Author:
Almutairi, Basim S

Title:
Investigating the neural substrates of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME)
A Structural and Functional MRI study

Investigating the neural substrates of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME): A Structural and Functional MRI study.

Basim Almutairi

A dissertation submitted to the University of Bristol

in accordance with the requirements for the award of the degree of PhD in the Faculty of Health Sciences, Bristol Medical School

March 2021

Summary / Abstract

Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) is characterised by continuous fatigue and has many diagnostic criteria. Cognitive dysfunction affects 86-94% of adults with CFS/ME. This thesis used MRI applications to investigate brain structure and function in CFS/ME. This thesis hypothesised to find brain volume differences, functional connectivity differences in brain networks, and functional differences measured by Blood Oxygenation Level Dependant (BOLD) signal activation during working memory task performance. The working memory paradigm was designed to investigate working memory components, processing and storage separately and combined. The relationship between fatigue and performance was assessed. This thesis's original contribution provides evidence that the salience network might have altered resting-state functional connectivity in CFS/ME in the absence of morphological differences. The salience network is involved in detecting and integrating salient sensory information; therefore, disruption in this network might disrupt incoming cognitive stimuli and influence other networks' connectivity, involved in fatigue and impaired memory. In the more demanding task, participants with CFS/ME were slower and less accurate but used the same working memory network as healthy controls. No brain volume differences, nor atrophy were found. The differences between these findings compared to previous studies might be due to different study designs, analysis methods, sample sizes with different symptoms, including illness duration, physical inactivity and sleep disturbance.

The salience network alteration could potentially have a significant role in CFS/ME, as we cannot determine cause and effect with current experimental design the association with fatigue and other CFS/ME symptoms remains unclear. Using longitudinal studies that account for neurologically relevant confounders are needed in CFS/ME to further investigate the role of salience network.

Acknowledgements

I would like to thank the Saudi ministry of health for funding this research and making this thesis possible.

I would like to give special thanks to my supervisors' Dr Jade Thai, Professor Esther Crawley Dr Elanor Hinton.

Dr Thai deserves exceptional thanks. She has guided me through my thesis and helped me to develop skills that will enable me to excel in my future academic career. Furthermore, she has become a dear friend and confidant for me. Professor Esther Crawley has been invaluable at developing my knowledge towards Chronic Fatigue Syndrome. DR. Elanor Hinton has been generous and showed her support when agreeing to be my supervisor in these situations.

A special thank you also to the CFS/ME team at the Cossham hospital: Dr Peter Gladwell, Dr Flora Wilson, Aysha Adrissi, Fiona Hetherington for their help in patient recruitment. Without their incredibly hard work, this research would not be possible.

Thank you to Dr Christelle Langley for her continued support and friendly discussions as well as assistance in scanning at all odd hours of the week.

Thank you to Aileen Wilson for her help in operating the 3T Skyra; without this skill set, I would have struggled to collect all the data.

A thank you to all who assisted on data collection, particularly for the MRI scans, Valeria Oliva, Cathy Fleet, Esther Saunders-Jennings, Alfie Wearn, Jaime Thakrar, Carlos Munoz Neira.

Thanks to the CFS/ME research team at the Oakfield house for the informative meetings and support.

I would like the thank my parents, Sallah and Modhi. They have made tremendous sacrifices to enable me to immigrate to the UK and pursue a PhD.

A very special thank you to my wife, Norah, for her continued emotional and personal support throughout this thesis. And thank you to my kids, Modhi, Abdullah and Diyam, for their unconditional love only a kid can give.

Finally, I thank the study participants from whom I have learned very much and who have enabled me to perform this research.

Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others is indicated as such. Any views expressed in the dissertation are those of the author.

Signed: ____Basim Sallah Almutairi_____ Date:____First submission_28/02/2021

Second submition_15-11-2021

Contribution Statement

Part of this research has been carried out by a research team which included:

Dr Jade Thai (JNT)

Developed the idea for this PhD. Advised on developing protocols and topic guides, interpretation of results, and drafting the systematic review publication (Chapter 2) and thesis.

Professor Esther Crawley (EC)

Developed the idea for this PhD. Advised on developing protocols and topic guides and interpretation of results and commented on drafts of systematic review publication (Chapter 2) and thesis.

Dr Elanor Hinton (ECH)

Commented on individual chapters and the thesis as a whole.

Dr Christelle Langley (CL)

CL read the titles and abstracts in the systematic review (Chapter 2) and help throughout the thesis in data analysis and interpretation.

Publications and Contributions

Chapter 2: Systematic Review and Quality Analysis

Published: (Published)

Contributions

Basim Almutairi (BA) led protocol and search strategy development, undertook the searches, screening, data extraction, critical appraisal, synthesis and writing of the paper. JNT, CL and EC contributed to the protocol and search strategy development. CL helped to double screen studies. BA led the writing of the paper, and all authors contributed to the interpretation of results and to drafting the paper. All authors read and approved the final version of the paper.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AAL	Automated Anatomical Labelling
ACC	Anterior Cingulate Cortex
AFNI	Analysis of Functional Neuroimaging software
ALE	Activation Likelihood Estimation
ANOVA	Analysis of Variance
B0	Applied Magnetic Field
B1	Rotating Magnetic Field
BA	Brodmann Area
BG	Basal Ganglia
BOLD	Blood Oxygenation Level-Dependent
CAT12	Computational Anatomy Toolbox
CBF	Cerebral Blood Flow
CBT	Cognitive Behaviour Therapy
CDC	Centre for Disease Control and Prevention
CEN	Central Executive Network
CFQ	Chalder Fatigue Questionnaire
CFS/ME	Chronic Fatigue Syndrome/Myalgic Encephalomyelitis
CRIC	Clinical Research and Imaging Centre
CSF	Cerebral Spinal Fluid
DCM	Dynamic Causal Modelling
DMN	Default Mode Network
dlPFC	Dorsolateral Prefrontal Cortex
DTI	diffusion tensor imaging
EEG	Electroencephalography
EMG	Electromyography
EPI	Echo Planar Imaging
FC	functional connectivity
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FPN	Frontoparietal Networks
FSL	FMRIB Software Library
FSS	Fatigue Severity Scale
FEW	Family Wise Error
GE	Gradient Echo
GLM	General Linear Model
HADS	Hospital Anxiety and Depression Scale
HC	Healthy Control
ICA	Independent Component Analysis
LFP	Local Field Potential
LTM	Long Term Memory
MD	Mean Diffusivity
MFI	Multidimensional Fatigue Inventory
MPRAGE	Magnetisation Prepared Rapid Acceleration Gradient Echo
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MS	Multiple Sclerosis
Ms	Millisecond
MUA	Multi-Unit Activity
NICE	National Institute for Health and Care Excellence
NMR	Nuclear Magnetic Resonance
NREC	National Research Ethics Committee

O-OER	Object-Oriented Episodic Record
Ospan	Operation span
PASAT	Paced Auditory Serial Addition Test
PEM	Post-Exertional Malaise
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
RF	Radio Frequency
ROI	Region of Interest
PSQI	Pittsburgh Sleep Quality Index
rs-fMRI	resting-state functional MRI
RSNs	Resting-State Networks
SE	Spin Echo
SMN	Sensory-Motor Network
SNR	Signal to Noise Ratio
SPECT	Single-Photon Emission Computerised Tomography
SPM	Statistical Parametric Mapping
sMRI	Structural MRI
SN	Salience Network
SWP	Small World Propensity
SymmSpan	Symmetry span
TIV	Total Intracranial Volumes
TR	Repetition Time
TE	Time to Echo
VBM	Voxel-Based Morphometry

1. Chapter 1: Introduction

1.1. Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME)

Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) is a common, enigmatic condition defined by physical and mental fatigue. CFS/ME is an acquired disorder that is characterised by various symptoms and affects multiple body systems. It is an illness characterised mainly by continuous fatigue, which lasts (depending on the diagnostic criteria used) for at least four to six months; that is not explained by any other condition and associated with other secondary symptoms [1]. These symptoms include post-exertion malaise, which lasts for more than 24 hours, significant short-term memory impairment, un-refreshing sleep, headache, muscle pain, tender lymph nodes and frequent or recurrent sore throat [1]. It is often triggered by an infection, flu-like and upper respiratory infections [2]. Commonly, CFS/ME patients suffer headaches, vision impairments, back pains, numbness, and fatigue and sleep dysfunction. Depending on the severity of the symptoms, CFS/ME may leave patients bedridden [3]. Most research studies theorise CFS/ME disorder as a result of genetically related factors or abnormalities in the immune system affecting the body and brain function [2].

