cardiff University Brain Research Imaging Centre, Cardiff, UK
[b] Department of Psychological Medicine, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK
[c] Biological Psychiatry Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy
[d] Department of Gastroenterology, Brighton & Sussex University Hospitals, Brighton, UK
[e] Department of Psychiatry, University of Cambridge Clinical School, Cambridge, UK
[f] Clinical Imaging Sciences Centre, Brighton and Sussex Medical School, Brighton, UK

ARTICLE INFO

*Keywords:*
Inflammation
Interferon-α
Fatigue
mRNA
Gene expression
Protein to protein interactions

ABSTRACT

*Introduction:* Interferon-alpha (IFN-α) is a key mediator of antiviral immune responses used to treat Hepatitis-C virus (HCV) infection. Though clinically effective, IFN-α frequently induces functionally impairing mood and motivation symptoms, particularly fatigue. Unlike mood impairment, which typically emerges after weeks of treatment, fatigue tends to emerge and evolve rapidly, typically within hours of the first IFN-α injection. Despite being a major source of functional impairment during IFN-α and other immune-based therapies, the biological mechanisms underlying fatigue remain poorly understood. Here, we aimed to identify acute immune-response signatures to IFN-α that could predict the later development of fatigue.

*Methods:* In this exploratory study, we analyzed whole blood transcriptomics in a longitudinal sample of 27 HCV patients initiating IFN-α and Ribavirin therapy. Blood samples were obtained at baseline and 4½ hours after the first IFN-α dose and transcriptomic data was obtained using Affymetrix Human Gene 1.1 ST Array Strips. Gene expression data visualization and quality control were assessed using Partek Genomics Suite V6.6 and protein–protein interaction networks using STRING and Ingenuity Pathway Analysis (IPA). A Fatigue Visual Analogue Scale (fVAS) was utilized to record fatigue symptoms at baseline, 4½ hours and 4 weeks after initiation of treatment.

*Results:* IFN-α was associated with an upregulation of 526 transcripts and a downregulation of 228 genes, indicating a rapid transcriptomic response in whole blood within 4½ hours of injection. 93 genes were significantly positively correlated with changes in fatigue, with gene expression changes measured from baseline to 4.5 h and increases in fatigue assessed from baseline to week 4 on the fVAS. We identified a novel network of predominantly cytosolic ribosomal units and ubiquitin proteins implicated in modulating mTOR signaling that was associated with the development of fatigue 4 weeks after initiation of IFN-α treatment ($p = 0.0078$).

*Conclusion:* Our findings suggest that acute activation of this anabolic/catabolic network by IFN-α may predispose to the experience of fatigue similar to evidence found in cancer-related fatigue. Further investigation is warranted to confirm the exploratory nature of these observations.

## 1. Introduction

Interferon-α (IFN-α) is a key mediator of antiviral immune responses used to treat Hepatitis-C virus (HCV) infection. Though clinically effective, chronic IFN-α administration frequently induces severe sickness responses, including functionally impairing fatigue and major depressive episodes (Capuron et al., 2002; Capuron & Miller, 2004; Maddock et al., 2005). To date, many studies have focused on

* Corresponding author at: Cardiff University Brain Imaging Centre, Cardiff CF24 4HQ, UK.
*E-mail addresses:* perichetomase@cardiff.ac.uk, eva.perichetomas@psych.ox.ac.uk (E. Periche-Tomas).

https://doi.org/10.1016/j.bbi.2024.09.038
Received 23 February 2023; Received in revised form 23 September 2024; Accepted 24 September 2024
Available online 15 October 2024

mechanisms of depression, which typically emerges 4 to 8 weeks after IFN-α treatment onset and affects approximately a third of patients (Whale et al., 2019). However, fewer studies have focused on fatigue which emerges far more quickly, usually within hours of the first injection and affects almost all treated patients (Capuron et al., 2002; Dowell et al., 2016). Furthermore, fatigue is frequently cited as one of the most functionally impairing features of IFN-α-based therapy and can persist even after treatment completion (Russell et al., 2019).

Traditionally, study of fatigue has been split into central (brain) and peripheral (neuromuscular) mechanisms (Chaudhuri & Behan, 2004; Greenhouse-Tucknott et al., 2022). IFN-α has been implicated in both processes. Regarding central mechanisms, type-I interferons have been shown to enter the brain by saturable transport systems at the blood brain barrier (BBB) (Banks, 2005; Banks & Erickson, 2010) and IFN-α is readily detected in the cerebrospinal fluid (CSF) of humans undergoing systemic IFN-α treatment for Hepatitis-C infection (Raison et al., 2009). In rodents, intra-peritoneal administration of IFN increases neuronal expression of STAT1 (signal transducer and activator of transcription 1), a common pathway in IFN signaling, within hours of administration (Wang, 2009; Wang & Campbell, 2005), and has been shown to modulate neuronal firing rates within the hypothalamus and other deep brain grey matter structures (Dafny et al., 1996). Supporting this, human neuroimaging studies of patients receiving IFN-α have demonstrated changes in basal ganglia glucose metabolism (FDG-PET), spectroscopic indices of neuronal activity (glutamate/glutamine concentrations) and tissue microstructure, each of which correlates with IFN-induced changes in fatigue or motivational state (Capuron et al., 2007, 2012; Dowell et al., 2016, 2017; Haroon et al., 2014). Taken together, these findings suggest that one of the mechanisms through which IFN-α may alter brain function is by crossing the BBB and inducing transcriptional changes, particularly within deep grey matter structures, such as the basal ganglia. However, how these IFN mediated changes in neuronal gene expression and function relate to fatigue remain to be determined.

Regarding peripheral mechanisms, interferon-induced transcriptional changes in muscular tissue have also been implicated in peripheral fatigue, particularly in the context of cancer or cancer-treatment associated fatigue. A molecular pathway particularly implicated in cancer fatigue is the 'mammalian target of rapamycin' (mTOR) pathway (Fig. 1), which plays an important role in cell growth, survival and proliferation, protein synthesis, transcription and translation and ribosomal biogenesis (Dowling et al., 2010), has been shown to translationally regulate Type-I interferon responses (Ivashkiv & Donlin, 2014; Livingstone et al., 2015) and drive interferon-HIF-1α mediated epithelial-to-mesenchymal transition in cancer (Yeh et al, 2018). mTOR-inhibitors show potent anticancer properties, however they are also commonly associated with severe cancer treatment-related fatigue (Peng et al., 2015). In rodents, the mTOR pathway has been linked to healthy muscle structure, mass and composition (Maiole et al., 2019; Risson et al., 2009) and its ability to modulate ubiquitin proteins, particularly Ubiquitin-40S Ribosomal protein S27a (RPS27A) which has been specifically linked to cancer treatment-induced skeletal muscle weakness and atrophy (Sakai et al., 2020). IFN-α can also induce a rare, non-inflammatory myopathy that is also thought to relate to its catabolic effects (Stübgen, 2009). Together, these studies suggest a potential association between IFN-α-induced transcriptomic changes, mTOR, and the experience of fatigue.