1.1.1 CFS/ME definitions

CFS/ME is a clinical diagnosis based on clinical presentation. Table 1 [4] describes the five diagnostic criteria used, including the Centre for Disease Control and Prevention (CDC), Fukuda, Oxford, National Institute for Health and Care Excellence (NICE), and Canadian guidelines [1, 5-7]. Each criterion depends on the period of the presence of fatigue and secondary symptoms present before a diagnosis of CFS/ME can be made. For a patient to be diagnosed with CFS/ME, the following tests should be completed to exclude other causes of fatigue: urea and electrolyte's, urinalysis, thyroid and liver function, full blood count, C-reactive protein, serum creatinine, random blood glucose, plasma viscosity, creatine kinases serum calcium and screening blood tests for gluten sensitivity [6].

Using more than one case definition to diagnose an illness is rare. Clinically, the use of case definitions is used to give an appropriate diagnosis as well as guiding therapy and management, while in research, the use of these definitions is about selecting the appropriate study population. In CFS/ME, there are more than 20 different diagnostic criteria, most of which were produced to serve research purposes [8]. There is no world-wide agreement on which definition should be used clinically or for research. Most of the recent research on this illness uses CDC or the Canadian consensus [8]. Many similarities can be found between these

definitions, but the main differences are in the number of the required symptoms and whether post-exertional malaise (PEM) is present. The following table (table 1) [4] summarises the key features of three of the main definitions and diagnostic criteria for CFS/ME.

Table 1 shows a summary of key features of the case definitions for Chronic Fatigue Syndrome/Myalgic Encephalomyelitis. The table is taken from the centres for disease control and prevention website [4].

	1994 Fukuda / CDC [1]	**2003 Canadian Consensus [5]**	**2011 ME International Consensus [9]**
Overview of Inclusions	**Fatigue + 4 out of 8 case-defining symptoms:** • PEM lasting more than 24 hours • unrefreshing sleep • significant impairment of short-term memory or concentration • muscle pain • pain in the joints without swelling or redness • headaches of a new type, pattern, or severity • tender lymph nodes in the neck or armpit • a sore throat that is frequent or recurring	**Fatigue, post-exertional malaise ± fatigue:** • sleep dysfunction • pain; • have two or more neurological/cognitive manifestations and 1or more from 2 categories of autonomic, neuroendocrine and immune manifestations	**Post-exertional neuroimmune exhaustion:** • ≥1 symptom from 3 neurological impairment categories • ≥ 1 symptom from the immune/gastro-intestinal/genitourinary impairment categories • ≥ 1 symptom from energy metabolism/transport impairments
Duration	≥ 6 months (clinical evaluation starts at one month – prolonged fatigue)	≥ 6 months (preliminary diagnosis can be earlier)	Not included
Fatigue	≥ 6 months new-onset severe persistent or relapsing fatigue unexplained after clinical evaluation not explained by on-going exertion not substantially relieved by rest results in a substantial reduction in occupational, educational, social, or personal activities	Significant new-onset persistent or recurrent physical or mental fatigue unexplained after clinical evaluation substantially reduces activity level	Not included
Post-exertional malaise	Not required but one of the 8 case defining symptoms	Required	Required, renamed post-exertional neuroimmune exhaustion (PENE)
Minimum number of symptoms	5	8	8
Exclusions	An active medical condition that explains chronic fatigue – untreated hypothyroidism, sleep apnoea, narcolepsy, medication side effects Previous diagnosis not unequivocally resolved – chronic hepatitis, malignancy. Past or current major depressive disorder with psychotic or melancholic features, bipolar disorder, schizophrenia, delusional disorders, dementias, anorexia nervosa, bulimia nervosa	Active disease processes that explain symptoms specifies: Addison's disease, Cushing syndrome, hypo- or hyperthyroidism, iron deficiency, anaemia, iron overload, diabetes mellitus, cancer, sleep apnoea, rheumatoid arthritis, lupus, polymyositis, polymyalgia rheumatic, AIDS, multiple	Alternative explanatory diagnoses (untreated), primary psychiatric disorders, somatoform disorder, substance abuse

	Alcohol or substance abuse within 2 years of illness onset or any time after Severe obesity (BMI > 45)	sclerosis, tuberculosis, chronic hepatitis, Lyme disease, primary psychiatric disorders, substance abuse	
Accepted comorbidities	Fibromyalgia, somatoform disorders, anxiety disorders, nonpsychotic or melancholic depression, multiple chemical sensitivity disorder, neurasthenia, treated Lyme disease or syphilis before chronic sequelae, isolated unexplained lab or physical abnormality insufficient to suggest the exclusionary diagnosis	Fibromyalgia, myofascial pain, temporomandibular joint syndrome, irritable bowel syndrome, interstitial cystitis, irritable bladder syndrome, Raynaud's phenomenon, mitral valve prolapse, migraines, allergies, multiple chemical sensitivities, Hashimoto's thyroiditis, sicca syndrome, depression	Fibromyalgia, myofascial pain, temporomandibular joint syndrome, irritable bowel syndrome, interstitial cystitis, Raynaud's phenomenon, mitral valve prolapse, migraines, allergies, multiple chemical sensitivities, Hashimoto's thyroiditis, sicca syndrome, reactive depression

1.1.2 CFS/ME Prevalence

Recently, CFS/ME disorder is estimated to have a prevalence of 0.76% (95% CI 0.23% to 1.29%) according to the clinically confirmed cases in many countries [10]. It had been reported to affect at least 250,000 people in Britain [11], over a million Americans [12], 100,000 Australians [13] and over 400,000 people in Canada [14]. The prevalence rate for CFS/ME in Japan is 1.5% [15] 0.6% in Korea [16] and 0.58% in Nigeria [17]. The disorder is common among populations in the age brackets between 10-19 years and those between 30-39 years. In general, adolescent with CFS/ME have a recovery rate of 54-94% compared to adults with CFS/ME, which have a recovery rate of 22% [10]. The prevalence of CFS/ME is three times higher among women compared to men; it, however, affects any race in equal measure [18, 19].

A study carried by the UK and Dutch clinical cohorts indicated different results among CFS/ME patients of different age by generally categorising their patients as adults and children. Children and adolescent patients showed better physical functions and presenting lesser fatigue compared to adults. In this case, younger children mostly present a sore throat but with lesser cardinal problems such as memory or sleep dysfunction. These findings were found to be generalisable across different regions [20].

1.1.3 CFS/ME Treatments

There is no pharmacological treatment for CFS/ME. However, the various methods used to treat CFS/ME include cognitive behavioural therapy, graded exercise therapy that entails a structured exercise program [21, 22]. For patients experiencing nausea, the conventional treatment that entails eating practices sipping fluids and eating little and often are employed.

In the case that nausea is severe, antiemetic drugs can be prescribed [21]. A low-dose of tricyclic depressants or melatonin can be described to patients having sleep problems [20].

1.1.4 Cognitive Dysfunction in CFS/ME

Cognitive dysfunction is one of the most common secondary symptoms in CFS/ME illness [1] and was reported in 86-94% of adults with CFS/ME studies in the UK, USA, Australia and Netherlands [23-28]. CFS/ME patients report difficulties with memory, learning, attention, and cognitive processing speed [29, 30].

One possible explanation is interference. More precisely, it has been suggested that CFS/ME causes cognitive impairment, including interference with the processing speed of verbal information as well as working memory [31, 32]. Baddeley and colleagues have fragmented the working memory components in the human brain into three main components which included; executive control to prevent distractors, attentional control to encode and recover related information and short term memory maintenance [33]. These components were suggested to be responsible for the cognitive difficulties in patients with CFS/ME by Marshall et al. 1997 [34]. However, various studies provide contradicting evidence regarding whether patients with CFS/ME have problems with working memory. According to DeLuca et al. (2004), CFS/ME patients without psychiatric comorbidity showed significantly reduced performance on information processing speed tests as opposed to working memory tests when compared to healthy individuals and those with rheumatoid arthritis [35]. However, these findings have been contradicted by recent and earlier studies, including Sulheim et al. (2015) and Marshall et al. (1997) [34, 36]. According to these studies, CFS/ME patients revealed impaired cognitive function in terms of both processing speed and working memory compared to healthy controls. Since there are inconsistent findings regarding the influence of CFS/ME on working memory, more research should, therefore, be conducted to clarify this effect.

1.2. Working memory

1.2.1 Working Memory Models

Working memory models have evolved in the last 50 years and had many theories behind them. They include; Cowan's Embedded Processes [37], the Object-Oriented Episodic Record (O-OER) model and the Feature Model [38]. Although the Baddeley model was used by the majority of studies, also in this study, it worth mentioning the other proposed models for working memory.

1.2.2 Baddeley Working Memory Model

Working memory is a structure of what was known as the short-term memory model, which was proposed by Baddeley and Hitch in 1992 with four main parts. These parts are the central executive and the three slaves' system: phonological loop, visuospatial sketchpad and episodic buffer (see figure 1). The central executive's role in this model is to organise the received information from the slave systems. The phonological loop is a storage system for auditory information which can be divided into the phonological store and articulatory process. Moreover, the phonological store is also known as the inner ear, which stores the information you hear, whereas the articulatory process, which is also known as the inner voice, repeats and rehearses words to keep them in working memory while needed. The next part of the slave system is the visuospatial sketchpad which is mainly responsible for dealing with both spatial as well as visual information using its two principal parts: the visual cache, used to store visual data like objects' colour and shape, and the inner scribe, which records the arrangement of objects as well as communicating with the central executive to transfer information. The working memory slave system's final part is called the episodic buffer. There is less known about this system compared to the previous two systems, but theorists have included it to account for communication and interaction between working memory and long-term memory [39, 40].

According to the Baddeley model, verbal working memory characterises an integrated network made up of a central executive and an articulatory loop. This model postulates that incoming information is encoded and maintained in the articulatory loop, low capacity and temporary phonological store through subvocal rehearsal. The information is then manipulated for further use by the help of the central executive function [40]. Encoding of verbal information is usually correlated with dorsolateral prefrontal regions [41], active maintenance, as well as information storage with ventrolateral prefrontal areas, and supplementary premotor and motor cortices

[41-44]. The central executive has been confined to a small area in the dorsolateral prefrontal cortex [41].

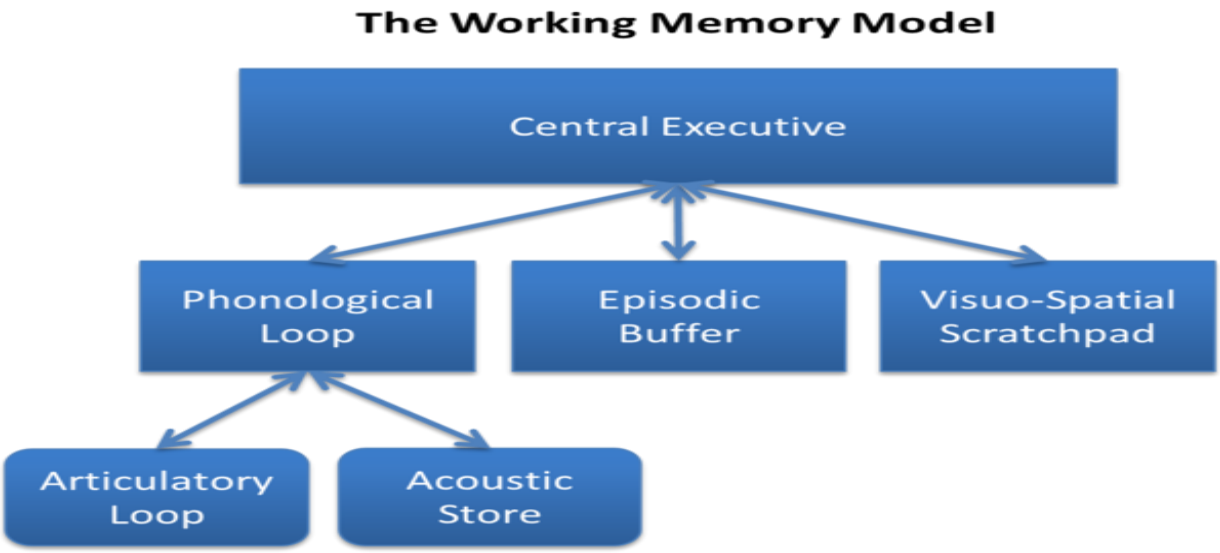


Figure 1 shows the working memory model. Figure from Baddeley and Hitch [30].

Neuroimaging studies have shown that the Baddeley model components have specific neural representations in the brain. They were able to distinguish between the phonological loop and the verbal and visuo-spatial maintenance subsystems as well as showing the disassociation (separation) of storage and rehearsal in verbal maintenance [43] and distinguishing between the central executive processor from the maintenance systems [45, 46]. Many studies have supported that the dorsolateral prefrontal cortex, the parietal network as well as the anterior cingulate cortex are the working memory network [47-49]. It has been shown that the phonological loop is linked to the activation in the temporal lobe where the visuo-spatial sketchpad is linked to the parieto-occipital region of both hemispheres and depends on the task [50, 51].

1.2.2.1 Strengths:

This model offers an explanation for parallel processing, where processes that are involved in a cognitive task occur at once. It gives an explanation for both the processing and storage of information, unlike some other models. It was developed using laboratory experiments in which confounders can be controlled. This is important to produce reliable results which can be replicated. New hypothesis and predictions can be tested easily since this model proposes separate and specific functions and subsystems. The model suggests that short-term phonological and visual memories are managed by separate short-term stores, which were supported by the famous KF study by Shallice and Warrington (1974) or LH by Farah et al. (1988) [52, 53]. According to Shallice and Warrington (1974), patient KF was able to recall verbal but not visual information immediately after its presentation. This finding supports the idea that separate short-term stores manage short-term phonological and visual memories [52]. On the other hand, patient LH suffered from brain damage due to a car accident and resulted in losing his ability to remember colours and shapes. However, the patient maintained a good memory only for spatial information. They suggested that the inner scribe, responsible for objects in the visual field, was not damaged, but the visual cache, responsible for form and colour, was [53]. The Baddeley model integrates a huge number of research findings. It has been supported by studies on patients with brain damage as well as experimental evidence [54, 55].

1.2.2.2 Weaknesses:

Although it gives more details of short-term memory, Baddeley model main weakness is that it is very simple and vague. It did not specify what central executive is or the role of attention is in working memory [56]. There is probably more than one component of the central executive, therefore, the notion that the Baddeley model has only one central executive might not be valid [57]. Arguably the results from laboratory-based experiments used have low ecological validity [56]. However, it is difficult to see how testing can be done in a normal environment whilst scanning and therefore, it is unlikely there is a better model. It has been argued that some tasks that require repeating, e.g. ‘the the the’ do not represent our everyday activity [56]. Furthermore, evidence from studies that involve brain-damaged participants are not able to perform a “before and after” comparison making it difficult to decide whether the changes found were a result of the damage [57].