Ascertaining the molecular mechanisms by which IFN-α induces fatigue in both brain and muscle tissue in humans is challenging due to lack of non-invasive methods. Consequently, our understanding of IFN-α induced fatigue remains limited. Whole blood transcriptomics may provide a minimally invasive approach for inferring brain transcriptional effects based on changes in peripheral blood gene expression, given that some genes have shown correlated gene expression between blood and brain (though it should be noted that the majority of peripheral blood transcripts do not correlate with brain gene expression) (Basu et al., 2021; Hess et al., 2016; Qi et al., 2018; Sullivan et al., 2006; Tylee et al., 2013). There is therefore a pressing need to identify peripheral blood transcriptomic biomarkers that are predictive of fatigue and provide insights into the underlying molecular mechanisms (either through mimicry of CNS transcriptional dynamics, or as causal intermediates that then enter the CNS to affect its activity).

We, therefore, undertook an exploratory prospective cohort study in which 27 HCV patients initiating IFN-α based therapy completed whole blood transcriptomic analyses at baseline and again 4½ hours after their first IFN-α injection. Patients were followed up with regular assessment of fatigue and sickness response to determine whether we could identify

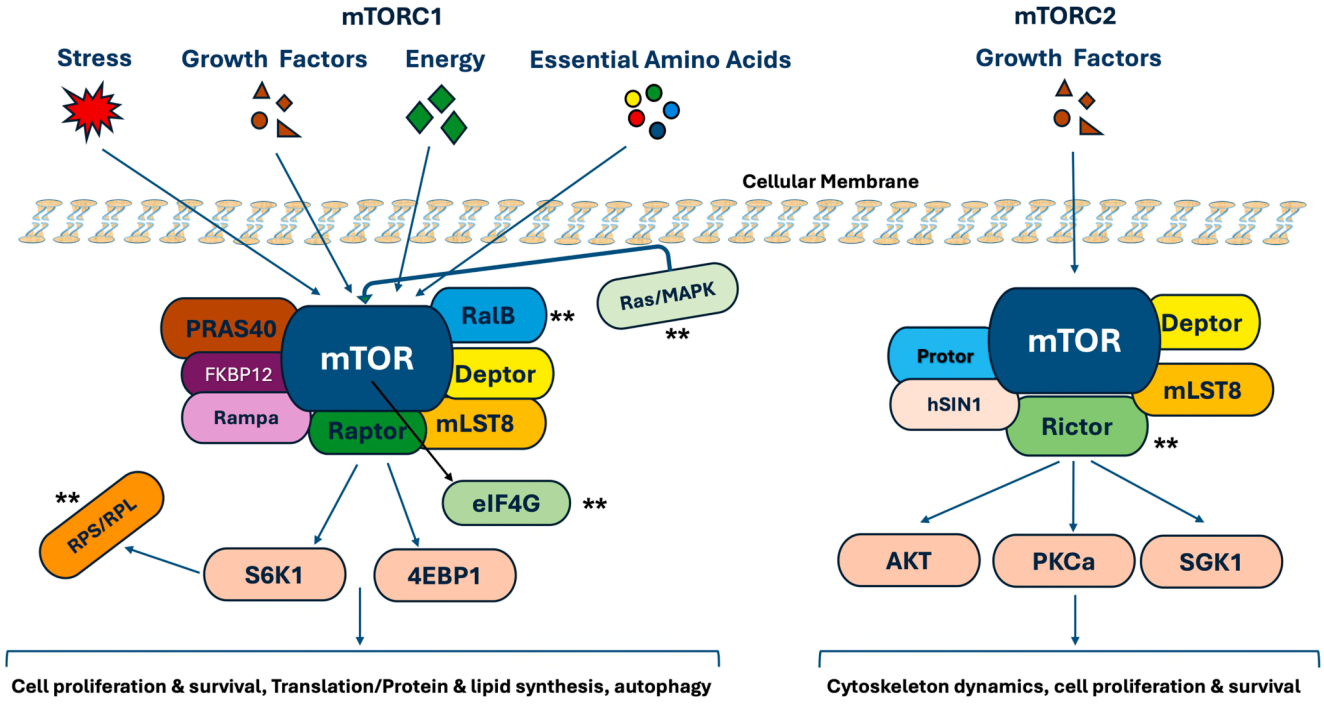


**Fig. 1.** Overview of the mTOR pathway.

acute blood-based response signatures to IFN-α that could be associated with the development of severe fatigue 4-weeks after initiation of treatment.

## 2. Materials and methods

### 2.1. Participants

We recruited twenty-seven patients (21 male, mean 50.4 +/- 11.1 years) with Hepatitis-C due to commence combination antiviral therapy with IFN-α and ribavirin. Participants were aged 30–68 years, fluent in English and fulfilled NICE guidelines for initiating IFN-α based therapy. Participants' current mental state and previous psychiatric history were evaluated at baseline using the Mini-International Neuropsychiatric review (MINI)(Sheehan et al., 1998). Exclusion criteria included history of psychotic illness or autoimmune disease, treatment for depression at study enrolment, if they had not abstained from substance abuse for at least 6 months, were co-infected with human immunodeficiency virus (HIV) or had any cause for liver disease other than Hepatitis-C. Ethical approval was granted by the Cambridge Central National Research Ethics Committee (12/EE/0491) and all individuals provided written informed consent.

### 2.2. Study design

In this exploratory prospective cohort design, participants were evaluated at baseline (mean 7 days before treatment), 4½ hours after their first IFN-α injection and 4, 8 and 12 weeks into IFN-α based treatment. Blood samples for whole-blood mRNA analysis were collected in PAXgene Blood RNA tubes (PreAnalytiX, Switzerland) at baseline and 4½ hours after the first IFN-α injection (but before the first dose of ribavirin) following standard protocols. Fatigue symptoms were assessed at each visit using a fatigue Visual Analogue Scale (fVAS) (Gift, 1989). The Hamilton Depression Rating Scale (HAMD) (Hamilton, 1960), State and Trait Anxiety Inventory (STAI) (Spielberger, et al., 1983) and the Epworth Sleepiness Scale (ESS) were also completed to describe the broader psychological response to IFN-α based therapy. Demographic data are summarized in Table 1.

### 2.3. Behavioral analysis

Effects of IFN-α on fatigue symptoms, defined as the difference in fVAS between baseline and 4 weeks after starting treatment, was used as the primary outcome variable. The fVAS was designed as a 100-mm-long horizontal line with two vertical anchoring lines labelled 'no fatigue' and 'extremely fatigued' at the left (0 mm) and right ends (100 mm) respectively. Participants were asked to rate their fatigue level by marking the point on the line that best represented their current perception of fatigue. Effects of IFN-α on fatigue symptoms were analyzed in R (v4.0.2) using repeated measures ANOVA with Holm correction for multiple comparisons.