1.2.3 Other models

1.2.3.1 The Embedded-Processes Model by Cowan

Cowan (1999) defines working memory as "cognitive processes that are maintained in an unusually accessible state" (Cowan 1999, p. 62) [37] (see figure 2). In general, this theory matches the Baddeley model, only differing in terminology and current focus areas [58]. The theory comprises a limited-capacity attentional focus which functions through areas of activated Long Term Memory (LTM). This model's main concentration is to use one framework to account for a wide range of experimental findings in working memory and attention fields. Cowan has described WM as a functional system that recognises both new and old information to carry out and manipulate mental operations such as problem-solving. According to the model, when a stimulus happens, it is stored for a brief moment in a sensory store. The sensory store then sends the stimulus to either an activated portion of LTM or the focus of attention. If the stimulus is unchanged, the sensory store tends to move to the activated LTM, but if it is a novel stimulus and voluntarily attended stimulus, it tends to stay within the focus of attention [37]. In this model, short-term memory keeps the needed information to complete the task, which is the activated portion of LTM [37]. In turn, short-term memory is responsible for holding information related to the on-going task. Then, the brain focus of attention holds a subset of these activations. After that, the central executive collects these representations for manipulation and processing. This approach proposed that there is a single central WM capacity which helps to explain the individual differences found in performance on WM tasks, by suggesting limitations in both LTM and attention. The measures that quantify visual and verbal WM capacity are equally predictive of general cognitive ability. Therefore, Cowan suggested that both verbal and visual working memory tasks are underlined by the same central capacity limit. While attention is limited to its ability to hold a certain amount of information, LTM contribution is limited to the number of representations that it can hold active in short-term memory. Those representations are subjected to decay over time and will not be available for processing by the central executive [37].

Cowan showed evidence that LTM can keep these representations active for a certain time and suggested 10-20 seconds as a decay time [37]. He also suggested that participants can hold 4±1 items in their WM if they are not rehearsing or chunking, unlike Baddeley's suggestion of seven items [37, 39]. His model also provides a rationale for using warm-up activities to activate related information earlier to the teaching of new skills [37]. This is done by activating LTM to look for a related and familiar concept to allow children to access and then connect

new information [37]. His model also suggests that continues projection of a set of memories to the focus of attention can serve as a refreshment to their representations [37, 59]. This strategy has been referred to as attentional refreshing by Lewandowsky & Oberauer later in 2008 [60]. A crucial issue of this theory is to specify the capacity of the proposed attentional focus, which means the capacity of WM [58].

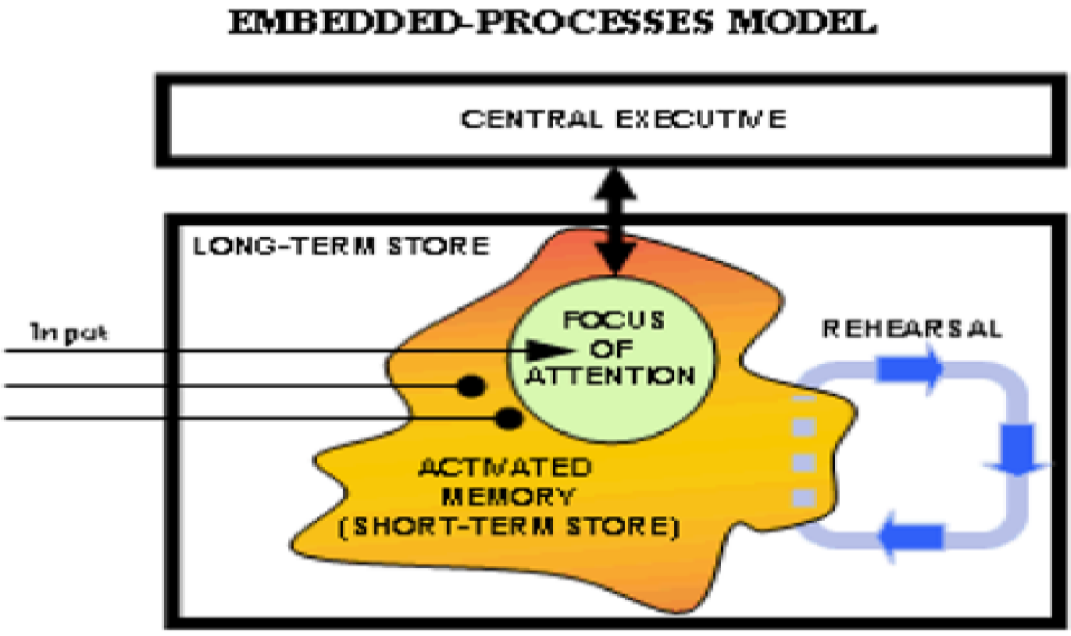


Figure 2 shows the Embedded-processes model. Figure from Cowan (1999) [40].

1.2.3.2 Object-Oriented Episodic (O-OER) Model

The O-OER model is the creation of Jones and his colleagues and avoided the modulatory assumptions that many traditional models proposed [38]. The theory is based on the assumption that temporary storage occurs on some unitary representational space in which all events relating to perceptions and cognitions appear in the form of a modal, abstract representation of events or objects. In this model, it argues that all information is stored temporarily as an abstract representation in only one location. It also suggests that the location is independent to the type of stimulus (e.g., visual, auditory or spatial), which means that either on-going or primary memory task demands will be stored in the same location and only activated when needed regardless of the type of information. Also, the theory provides that in the case of auditory stimuli, there is an automatic object as a result of mental processes detecting boundaries through audio sensitivity. Also, the process establishes unique objects in case of a sufficient change in incoming information energy, which indicates the beginning of a different event, a phenomenon representing a changing-state hypothesis [61]. The first disadvantage of the O-OER model is that the model's ability to retrieve a set of items is dependent on the maintained

integrity of the cues [62]. The maintained integrity of the cues was thought to be vulnerable to temporal decay [62]. Another disadvantage is that it assumes a single-multi modal system which is contradictory with the data taken from other research groups [39].

1.2.3.3 The Feature Model

The feature model derives its name from the consideration of a variety of elements in characterising information in memory. It was first described in 1990 by Nairne, and its primary feature is the use of signs for short term memory as well as long term memory [63]. According to the model,

When the memory trace is created, it immediately encoded into two separate memory systems in which each system has its own properties. During the on-going cognitive processes, the encoded primary memory tracers are subjected to interference from other tracers or from the encoding process of subsequent items [63]. The tracers, or usually called cues, become linked to both primary and secondary memory and can be retrieved later [63]. After associating these cues to form a memory, these cues form a group of nerves to store the memory [63]. When these cues are present, the group of nerves gets activated, which enables short-term memory to work on this memory [63]. Nevertheless, during the presentation of the cues, only the tracers from the primary memory are presented to conscious awareness. Nairne has described the primary memory system as “a repository of cues” (Nairne, 2002, p. 286). Nairne illustrated that the repository of cues is responsible for maintaining feature traces that permit access to the intact traces preserved in secondary memory [63]. When these cues are no longer present or needed, the group will no longer be active and will be waiting in long term memory storage for these cues to be present again [63]. The success of the retrieval process depends on how well the primary memory available cues match the secondary memory unique traces. The model does not use the decay theory to explain the forgetting mechanism; it, however, focuses on item-based interference instead which means that other items which use the same or similar group might block or get in the way of remembering other memories [63]. The advantage of the feature model is that the two levels of memory possess different properties. The two are interrelated and play a critical role in determining the result of one’s memory. Another advantage of the feature model is that it can expose the consequences of individual experiments. The idea is that through the two distinct but interrelated memory levels, it is possible for the model to showcase the full extent of memory development. However, such exposure might, at times be misleading through confusion arising from the different levels of memory [64].

1.2.4 Measuring WM

Measurement is a critical aspect of determining the effectiveness, efficiency and sustainability of working memory in different aspects of life. Essentially, working memory is critical in cognition due to its role as a significant predictor of many characteristics of a higher-order function. Starting from the work of Daneman and Carpenter in 1980 [65], these WM tasks have shown their robustness in predicting everything from simple reading comprehension [65] to performing a difficult task, e.g. Stroop task [66]. Since working memory has different components such as processing and storage, measuring these components separately or as a whole is beneficial to identify if issues are in WM as a whole or in a particular component. The process of measurement involves many aspects of working memory which is of significant value. Measuring working memory allows a better understanding of human cognition, which occurs in everyday life. Some of the principal and most critical tasks for measuring working memory are operation span, symmetry span, and rotation span.