**Table 1**
Demographic data.

| | Baseline | 4-Wks | *P* value |
|---|---|---|---|
| Age (years) | 50.4 ± 11.3 | | |
| Sex | | | |
| Male | 21 | | |
| Female | 6 | | |
| f-VAS | 29.9 ± 26.3 | 61.2 ± 29.4 | <0.001 |
| HAM-D | 5.96 ± 7.00 | 13.6 ± 7.59 | <0.001 |
| STAI | 30.4 ± 7.40 | 38.4 ± 13.7 | <0.005 |
| ESS | 5.85 ± 3.37 | 9.18 ± 4.48 | <0.005 |

Data represent mean ± standard deviation. fVAS: Fatigue Visual Analogue score; HAM-D: Hamilton Depression Rating Scale; STAI: State and Trait Anxiety Inventory; ESS: Epworth Sleepiness Scale.

### 2.4. RNA isolation and transcriptomics analysis

Isolation of total RNA was performed using the PAXgene blood mRNA kit following the manufacturer's protocol (PReAnalytix, Hombrechtikon, CHE). RNA quantity and quality were assessed using the A260/280 and A260/230 ratios and evaluated using a Nanodrop spectrophotometer (NanoDrop Technologies, Delaware, USA). Samples were stored at −80 °C. RNA integrity number (RIN), assessed using the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA), was in the range of 7–10.

Microarray assays were performed following the protocol described in the Affymetrix GeneChip Expression Analysis technical manual (Affymetrix, California, USA). Briefly, 250 ng RNA were used to synthesize cDNA with the Ambion WT Expression Kit (ThermoFisher Scientific), which was then purified, fragmented, labeled, and hybridized onto Human Gene 1.1 ST Array Strips (Affymetrix). Gene expression data visualization and quality control were assessed using Partek Genomics Suite V6.6 using recommended approaches. Specifically, background correction was conducted using Robust Multi-strip Average (RMA) (Irizarry et al., 2003) to remove noise from autofluorescence. Briefly, after background correction, Quantiles normalization (Bolstad et al., 2003) was applied to adjust the distribution of probe intensities across different microarray chips. Subsequently, a summarization step was conducted using a linear median polish algorithm (Tukey, 1977) to integrate probe intensities in order to compute the expression levels for each gene transcript.

Quality control and batch effects were assessed using Principal Component Analyses (PCA), with no outliers or batch effects observed. Genes modulated by IFN-α (expression profile at 4½ hours post-injection minus baseline) were identified with fold changes (FC) > 1.2 and < -1.2, and $p < 0.05$ (uncorrected). This relatively low FC threshold was selected to capture early responses to IFN-α within 4½ hours of the first injection, providing a clearer measure of IFN-α effects, unconfounded by later mood, appetite, or behaviour changes. Additionally, we opted not to filter low-expressed genes, consistent with our previous approach (Hepgul et al., 2016), as these genes, even when physiologically low, can be significantly modulated and contribute meaningfully to pathway analyses. This method ensured sufficient gene numbers for robust protein–protein interaction and pathway analyses.

Pearson's correlation analysis of IFN-α induced gene expression (delta log2 values: log2 4½ hours minus log2 baseline) and fatigue symptoms was performed in R (v4.0.2) and used to predict IFN-α induced fatigue 4 weeks after initiation of treatment.

Genes identified as differentially expressed (FC > 1.2 and < -1.2 and uncorrected $p < 0.05$) and genes that were acutely modulated by IFN at 4.5 h (minus baseline) and were associated with an increase in fatigue at 4 weeks (uncorrected $p < 0.05$), were then imported into STRING software (Search Tool for the Retrieval of Interacting Genes/Proteins, https://string-db.org/) and used to identify protein-to-protein interaction (PPI) networks that showed (1) a significant main effect of IFN-α or (2) were significantly associated with an increase in fatigue at 4 weeks respectively. Functional enrichment and network analysis in STRING were restricted to data from experimental, co-expression and protein homology studies (i.e. weaker data from text-mining were excluded) and a high confidence rating was used (i.e. > 0.7). STRING uses a graph-theoretic approach to provide statistical control (i.e. whether the complexity (number of edges) of observed networks of functionally interconnected transcripts are likely to arise through chance alone). Finally, Ingenuity Pathway analysis (IPA) was used to identify which upstream regulators may underpin the set of transcripts found to significantly predict IFN-α induced-fatigue (QIAGEN Inc., https://digitalinsights.qiagen.com/IPA).

## 3. Results

### 3.1. Fatigue symptoms

IFN-α significantly increased fatigue symptoms (fVAS: $F_{(4,104)} = 15.96$, $p < 0.001$) from a mean of 29.9 ± 26.25 at baseline to a peak mean of 62.62 ± 25.24 at 8 weeks. The increase in fatigue was rapid with a significant effect already observed at 4½ hours ($t_{26} = 11.46$, p = 0.016) and significant increases also observed at 4 ($t_{26} = 31.27$, p < 0.0001), 8 ($t_{26} = 32.70$, p < 0.001) and 12 weeks ($t_{26} = 30.68$, p < 0.001) (Fig. 2). Significant positive correlations were also observed between symptoms at 4½ hours after challenge and symptoms at 4 weeks (r = 0.52, p = 0.005) and 8 weeks after initiation of treatment (r = 0.56, p = 0.002) but not between symptoms at 4½ hours and 12 weeks of treatment (r = 0.28, p = 0.148).

### 3.2. Main effect of Interferon-α on gene expression

We identified genes modulated by IFN-α by comparing the expression profile of the sample at 4½ hours after the first injection with the profile at baseline. IFN-α modulated 754 genes, 526 were upregulated (at an uncorrected p < 0.05; FC > 1.2) and 228 were downregulated (at uncorrected p < 0.05; FC < -1.2).

STRING was then used to explore protein-to-protein interactions (PPI) and perform functional enrichment analysis of the upregulated genes. The PPI network generated by STRING consisted of 520 nodes and 546 edges with an average node degree of 2.1 (average number of connections per node) and average clustering coefficient of 0.31 (the degree to which nodes in the graph tend to cluster together) which are both higher than expected by chance alone. The number of edges was significantly larger than expected for a random network of the same size ($p < 1x10^{-16}$). As expected, STRING curated databases Reactome and KEGG revealed enrichment of a number of pathways associated with immune processes (Reactome pathways: HSA-913531: Interferon signaling, $p = 1.29 \times 10^{-35}$; HSA-168256: Immune system, $p = 7.97 \times 10^{-33}$; KEGG pathways: hsa04621: NOD-like receptor signaling pathway, $p = 1.94 \times 10^{-11}$; hsa05164: Influenza A, $p = 9.32 \times 10^{-11}$). IFN-α also modulated several genes associated with mTOR signalling, including members of the EIF4 and MAPK families, as well as RALB and RICTOR (Fig. 3) consistent with findings reported following IFN-α challenge in cancer (Yeh et al., 2018) and neural stem cell lines (Carvajal Ibañez et al., 2023).

PPI analysis was also performed for the IFN-α downregulated genes, however, no significant enrichment was detected (204 nodes, 12 edges).

### 3.3. Changes in gene expression modulated by IFN-α and the development of IFN-α induced fatigue

Early changes in gene expression induced by IFN-α were correlated with an increase in fatigue symptoms at 4 weeks (minus baseline). Correlation coefficients at 4 weeks were computed in R (cor function at index cut-off r > 0.5, p < 0.05). A total of 178 IFN-α modulated genes (4½ minus baseline) correlated with fatigue symptoms at 4 weeks.