1.2.4.1 Operation Span (Ospan)

In the operational span task [67], participants are asked to verify whether or not a math operation is correct or not then try to remember the letter presented at the end of the math operation (i.e., Is (3x1)-1=4? B). Ospan relates to the sustainability of memory and its ability to work for a long time. The idea is that many people require systems able to work for long enough so that they can get the most out of the process, and that does not expire before the stored information is fully utilised. The operation span refers to the full life expectancy of the system after which it fails to work, and the information stored might be at risk of getting lost. Ospan has an advantage that it gives the participant the time to read and calculate at their own pace. If it was a fixed time, some participants might not have enough time to finish reading or additional time to rehearse, which eventually leads to a false measure of WM [67, 68]. However, the Ospan task has a major disadvantage over other tasks. It is very time consuming for the experimenter, as well as attention-demanding for the participant [69, 70].

1.2.4.2 Symmetry Span (SymmSpan)

Any researcher who operates a project must consider measuring the symmetry span of working memory capacity. In the SymmSpan task [71], participants are asked to remember the location of the presented red squares in order and determine if the stimulus is symmetrical or not. The flexibility of the model is critical as it allows the researcher to understand the memory in detail. This span, therefore, tests the ability of a memory model to work in diverse environments and

relate to many other different factors that might be present [71]. However, SymmSpan as well is time-consuming and attention-demanding for the experimenter [69, 70].

1.2.4.3 Rotation Span

Another critical aspect whose measurement is essential in measuring WM is the rotation span which relates to the ability of the memory to rotate in cycles and work even after it expires for the first time. In the rotation span task [72], participants are presented with a series of letters and arrows on the screen. They are asked to indicate if the letter is rotated or a mirror image and to remember the direction of the arrows for a later test. Memory capabilities undergo several life cycles and can always reignite their capacities even after the first round comes to an end [72]. The idea behind measuring the three working memory components is to have a working memory model that considers all aspects of the memory. The holistic way of making decisions promotes the sustainability of the memory and therefore enhances its lifespan and promotes the achievement of desired goals [73]. However, as the previously mentioned tasks, the rotation span is time-consuming and attention-demanding for the experimenter [69, 70].

1.2.4.4 N-Back

Introduced by Wayne Kirchner in 1958 [74], this task is commonly used in cognitive neuroscience and considered as a continuous performance task to measure a part of working memory and working memory capacity [75]. In detail, a sequence of stimuli are presented to a subject, and then the subject indicates if the stimulus matches the one from n steps earlier in the sequence [74]. It can be visual, auditory or both, which is called a dual n-back task. The letter N represents the position of the letter that the subject needs to attend to, or how far back they need to remember since each letter is presented individually. In the simplest n-task, also known as 1-N, the subject needs to state if the stimulus matches the immediately matching stimulus, i.e. a letter “B” followed by a letter “B”. The task can be more or less challenging by adjusting the load factor of n [74]. Although this task needs to maintain and manipulate information in working memory [75], it is argued that it is not purely a measurement of working memory but might be applied to detect differences in cognitive functioning [76].

1.2.4.5 Bayliss et al. (2003) Task

In an earlier study, a complex span task was designed by Bayliss et al. (2003) to measure the performance of working memory in terms of processing and storage components separately [33]. Bayliss et al. (2003) presented two studies that investigated the underlying constrains of WM performance in children and adults [33]. They used independent measures to measure

processing efficiency and storage capacity in order to measure working memory capacity as they established their relative importance in predicting performance on complex span tasks. In the first study, they used four complex span tasks in children. These four tasks aimed to cross two types of processing (verbal and visuospatial) with two methods of storage (verbal and visuospatial). On a grey computer screen background, nine distinct coloured circles were presented with a digit from 1 to 9. In the verbal processing task, the children were required to choose the colour that better represent the auditorily presented object names. The children were asked to scan the screen in order to locate a visually distinctive circle as a part of the visuospatial processing tasks. To compare this task with traditional short-term memory tasks, children were asked to recall the digits presented in the centre of the circle in the verbal storage tasks or, in the visuospatial storage tasks, they were required to recall the locations of the target circles. The second task was a modified and more demanding version of the previous task but on adults. However, in this task, there were nine coloured squares with four big ones and three smaller ones. In the search task, participants were asked to locate the big square with the bevelled edge. The processing efficiency task, as well as the storage task, were the same as the children ones. Reaction times in these data were used to associate processing difficulty across the span levels within each task [33].

This study showed that there are two primary keys, short-term memory capacity and processing efficiency, to limit the working memory performance [33]. It also showed an unexplained residual variance in working memory function [33]. They proposed that this residual variance represents an executive component because it comes from the necessity to combine processing and storage components in a single task [33]. Nevertheless, they did not use a direct measure to test if the residual variance was executive in nature [33]. Another study, Bayliss and Jarrold (2015), proposed that this represents the forgetting rate [77]. Therefore, the debate is still open, whether it represents an executive component or other factors.

The version of Bayliss et al (2003) tasks used in this thesis consists of three working memory tasks, processing, storage and complex tasks [33]. In the processing task, participants would hear a word that represents a colour of the four different coloured squares presented. They needed to decide which colour represents best the heard word. For instance, if the word was "Strawberry" the accurate response was pressing the button that represents the red square. In the storage task, a series of numbers (4 digits) were presented to the participant to memorise then another series was presented, and the participants needed to decide whether they were the same sequence of numbers or not. In the complex task, participants were presented with four squares with different colours and numbers. Participants would hear a word that represent a

colour and they had to choose the square that best represent that word and memorise the number inside that square. After every four trials, the participants would be presented with a series of numbers then they needed to decide whether the sequence of numbers was the same or different (for further details see p83).

1.3 Neuroanatomy of Working Memory

The brain plays a critical role in the memory process and is the organ of the human body where memory and information are stored. The concept of memory relates to the ability of the brain to keep information that can be used later to give reference to an event that took place earlier. Therefore, it is critical to understand how the brain helps in the memory process. The functions of the brain depend on the communication between neurons which are responsible for coding information and storing them in a manner that they are easily retrieved when similar codes are ignited at a later date [73]. Working memory is supported by a complex network in the central nervous system. This includes the prefrontal cortex, temporal lobe, limbic system, and cerebellum (see figure 3) [73].