Among those 178 genes, 93 positively correlated with fatigue symptoms. PPI analysis of these 93 transcripts produced a network that was significantly more complex than expected for a random network of the same size ($p < 6.46 \times 10^{-8}$) consisting of 83 nodes and 62 edges (average node degree: 1.49; cluster coefficient: 0.323) centered on two functionally interconnected main nodes interconnected by RSP27a, a stress sensor translationally regulated by the mTOR pathway". (Fig. 4). The pathways that were most enriched among genes coding proteins in this network involved: Reactome pathways: HSA-168255: Influenza infection, $p = 2.28 \times 10^{-07}$; HSA-156902: Peptide chain elongation, $p = 2.36 \times 10^{-07}$, HAS-168256: Immune system, $p = 2.36 \times 10^{-07}$; KEGG pathways: hsa03010: Ribosome, $p = 2.44 \times 10^{-06}$; hsa05131: Shigellosis, p = 0.00081, hsa04120: Ubiquitin mediated proteolysis, p = 0.0334).

Among the upstream regulators of IFN-α modulated genes (4½ hours minus baseline) that were positively correlated with fatigue, identified by IPA, 3 of the top 4 regulators were associated with the mTOR pathway (p-value of overlap = $1.04 \times 10^{-11}$, $8.95 \times 10^{-10}$, and $1.91 \times 10^{-07}$) (Table 2). PPI analysis did not show any significant enrichment

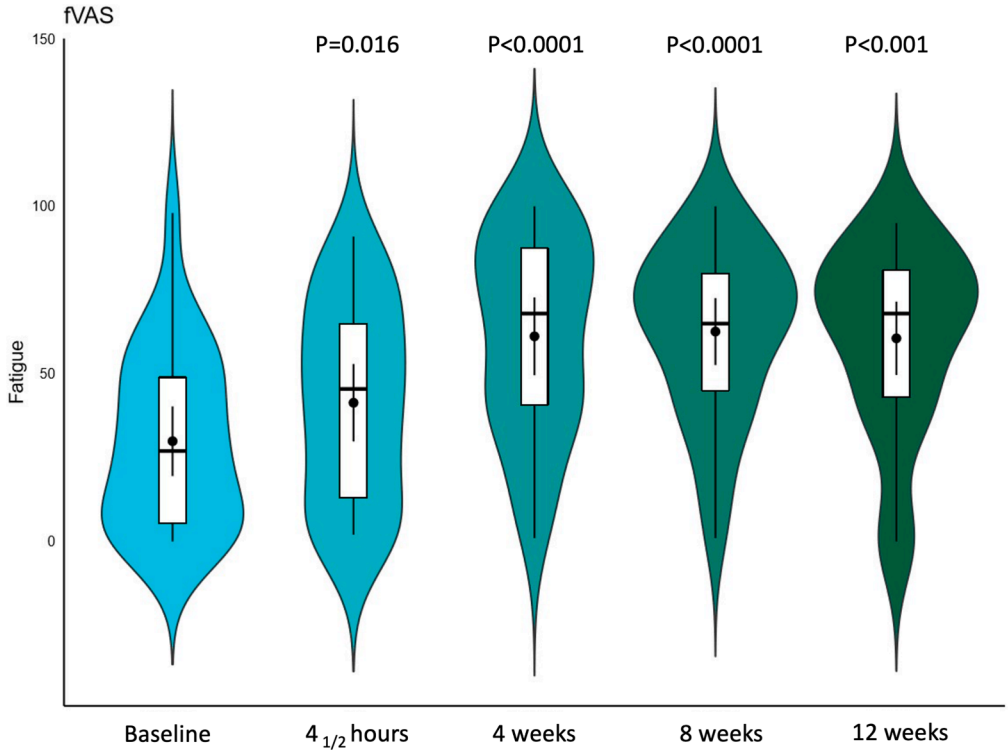


**Fig. 2.** Fatigue response to interferon-alpha.

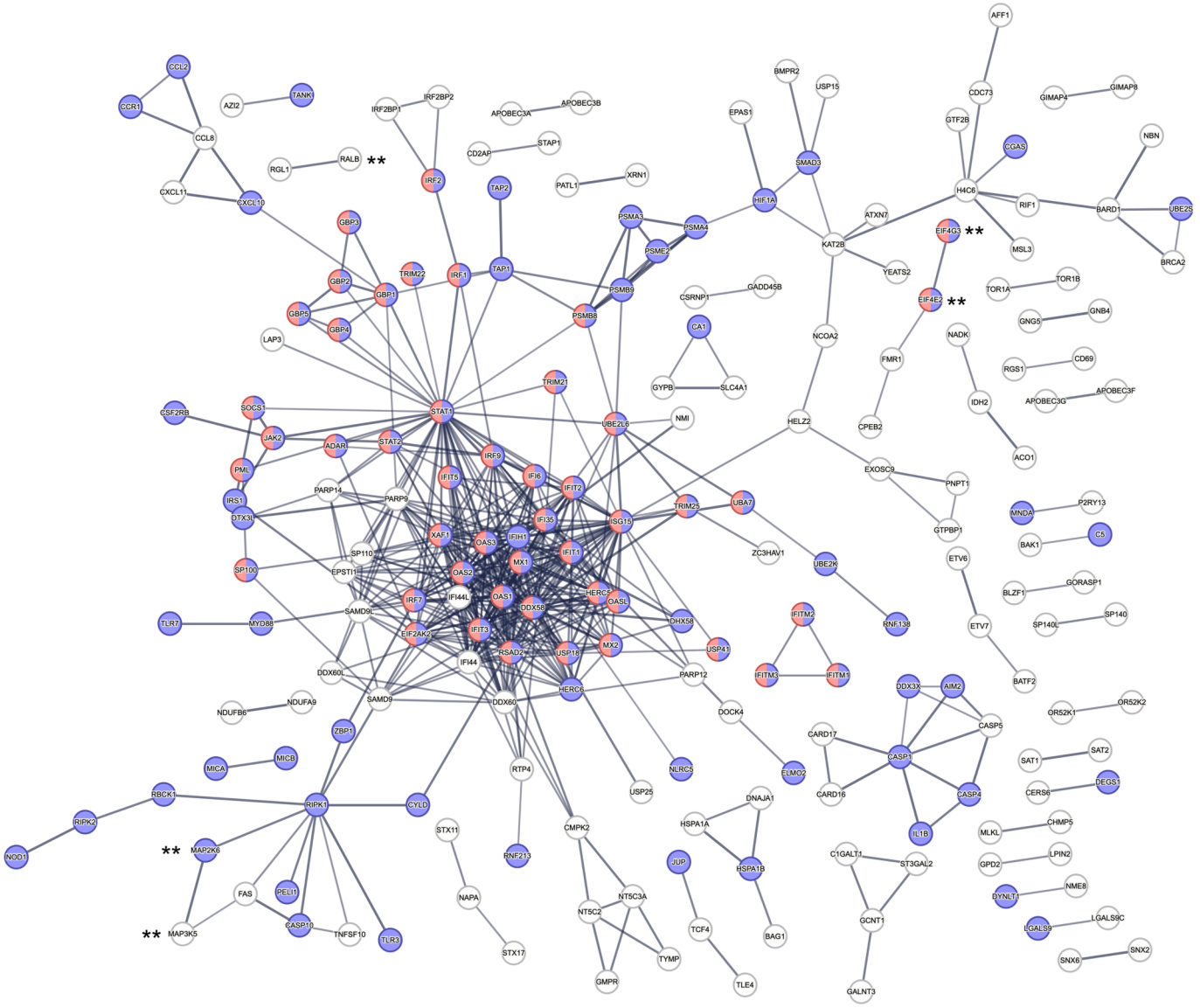


**Fig. 3.** Main effect of interferon-alpha.

for the genes negatively associated with fatigue (66 nodes, 3 edges).