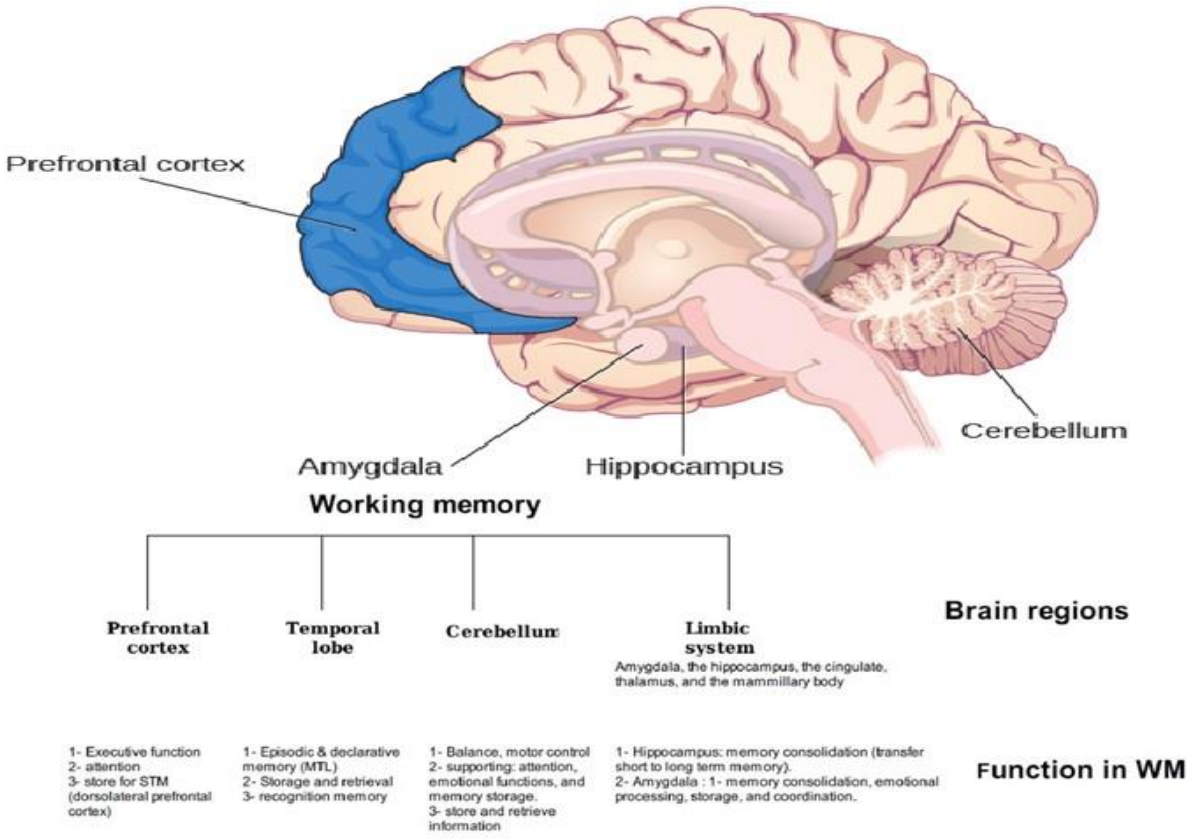


Figure 3 shows the cerebellum which plays a role in attention, emotional functions, memory storage and processing procedural memories, such as how to play the piano. The prefrontal cortex seems to play a role in remembering semantic tasks. The hippocampus is linked with episodic and declarative memory as well as recognition memory. Finally, the amygdala is suggested to be involved in fear and fear-related memories. The top part of the figure is taken from Constantinidis & Klingberg (2016) [73].

1.3.1 Prefrontal Cortex and Parietal Lobe

Notably, the cerebral prefrontal cortex plays a primary role in memory and attention in the sense that it is responsible for high mental functions [78-80]. An accepted theory, by Jacobsen (1936), that the function of the prefrontal cortex is to serve as a store for short-term memory and damage to the primate prefrontal cortex initiated short-term memory deficit [81]. More precisely, dorsolateral prefrontal cortex (dlPFC; Brodmann area (BA46)) was identified to be the brain region responsible for this deficit [82]. According to Miller and Cohen (2001 p. 168), "The PFC is critical in situations when the mappings between sensory inputs, thoughts, and actions either are weakly established relative to other existing ones or are rapidly changing". It is assumed to act as a high-level filtering mechanism that is needed to enhance goal-directed activations and suppress any irrelevant activations [83]. By using this filtering mechanism, it enables executive control at different levels of processing. These processing levels include selecting, updating, maintaining and rerouting activations [83]. As indicated by Constantinidis & Klingberg 2016, the prefrontal cortex processes short term memories, while the parietal lobe involves in the processing of long term memories [73]. The parietal lobe has a role in mediating attention when needed, as well as providing spatial awareness and navigational skills [84]. Moreover, the parietal lobe is responsible for focusing our attention on different stimuli at the same time [84]. It also plays a role in assisting verbal short-term memory and damage to the supramarginal gyrus can cause short-term memory loss [85].

1.3.2 Temporal Lobe

According to Baddeley's working memory model, the medial temporal lobe plays a critical role in not only the episodic but also declarative forms of memory [64]. The lobe is located in between the left and right hemispheres, making it closer to the signals coming from both sides. The theory indicates that the central positioning of the lobe makes it easy for information to be received and sent out of its system with ease [73]. Therefore, it plays a critical role in managing the flow and storage of information in the brain. It also plays a role in recognition memory, which means that the capacity to identify an object as a recently encountered object [86]. Damage to the important temporal lobe can cause disturbance of selective attention of auditory and visual input, auditory sensation and perception, visual perception, language comprehension, impaired organisation and categorisation of verbal material, and altered personality [87]. The damage can also cause long term memory impairment in which semantic knowledge such as the ability to recognize family and friends, information learned in school and knowledge of historical events could be affected [87].

1.3.3 Cerebellum

While the cerebellum is a vital part of the brain responsible for balance and motor control, it also plays a critical role in supporting cognitive functions including attention, emotional functions, and memory storage [88, 89]. The cerebellum can store and retrieve information whenever they are needed. The cerebellum, with the help of other brain parts, such as the hippocampus, and amygdala, helps in the processing and storage of different types of memories [88, 89]. The cerebellum may be involved in cognitive functions like language and attention as well as involved in regulating fear and pleasure responses [90]. The amygdala may be involved in determining what memories should be stored [88, 89].

1.3.4 Limbic System

The limbic systems are located at the heart of the medial temporal lobe and encompass a series of brain regions working together to process memory. Some of these sub-regions include the amygdala, the hippocampus, the cingulate, the thalamus, and the mammillary body among other contributors that interlink with memory processing. For instance, the hippocampus, which is a small organ in the brain's medial temporal lobe that is a critical portion of the limbic system. The hippocampus is involved in emotional processing by being part of a network that plays a critical role in the transference process that changes a sample memory node from short term to long-term signals [73, 91]. This means that the hippocampus is responsible for the transference process, which is called memory consolidation. The idea is that it has the capability of controlling relevant spatial memory and behaviour, which shifts the longevity of memory to the long run [73]. Amygdala and hippocampus are heavily interconnected [92] and their interaction is necessary for the retrieval of emotional memories. In addition, there is evidence that the hippocampus and amygdala network is critical to encoding and consolidation of emotional memories [93, 94]. There is converging evidence from lesion studies, neuroimaging studies, and single-unit recording studies employing various behavioural methods designed to isolate performance based on recollection and recognition that has challenged the view that the hippocampus supports only recollection and the perirhinal cortex supports the recognition memory [95]. For example, Merkow et al. (2015) showed the contribution of the hippocampus to recollection and familiarity components of recognition memory after measuring the high-frequency activity (HFA) in spatiotemporally precise signal of neural activation that is measured in participants undergoing direct brain recordings of the hippocampus [96]. With the advent of neuroimaging studies and whole brain network analysis over the last two decades neuroscience is progressing away from a localisation model of brain

function where one function is attributed to one brain region. A brain network and functional connectivity approach with evidence across multiple modalities is required to attain a true understanding of complex brain function.

According to Cortis (2015), the hippocampus can grow new neurons, unlike many other parts of the brain. However, this ability is often affected by glucocorticoids relating to stress issues [62]. Damage to this area of the brain would leave the person unable to process new declarative memories as in the famous case of H.M., who had both his hippocampi removed to treat his seizure [73, 97]. In rats, the hippocampus plays a crucial role in various tasks of memory, such as maze running and object recognition. Therefore, it is essential in spatial memory as well as its role in recognition memory [98]. According to Cortis (2015), the amygdala also plays a primary function on memory and emotional processing which makes it a critical region in the development, storage and retrieval of memory [62]. Additionally, it is involved in memory consolidation in which it enables encoding memories at a deeper level in emotionally arousing events [99]. See figure 3 for a summary of the brain regions and their functions in working memory.

1.4 Modalities Measuring Working Memory

Neuroimaging modalities have investigated working memory using transcranial magnetic stimulation, electroencephalography, magnetoencephalography, fMRI [100, 101], SPECT [102, 103], and Positron Emission Topography (PET) [104-106]. Each technique had its drawback making it difficult to be applied. While transcranial magnetic stimulation tends to affect a large area of the brain and is limited to the areas near the surface, electroencephalography records the on-going activity in an intricate pattern which eventually makes it difficult to identify. Magnetoencephalography suffers from poor spatial resolution, which makes it hard to know where the activity originated from. Unlike MRI, SPECT and PET have major disadvantages. Both SPECT and PET are invasive and need the use of radioactive materials. PET is also extremely expensive as well as it preferably requires a cyclotron on site. MRI provides better temporal resolution as well as better spatial resolution and it is a non-invasive modality [107]. MRI, on the other hand, is not invasive, less expensive than PET, and does not use any radioactive material nor need for a cyclotron in the site [107].

1.5 Investigating Working Memory and Basal Ganglia in Individuals with CFS/ME Using fMRI

Four studies have investigated CFS/ME using working memory tasks and fMRI [108-111]. Lange et al. (2005) showed that CFS/ME patients process auditory information that is challenging as accurately as healthy controls, but they use more extensive regions of the working memory network [108]. In their study, they used Paced Auditory Serial Addition Test (PASAT) in which participants are asked to add the digits they hear to the one preceding it. For instance, if they heard (1, 3, 5) they first add the first two (1+3) and respond with the number 4. Then they add the following two (3+5) and respond with the number 8 [112]. Moreover, during the PASAT task, participants with CFS/ME demonstrated a substantial increase in BOLD signal in bilateral premotor and left superior parietal regions. However, the healthy controls group did not reveal any BOLD signal elevation when compared to the CFS/ME group in any of the studied regions of interest [108].

These findings have been supported by Caseras et al. (2006) who revealed that although both participating groups performed comparatively well and in all task levels they were able to activate the verbal working memory network, CFS/ME patients showed greater activation in medial prefrontal regions such as anterior cingulated gyrus compared with healthy controls during 1-back condition [109]. In conditions that were more challenging, CFS/ME patients had reduced activation in dorsolateral parietal and prefrontal cortices. This reduction in activation was interpreted as when the task gets more difficult; patients with CFS/ME failed to recruit working memory regions to the same level as the healthy controls [109]. While both groups performed comparably well in terms of activating the verbal working memory network while performing all task levels, participants with CFS/ME, in the 1-back condition showed more activation than control subjects in medial prefrontal regions which includes the anterior cingulate gyrus. However, participants with CFS/ME showed a reduction in activation in the left lateral and dorsolateral prefrontal cortex and in the left parietal lobule compared to HC. On the 3-back condition, participants with CFS/ME also showed a reduction in activation in the right and left superior parietal regions but an increase in activation in the right inferior temporal gyrus. These results were interpreted as participants with CFS/ME have different activations of the working memory network compared as compared to HC. The authors argue that these differences in activation could be a result of a compensatory strategy of the working memory system when impaired or saturated [109].

In a study seeking to investigate the neural correlates of working memory in CFS/ME patients when compared to healthy controls, Cook et al. reported no differences between CFS/ME and healthy controls in simple tasks like finger tapping or auditory monitoring in their earlier (2007) and recent (2017) studies [110, 111]. However, CFS/ME participants showed significantly increased activation in several cortical and subcortical regions throughout the fatigue-inducing cognitive task. Additionally, patients appear to exert greater efforts when processing auditory information as efficiently as healthy controls. These findings suggest that there exists a correlation between subjective mental fatigue feelings and brain responses while undergoing fatiguing cognition [111].

Basal Ganglia and Fatigue

Basal ganglia are grey matter masses found in the centre of the brain between the white matter hemispheres. Anatomically, they consist of the caudate nucleus, putamen, ventral striatum, globus pallidus, ventral pallidum, substantia nigra, and subthalamic nucleus [113]. This location allows basal ganglia to receive signals from several cortical regions, including both hemispheres and thus may allow them to integrate information coming from PFC and visual extrastriate cortices [114-116]. Literature shows that the basal ganglia are essential in learning behavioural requirements [117-121] and involved in global support of visual working memory processes [122]. These functions include cognition, procedural learning, eye movements, control of voluntary motor movements, habit learning and emotion [123, 124].

Basal ganglia, in neurological disorders such as CFS/ME, has been suggested to be implicated in fatigue [125]. Functional interruption of the usual process of activation between basal ganglia, thalamus, limbic system and higher cortical areas due to metabolic and structural lesions is implicated in central fatigue in CFS/ME [126]. This means that any interruption to these pathways, which connect these brain areas, would result in fatigue [126]. In a recent study, Nakagawa et al. (2016) investigated the basal ganglia association with fatigue and motivation [127]. They found that mean diffusivity (MD) was associated with fatigue and motivation in the right putamen, pallidum and caudate [127]. They also found an association between fatigue and basal ganglia structural changes. Therefore, they argued that altered brain structure could cause fatigue in clinical cases [127]. The increase in MD was interpreted by the authors to likely be due to fatigue mechanism as an abnormal function of parts of basal ganglia and motors systems [127]. Disruption of normal autonomic function would require additional energy to accomplish complex motor programmes which, could lead to loss of motivation [127]. This preliminary research showed the basal ganglia could have an important role in

central/cognitive fatigue. Further research is needed to elucidate the nature of BG involvement in fatigue.

1.6 Rationale and Aims for The Thesis

Cognitive impairment adversely affects daily functioning for patients with CFS/ME as memory deficits may lead to difficulties at work and in school [128]. Adults with CFS/ME may have difficulties at work with remembering tasks assigned to them, while students with CFS/ME may have difficulties in remembering information presented in classes [128]. Furthermore, studies have previously linked basal ganglia function to fatigue in CFS/ME [125, 127, 129-131]. Although the exact nature of this relationship is unknown, basal ganglia may be implicated in central fatigue associated with this illness. Bayliss et al. (2003) paradigm was utilised to investigate working memory [33]. Using automated analysis methods such as voxel-based morphometry in this illness showed inconsistent results. Results included differences in both grey matter volume [132-136], white matter volume reduction [136-140] or no differences at all between CFS/ME and healthy controls [141-143].

The four main thesis aims were: 1. To investigate whether there are brain volume differences (globally or regionally) between CFS/ME and healthy controls;

2. To investigate the involvement of basal ganglia in fatigue in this illness;

3. To investigate the involvement of brain networks in fatigue.

4. To investigate the impact of fatigue on cognitive function with tasked based fMRI [33].

1.7 Thesis Chapters

Chapter 2 provides a systematic review of the structural and functional MRI studies that investigated CFS/ME. It was performed to determine the type of paradigm to be used to assess the impact of fatigue on cognition in CFS/ME and to establish whether there is a consistent finding in previous literature. Chapter 3 provided a brief review of all the methodological approaches used in this thesis. The following chapters (chapters 4, 5, 6, and 7) will be introduced as separate papers. Chapter 4 is a voxel-based morphometry analysis to determine any structural morphometrical differences between the healthy controls and CFS/ME groups, with fatigue correlates of any differences explored. Chapter 5 illustrates the use of resting-state fMRI in investigating the functional connectivity of basal ganglia in this patient group and correlates it to fatigue scores. Chapter 6 presents the resting-state functional brain networks and their relation to fatigue as measured by the Chalder Fatigue Questionnaire. Chapter 7

provides details on the working memory task for fMRI, analysis, and results. It also illustrates the correlation between fatigue scores and differences in brain function between groups. Chapter 8 discusses the experimental findings in term of overall thesis aims. It also includes limitations of this thesis and recommendations for future studies.

2 Chapter 2: Using Structural and Functional MRI as A /Neuroimaging Technique to Investigate Chronic Fatigue Syndrome/ Myalgic Encephalopathy: A Systematic Review

2.1 Overview of Chapter

Chapter 2 shows the methods and results of a systematic review to identify and synthesise structural and functional MRI studies that investigated CFS/ME. This includes the results as well as the quality assessment of those studies.

2.2 Introduction

Chronic Fatigue Syndrome (CFS), also known as Myalgic Encephalomyelitis/Myalgic Encephalopathy (ME) is a disorder characterized by persistent fatigue which lasts for at least 4 or 6 months (depending on the diagnostic criteria) and is associated with a variety of symptoms. One of the most common symptoms is cognitive dysfunction which is reported in >94% of adults [144]. CFS/ME is relatively common. A recent meta-analysis performed by Johnston et al. (2014) indicates a prevalence of 0.76% (95% CI 0.23% to 1.29%) based on clinically confirmed cases in several countries [10]. Over 50% of adults who access specialist care are unemployed because of CFS/ME [145]. The aetiology and pathophysiology of CFS/ME are not known, and the underlying mechanism for cognitive dysfunction is not understood.

Imaging techniques, such as magnetic resonance imaging (MRI), have been used to aid clinical diagnoses for decades. A variety of MRI techniques have been used in neurology from structural MRI (sMRI) in lesion detection to functional MRI (fMRI) applications for neurosurgical planning. Recently MRI has been used to examine fatigue and cognition in CFS/ME. This systematic review aims to evaluate the use of sMRI and fMRI to investigate CFS/ME. We also aim to provide an insight into what MRI can offer to our understanding of cognitive dysfunction in CFS/ME. Finally, we will make suggestions for future directions of research.