## 4. Discussion

In this study, we employed microarrays to explore acute interferon-induced changes in the whole transcriptome, and to ascertain their relationship with the subsequent development of fatigue symptoms in a sample of 27 patients undergoing IFN-α treatment for Hepatitis-C. Firstly, IFN-α administration was associated with a rapid transcriptomic response in whole blood, as differential gene expression analysis revealed 526 upregulated and 228 downregulated transcripts, within 4½ hours of injection; which coincided with the onset of IFN-α-induced fatigue. This finding is consistent with previous studies which report a rapid stimulation of interferon-sensitive genes in blood following peripheral administration of IFN in humans (Yeh et al., 2018). Our second finding was that a network of transcripts, which comprised predominantly ubiquitin and ribosomal genes, was significantly associated with the subsequent development of fatigue 4 weeks after the first IFN injection.

This connected network consisted of two tightly connected clusters of genes which are interconnected by the ubiquitination ribosomal gene RPS27A, a stress sensor that is translationally regulated by the mTOR pathway and interestingly previously associated with cancer treatment-induced muscle atrophy in murine models (Sakai et al., 2020). The first cluster in our network was composed of genes expressing protein degradation units belonging to the E2 (UBE2D1, UBE2D2, UBEV2) and E3 (FBXW11, BTRC, RLIM) ubiquitin ligase family. The UBE2D family are E2-Ub conjugating enzymes, that interact highly with E3s providing them with catalytic activity. Interestingly, these proteins have previously been shown to play a role in muscle wasting across a range of different physiological situations (Polge et al., 2015). Furthermore, E3 protein ligases such as F8XW11 and BTRC are also involved in the degradation of DEPTOR, an endogenous regulator of the mTOR pathway, whereby inhibition of DEPTOR by E3 ligases promotes mTOR expression (Jiang et al., 2019; Zhao et al., 2011). The second cluster of genes identified in our network were RPL and RPS ribosomal units. The mTOR pathway regulates gene expression of ribosomal biosynthesis by promoting the translation of RP mRNAs (Mayer & Grummt, 2006) and activation of mTOR signaling can result in both acute and long-term upregulation of protein synthesis (Bolster et al., 2004). mTOR is a dual regulator of anabolism and catabolism in muscle mass (Yoon, 2017). Disruption of the fine balance between protein synthesis and

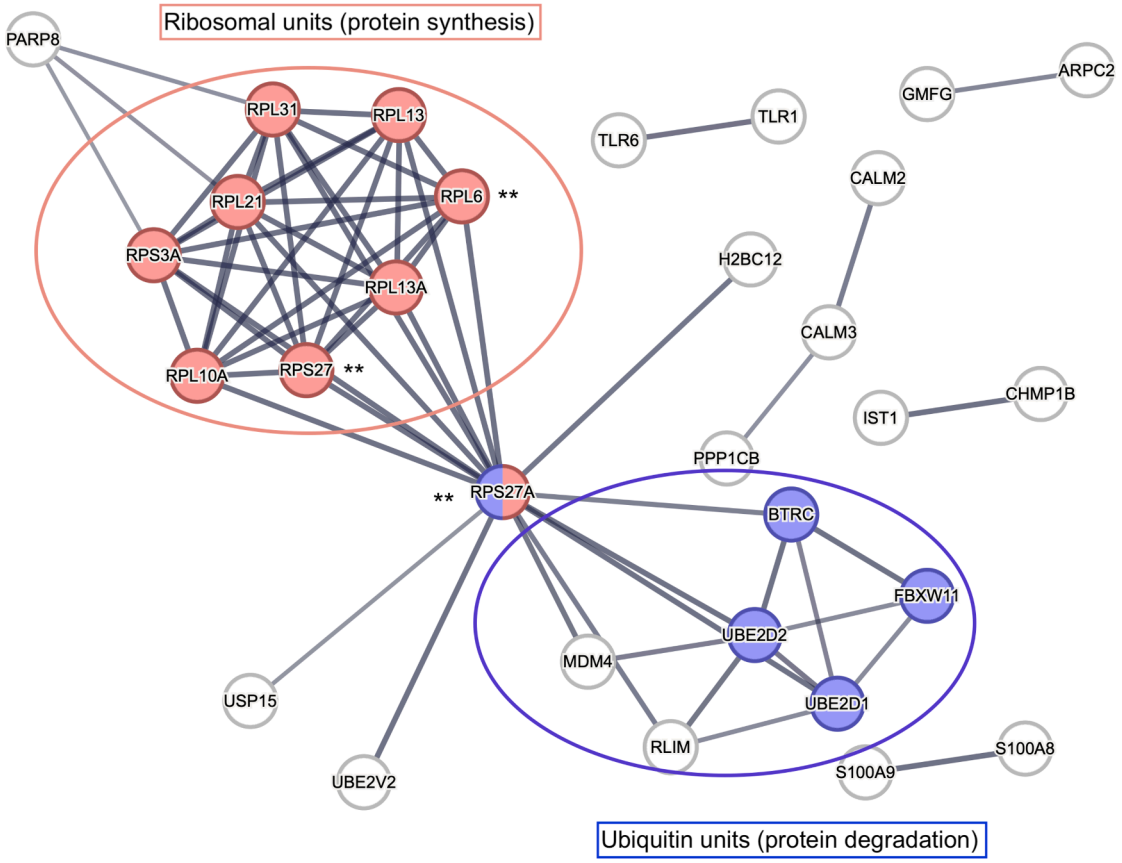


**Fig. 4.** Interferon-alpha induced fatigue association.

**Table 2**
Upstream Regulators.

| Upstream Regulator | Target Molecules |
|---|---|
| **LARP1** | **RPL10A,RPL13,RPL13A,RPL21,RPL31,RPL6,RPS27, RPS27A,RPS3A** |
| **Torin1** | **ATP6V1E1,RPL10A,RPL13,RPL13A,RPL21,RPL31,RPL6, RPS27, RPS27A,RPS3A** |
| Acyline | BCL6,RPL10A,RPL13,RPL13A,RPL21,RPL31,RPL6 |
| **Sirolimus** | **IL16,RAN,RPL10A,RPL13,RPL13A,RPL21,RPL6,RPS27, RPS27A, RPS3A,USP15,VMP1,ZNF845** |
| MLXIPL | RPL10A,RPL13,RPL13A,RPL21,RPL31,RPL6,RPS27A |
| IL17RA | CSF3R,CXCR2,S100A8,S100A9 |
| Mir-802 | ARRDC3,GNG12,OAZ2,PPP1CB,RAN,RAP1B |
| MRGPRX3 | CSF3R,CXCR2,S100A8,S100A9 |
| MYCN | RPL13,RPL13A,RPL21,RPL31,RPL6,RPS27,RPS3A,UBE2V2 |
| Interferon beta 1-a | IL16,RAN,RPS27,S100A9,TLR1 |

List of the first 10 upstream regulators identified by IPA. In bold: upstream regulators associated with the mTOR pathway: LARP1, torin1 and sirolimus, p-value of overlap = $1.04 \times 10^{-11}$, $8.95 \times 10^{-10}$, and $1.91 \times 10^{-07}$ respectively.

protein degradation, likely disrupts muscle function, which suggests a plausible mechanism through which IFN-α may induce fatigue.

Type-I IFN-α/β (IFNAR) receptors are ubiquitously expressed across almost all tissue types. Furthermore, peripherally administrated IFN-α readily crosses the vascular endothelium and blood brain-barrier (BBB) to gain access to the CNS and peripheral neuromuscular tissue (Banks, 2005). It has been shown that interperitoneal IFN injection is followed by a marked increase in the expression of Interferon sensitive genes in the brain parenchyma of murine models (Wang et al., 2008; Wang & Campbell, 2005). Recently, IFN has also been implicated in mediating neural stem cell homeostasis (Carvajal Ibañez et al., 2023), and processes such as anti-apoptosis activity, cellular metastasis and vasculogenic mimicry in tumour microenvironments (Yeh et al., 2018) Within the brain, IFN has a particular predilection for subcortical structures such as the basal ganglia (Goutières et al., 1998) and IFN-induced fatigue/ motivational impairment has been associated with disruption of basal ganglia glucose metabolism, dopamine turnover (Capuron et al., 2007; Nakagawa et al., 2016), glutamate/glutamine concentrations (Haroon et al., 2014) and tissue microstructure (Harrison et al., 2015). Interestingly, there is also a small literature suggesting that mTOR expression in the basal ganglia may play a role in motor learning skills and motor memory (Bergeron et al., 2014).

Though offering potential new insights into the pathophysiology of IFN-induced fatigue our study has a number of limitations. Firstly, although certain gene transcripts exhibit a correlated expression pattern in both blood and brain (Basu et al., 2021; Hess et al., 2016; Qi et al., 2018; Sullivan et al., 2006; Tylee et al., 2013) and peripheral blood transcriptomic approaches have been successfully applied to identify molecular signatures and changes in gene expression during chronic IFN-α treatment (Felger et al., 2012; Hepgul et al., 2016), it is important to note that transcripts detected in peripheral blood may be substantially different and not align with gene expression in the brain. Furthermore, this study is unable to capture IFN-mediated tissue-specific changes in gene expression. In addition, whole blood encompasses multiple cell types and this study accessed how IFN mediates changes at the global level and therefore cannot provide information about which specific cells mediate the observed effect. A more in-depth characterization of cell-specific mRNA profile should be considered for further studies. Secondly, patients underwent combination therapy with IFN-α and the antiviral drug ribavirin. Arguably, this pharmacological agent may have had an influence in some of the behavioral changes observed though it is worth noting that it will not have influenced the transcriptional changes

we observed as the first dose of Ribavirin was administered after both blood samples were taken.

Both STRING and IPA, the tools used in our analysis, do not inherently control for correlations among genes, treating each gene as an independent entity. In reality, genes are interconnected, with their expression often being correlated. This limitation might have led to higher false positive rates, overrepresentation of certain pathways, or a failure to detect complex gene-gene interactions. However, the fact that STRING considers several types of evidence for interaction, and not just co-expression, somewhat mitigates the potential effects of correlation among genes. Of note, muscle mass and function were also not measured as part of this study. Inclusion of this in future studies could help further evaluate evidence linking IFN-α induced fatigue to mTOR.

Additionally, our results suggest a complex association between mTOR and IFN-α induced fatigue and though the evidence currently best supports a link between mTOR inhibition and fatigue, definitively determining the directionality between mTOR activity and fatigue will require further investigation. Given the multifaceted role of mTOR in regulating anabolic and catabolic processes and the potential for IFN-α to disrupt this balance, further investigation is warranted to elucidate the precise role of mTOR activity in IFN-α induced fatigue.

Finally, though the repeated measures design adds power, our sample size was relatively modest and did not account for clinical covariates, therefore our findings must be viewed as hypothesis-generating and requiring confirmation in future larger studies.

To conclude, this exploratory study provides evidence of an acute immune-response signature to the administration of IFN-α that is associated with the subsequent development of fatigue. Identification of this novel network of predominantly cytosolic ribosomal and ubiquitin transcripts implicated in modulating mTOR signaling may provide new insights into how interferons induce fatigue and highlight a potential novel therapeutic target for virus-induced fatigue.

## CRediT authorship contribution statement

**Eva Periche-Tomas:** Writing – original draft, Investigation, Conceptualization. **Annamaria Cattaneo:** Methodology. **Nadia Cattane:** Methodology. **Claudia Bone:** Investigation. **Jeremy Tibble:** Conceptualization. **Edward T. Bullmore:** Funding acquisition, Conceptualization. **Carmine Pariante:** Conceptualization, Methodology. **Neil A. Harrison:** Review & editing, Methodology, Investigation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This study was partially funded by the MRC Immunopsychiatry Consortium grant (RG71546) to E.B

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2024.09.038.

## Data availability

Data will be made available on request.

## References

Banks, W., 2005. Blood-brain barrier transport of cytokines: a mechanism for neuropathology. Curr. Pharm. Des. 11 (8), 973–984. https://doi.org/10.2174/1381612053381684.

Banks, W.A., Erickson, M.A., 2010. The blood–brain barrier and immune function and dysfunction. Neurobiol. Dis. 37 (1), 26–32. https://doi.org/10.1016/j.nbd.2009.07.031.

Basu, M., Wang, K., Ruppin, E., Hannenhalli, S., 2021. Predicting tissue-specific gene expression from whole blood transcriptome. Sci. Adv. 7 (14), eabd6991. https://doi.org/10.1126/sciadv.abd6991.

Bergeron, Y., Chagniel, L., Bureau, G., Massicotte, G., Cyr, M., 2014. mTOR signaling contributes to motor skill learning in mice. Front. Mol. Neurosci. 7, 26. https://doi.org/10.3389/fnmol.2014.00026.

Bolster, D.R., Jefferson, L.S., Kimball, S.R., 2004. Regulation of protein synthesis associated with skeletal muscle hypertrophy by insulin-, amino acid- and exercise-induced signalling. Proc. Nutr. Soc. 63 (2), 351–356. https://doi.org/10.1079/PNS2004355.

Capuron, L., Lawson, D.H., Nemeroff, C.B., 2002. Neurobehavioral effects of interferon-_ in cancer patients: phenomenology and paroxetine responsiveness of symptom. Dimensions. 26 (5), 10.

Capuron, L., Miller, A.H., 2004. Cytokines and psychopathology: lessons from interferon-α. In. Biol. Psychiatry 56. https://doi.org/10.1016/j.biopsych.2004.02.009.

Capuron, L., Pagnoni, G., Demetrashvili, M.F., Lawson, D.H., Fornwalt, F.B., Woolwine, B., Berns, G.S., Nemeroff, C.B., Miller, A.H., 2007. Basal ganglia hypermetabolism and symptoms of fatigue during interferon- α therapy. Neuropsychopharmacology 32 (11), Article 11. https://doi.org/10.1038/sj.npp.1301362.

Capuron, L., Pagnoni, G., Drake, D.F., Woolwine, B.J., Spivey, J.R., Crowe, R.J., Votaw, J.R., Goodman, M.M., Miller, A.H., 2012. Dopaminergic Mechanisms of Reduced Basal Ganglia Responses to Hedonic Reward during Interferon Alfa Administration. in *Arch Gen Psychiatry* (10 Vol. 69, 1044–1053.

D. Carvajal Ibañez M. Skabkin J. Hooli S. Cerrizuela M. Göpferich A. Jolly K. Volk M. Zumwinkel M. Bertolini G. Figlia T. Höfer G. Kramer S. Anders A.A. Teleman A. Marciniak-Czochra A. Martin-Villalba Interferon regulates neural stem cell function at all ages by orchestrating mTOR and cell cycle EMBO Mol. Med. 15(4), e16434 2023 10.15252/emmm.202216434.

Chaudhuri, A., Behan, P.O., 2004. Fatigue in neurological disorders. Lancet 363 (9413), 978–988. https://doi.org/10.1016/S0140-6736(04)15794-2.

Dafny, N., Prieto-Gomez, B., Dong, W.Q., Reyes-Vazquez, C., 1996. Interferon modulates neuronal activity recorded from the hypothalamus, thalamus, hippocampus, amygdala and the somatosensory cortex. Brain Res. 734 (1–2), 269–274.

Dowell, N.G., Cooper, E.A., Tibble, J., Voon, V., Critchley, H.D., Cercignani, M., Harrison, N.A., 2016. Acute changes in striatal microstructure predict the development of interferon-Alpha induced fatigue. Biol. Psychiatry 79 (4), 320–328. https://doi.org/10.1016/j.biopsych.2015.05.015.

Dowell, N. G., Bouyagoub, S., Tibble, J., Voon, V., Cercignani, M., & Harrison, N. A. (2017). *Interferon-alpha-Induced Changes in NODDI Predispose to the Development of Fatigue*. 7.

Dowling, R.J.O., Topisirovic, I., Fonseca, B.D., Sonenberg, N., 2010. Dissecting the role of mTOR: lessons from mTOR inhibitors. Biochim. Biophys. Acta Protein Proteomics 1804 (3), 433–439. https://doi.org/10.1016/j.bbapap.2009.12.001.

Felger, J.C., Cole, S.W., Pace, T.W.W., Hu, F., Woolwine, B.J., Doho, G.H., Raison, C.L., Miller, A.H., 2012. Molecular signatures of peripheral blood mononuclear cells during chronic interferon-α treatment: relationship with depression and fatigue. Psychol. Med. 42 (8), 1591–1603. https://doi.org/10.1017/S0033291711002868.

Gift, A.G., 1989. Visual analogue scales: measurement of subjective phenomena. Nurs. Res. 38 (5), 286–288. https://doi.org/10.1097/00006199-198909000-00006.

GoutièRes, F., Aicardi, J., Barth, P.G., Lebon, P., 1998. Aicardi-Goutières syndrome: an update and results of interferon-α studies. Ann. Neurol. 44 (6), 900–907. https://doi.org/10.1002/ana.410440608.

Greenhouse-Tucknott, A., Butterworth, J.B., Wrightson, J.G., Smeeton, N.J., Critchley, H.D., Dekerle, J., Harrison, N.A., 2022. Toward the unity of pathological and exertional fatigue: a predictive processing model. Cogn. Affect. Behav. Neurosci. 22 (2), 215–228. https://doi.org/10.3758/s13415-021-00958-x.

Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiatry 23 (1), 56–62. https://doi.org/10.1136/jnnp.23.1.56.

Haroon, E., Woolwine, B.J., Chen, X., Pace, T.W., Parekh, S., Spivey, J.R., Hu, X.P., Miller, A.H., 2014. IFN-alpha-induced cortical and subcortical glutamate changes assessed by magnetic resonance spectroscopy. Neuropsychopharmacology 39 (7), 1777–1785. https://doi.org/10.1038/npp.2014.25.

Harrison, N.A., Cooper, E., Dowell, N.G., Keramida, G., Voon, V., Critchley, H.D., Cercignani, M., 2015. Quantitative magnetization transfer imaging as a biomarker for effects of Systemic inflammation on the brain. Biol. Psychiatry 78 (1), 49–57. https://doi.org/10.1016/j.biopsych.2014.09.023.

Hepgul, N., Cattaneo, A., Agarwal, K., Baraldi, S., Borsini, A., Bufalino, C., Forton, D.M., Mondelli, V., Nikkheslat, N., Lopizzo, N., Riva, M.A., Russell, A., Hotopf, M., Pariante, C.M., 2016. Transcriptomics in interferon-α-treated patients identifies inflammation-, neuroplasticity- and oxidative stress-related signatures as predictors and correlates of depression. Neuropsychopharmacology 41 (10), 2502–2511. https://doi.org/10.1038/npp.2016.50.

Hess, J.L., Tylee, D.S., Barve, R., de Jong, S., Ophoff, R.A., Kumarasinghe, N., Tooney, P., Schall, U., Gardiner, E., Beveridge, N.J., Scott, R.J., Yasawardene, S., Perera, A., Mendis, J., Carr, V., Kelly, B., Cairns, M., Unit, N.G., Tsuang, M.T., Glatt, S.J., 2016. Transcriptome-wide mega-analyses reveal joint dysregulation of immunologic genes

and transcription regulators in brain and blood in schizophrenia. Schizophr. Res. 176 (2–3), 114–124. https://doi.org/10.1016/j.schres.2016.07.006.
Ivashkiv, L.B., Donlin, L.T., 2014. Regulation of type I interferon responses. Nat. Rev. Immunol. 14 (1), Article 1. https://doi.org/10.1038/nri3581.
Jiang, Y., Su, S., Zhang, Y., Qian, J., Liu, P., 2019. Control of mTOR signaling by ubiquitin. Oncogene 38 (21), 3989–4001. https://doi.org/10.1038/s41388-019-0713-x.
Livingstone, M., Sikström, K., Robert, P.A., Uzé, G., Larsson, O., Pellegrini, S.P., 2015. Assessment of mTOR-Dependent translational regulation of interferon stimulated genes. PLoS One 10 (7), 1–20. https://doi.org/10.1371/journal.pone.0133482.
Maddock, C., Landau, S., Barry, K., Maulayah, P., Hotopf, M., Cleare, A.J., Norris, S., Pariante, C.M., 2005. Psychopathological symptoms during interferon-α and ribavirin treatment: effects on virologic response. Mol. Psychiatry 10 (4), 332–333. https://doi.org/10.1038/sj.mp.4001634.
Maiole, F., Giachero, S., Fossati, S.M., Rocchi, A., Zullo, L., 2019. mTOR as a marker of exercise and fatigue in octopus vulgaris arm. Front. Physiol. 10 (September), 1–9. https://doi.org/10.3389/fphys.2019.01161.
Mayer, C., Grummt, I., 2006. Ribosome biogenesis and cell growth: mTOR coordinates transcription by all three classes of nuclear RNA polymerases. Oncogene 25 (48), Article 48. https://doi.org/10.1038/sj.onc.1209883.
Nakagawa, S., Takeuchi, H., Taki, Y., Nouchi, R., Kotozaki, Y., Shinada, T., Maruyama, T., Sekiguchi, A., Iizuka, K., Yokoyama, R., Yamamoto, Y., Hanawa, S., Araki, T., Miyauchi, C.M., Magistro, D., Sakaki, K., Jeong, H., Sasaki, Y., Kawashima, R., 2016. Basal ganglia correlates of fatigue in young adults. Sci. Rep. 6 (1), 21386. https://doi.org/10.1038/srep21386.
Peng, L., Zhou, Y., Ye, X., Zhao, Q., 2015. Treatment-related fatigue with everolimus and temsirolimus in patients with cancer—A meta-analysis of clinical trials. Tumor Biol. 36 (2), 643–654. https://doi.org/10.1007/s13277-014-2669-3.
Polge, C., Attaix, D., Taillandier, D., 2015. Role of E2-Ub-conjugating enzymes during skeletal muscle atrophy. Front. Physiol. 6. https://doi.org/10.3389/fphys.2015.00059.
Qi, T., Wu, Y., Zeng, J., Zhang, F., Xue, A., Jiang, L., Zhu, Z., Kemper, K., Yengo, L., Zheng, Z., eQTLGen Consortium, Marioni, R. E., Montgomery, G. W., Deary, I. J., Wray, N. R., Visscher, P. M., McRae, A. F., & Yang, J. (2018). Identifying gene targets for brain-related traits using transcriptomic and methylomic data from blood. *Nature Communications*, *9*(1), 2282. 10.1038/s41467-018-04558-1.
Raison, C.L., Borisov, A.S., Majer, M., Drake, D.F., Pagnoni, G., Woolwine, B.J., Vogt, G. J., Massung, B., Miller, A.H., 2009. Activation of central nervous system inflammatory pathways by interferon-alpha: Relationship to monoamines and depression. Biol. Psychiatry 65 (4), 296–303. https://doi.org/10.1016/j.biopsych.2008.08.010.
Risson, V., Mazelin, L., Roceri, M., Sanchez, H., Moncollin, V., Corneloup, C., Richard-Bulteau, H., Vignaud, A., Baas, D., Defour, A., Freyssenet, D., Tanti, J.F., Le-Marchand-Brustel, Y., Ferrier, B., Conjard-Duplany, A., Romanino, K., Bauché, S., Hantaï, D., Mueller, M., Gangloff, Y.G., 2009. Muscle inactivation of mTOR causes metabolic and dystrophin defects leading to severe myopathy. J. Cell Biol. 187 (6), 859–874. https://doi.org/10.1083/jcb.200903131.
Russell, A., Hepgul, N., Nikkheslat, N., Borsini, A., Zajkowska, Z., Moll, N., Forton, D., Agarwal, K., Chalder, T., Mondelli, V., Hotopf, M., Cleare, A., Murphy, G., Foster, G., Wong, T., Schütze, G.A., Schwarz, M.J., Harrison, N., Zunszain, P.A., Pariante, C.M., 2019. Persistent fatigue induced by interferon-alpha: a novel, inflammation-based, proxy model of chronic fatigue syndrome. Psychoneuroendocrinology 100, 276–285. https://doi.org/10.1016/j.psyneuen.2018.11.032.
Sakai, H., Ikeno, Y., Tsukimura, Y., Inomata, M., Suzuki, Y., Kon, R., Ikarashi, N., Chiba, Y., Yamada, T., Kamei, J., 2020. Upregulation of ubiquitinated proteins and their degradation pathway in muscle atrophy induced by cisplatin in mice. Toxicol. Appl. Pharmacol. 403 (June), 115165. https://doi.org/10.1016/j.taap.2020.115165.
Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl 20), 22–33.
G. Spielberger R.L., Lushene, R. E, Vagg, P.R., & Jacobs, G.A. State-trait anxiety inventory 1983 Mind Garden Palo Alto. CA.
Stübgen, J.-P., 2009. Interferon alpha and neuromuscular disorders. J. Neuroimmunol. 207 (1), 3–17. https://doi.org/10.1016/j.jneuroim.2008.12.008.
Sullivan, P. F., Fan, C., & Perou, C. M. (2006). *Evaluating the comparability of gene expression in blood and brain*. 261–268.
Tylee, D. S., Kawaguchi, D. M., & Glatt, S. J. (2013). On the outside, looking in: A review and evaluation of the comparability of blood and brain '-omes'. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics*, *162B*(7), 595–603. 10.1002/ajmg.b.32150.
Wang, J., 2009. Interferon-α, Molecular Signaling Pathways and Behavior. In: Siegel, A., Zalcman, S.S. (Eds.), The Neuroimmunological Basis of Behavior and Mental Disorders. Springer, US, pp. 71–85. https://doi.org/10.1007/978-0-387-84851-8_5.
Wang, J., Campbell, I.L., 2005. Innate STAT1-dependent genomic response of neurons to the antiviral cytokine alpha interferon. J. Virol. 79 (13), 8295–8302. https://doi.org/10.1128/JVI.79.13.8295-8302.2005.
Wang, J., Campbell, I., Zhang, H., 2008. Systemic interferon-a regulates interferon-stimulated genes in the central nervous system. Mol. Psychiatry 9.
Whale, R., Fialho, R., Field, A.P., Campbell, G., Tibble, J., Harrison, N.A., Rolt, M., 2019. Factor analyses differentiate clinical phenotypes of idiopathic and interferon-alpha-induced depression. Brain Behav. Immun. 80, 519–524. https://doi.org/10.1016/j.bbi.2019.04.035.
Yeh, Y.-H., Hsiao, H.-F., Yeh, Y.-C., Chen, T.-W., Li, T.-K., 2018. Inflammatory interferon activates HIF-1α-mediated epithelial-to-mesenchymal transition via PI3K/AKT/mTOR pathway. J. Exp. Clin. Cancer Res. 37 (1), 70. https://doi.org/10.1186/s13046-018-0730-6.
Yoon, M.-S., 2017. mTOR as a key regulator in maintaining skeletal muscle mass. Front. Physiol. 8. https://www.frontiersin.org/articles/10.3389/fphys.2017.00788.
Zhao, Y., Xiong, X., Sun, Y., 2011. DEPTOR, an mTOR inhibitor, is a physiological substrate of SCFβTrCP E3 ubiquitin ligase and regulates survival and autophagy. Mol. Cell 44 (2), 304–316. https://doi.org/10.1016/j.molcel.2011.08.029.