2.3 Aim and Objectives

2.3.1 Aim:

The aim was to undertake a systematic review of the structural and functional MRI studies as well as assessing their quality using Nichols et al. (2017) [146].

2.3.2 Objectives

1- Systematically identify all structural and functional MRI studies that investigated CFS/ME.
2- Describe the findings of structural and functional MRI studies that investigated CFS/ME as well as describing their study design.
3- Identify future research directions for MRI studies in people with CFS/ME in order to guide the development of a new MRI protocol.

2.4 Material and methods

2.4.1 Protocol

The protocol was published on PROSPERO (Available at: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=50569

2.4.2 Selection Criteria

We searched Medline and Ovid and included articles from 1991 (Oxford diagnostic criteria for CFS/ME) to April 2019. We included all English language studies using MRI to investigate chronic fatigue syndrome. We used the following keywords (and abbreviations) for CFS/ME: “chronic fatigue syndrome”, “fatigue syndrome, chronic”, “myalgic encephalomyelitis”, “myalgic encephalopathy”, “CFS”, “ME” or “CFS/ME”. To detect all structural and functional studies which used magnetic resonance imaging in participants with CFS/ME, we used the following keywords for imaging techniques: “magnetic resonance imaging”, “MRI”, “structural MRI”, “sMRI”, “functional magnetic resonance imaging”, “functional MRI”, “fMRI”, “resting state functional magnetic resonance imaging”, “resting-state functional magnetic resonance imaging”, “resting-state functional MRI”, “resting state functional MRI”, “rsfMRI” and “rs-fMRI”. The full search strategy is provided in the supplementary file “SearchFile MEDLINE and OVID”.

We included all studies which used one of the five major CFS/ME definition criteria; Fukuda, Centre for Disease Control (CDC), National Institute of Health & Care Excellence (NICE), Canadian or Oxford Criteria and there was no age restriction. Duplicates, case study articles

and editorials were excluded. Two reviewers (BA, CL) independently reviewed the titles and abstracts of identified studies, and potentially relevant articles were identified for full-text review. Two reviewers independently reviewed full-text articles to determine which articles were eligible. Disagreements were resolved by discussion until consensus was reached.

2.5 Risk Of Bias Assessment

We assessed study quality and risk of bias using the following criteria outlined in Nichols et al. (2017): clearly stated research objective; recruitment procedure; inclusion/exclusion criteria; description of sample demographics; reporting of imaging methodology; and whether comparison groups were used. These criteria have been set with the aim to increase the reproducibility of research for neuroimaging studies using MRI. We chose Nichols et al. (2017) tools over other assessment tools (eg Cochrane risk of bias 2.0) as it specifically assesses the risk of bias in neuroimaging studies. Stating clearly the recruitment procedure, inclusion/exclusion criteria, population demographics and comparison group enables a critical reader to evaluate the study and to determine whether the sample may be susceptible to bias and whether the results are generalizable [146]. If a study fully reported all criteria, it was considered a high-quality study with low risk of bias. If it failed to report one criterion, it was considered a medium quality paper. Finally, if it failed to report two or more criteria, it was considered a low-quality study with a high risk of bias. Given the low number of identified studies, we did not exclude any studies based on quality assessment, but we reviewed the results taking study quality into consideration. We did not submit this work to an ethics committee because it is a systematic review of the literature.

2.6Patient and Public Involvement

No patient or public involved

2.7Results

A total of 824 papers were identified. Of these, 132 were duplicates, and three were not in English (see figure 4). Of the remaining 689, there were 629 that did not fit our eligibility criteria, leaving 60 papers for full-text review. Of these, 20 studies were excluded because they did not use structural or functional MRI applications. A further five were excluded because the CFS/ME diagnostic criteria used were not clear. Therefore, we extracted data from 35 studies. Of the papers included, 19 were structural MRI, and 16 were functional MRI studies.

2.7.1 CFS/ME Diagnostic Criteria and MRI Images Acquisition

Details on the diagnostic criteria as well as MRI images acquisition are available in table 3 for sMRI and table 4 for fMRI studies.

2.7.2 Image Analysis

sMRI

In 13 studies, quantitative computational analysis, such as Statistical Parametric Mapping (SPM) and FMRIB Software Library (FSL), of images was carried out by utilization of an automated technique [132-139, 142, 143, 147, 148]. However, six studies in sMRI relied on visual inspection by two radiologists and where there was disagreement a third radiologist was involved [141, 149-153].

fMRI

All of the 16 studies used quantitative computational analysis, such as Statistical Parametric Mapping (SPM) [108, 131, 139, 154-161], Analysis of Functional Neuroimaging software (AFNI), XBAM software [109, 162] and FMRIB Software Library (FSL) [163].

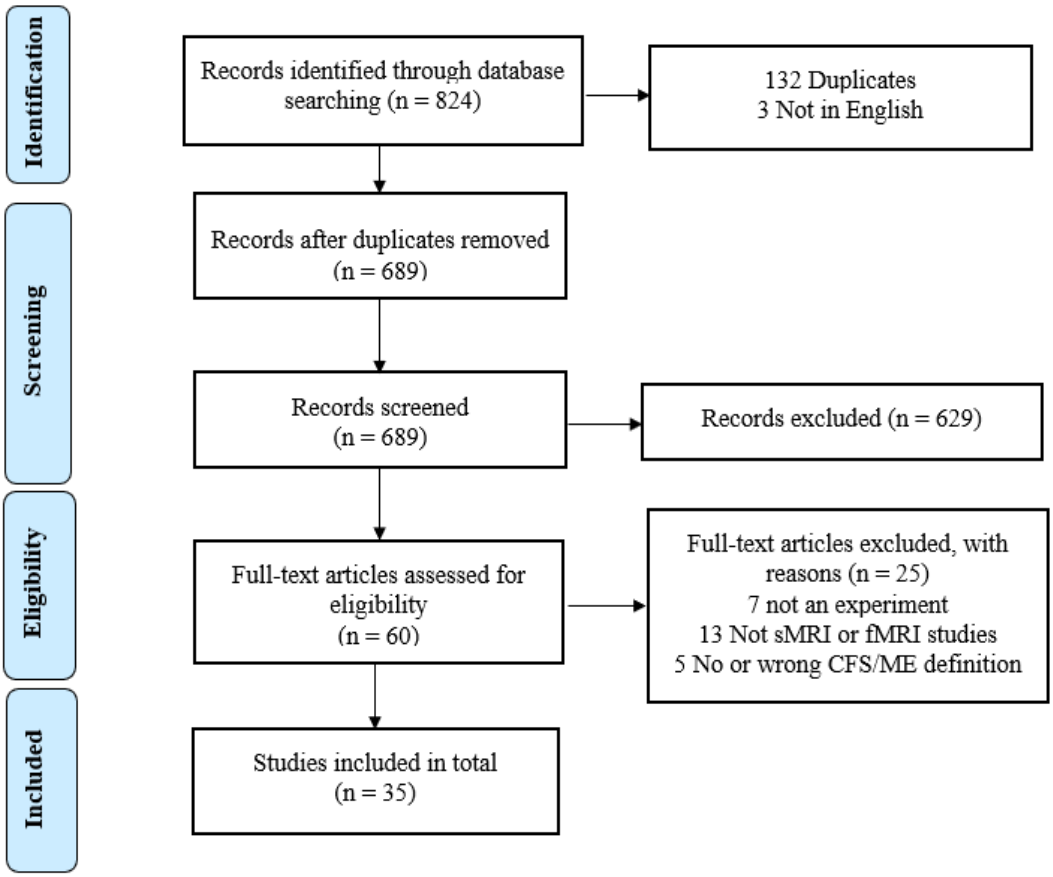


Figure 4: PRISMA flow chart showing the method followed.

2.7.3 Structural MRI Results

Details of the nineteen sMRI studies are available in table 2.

Table 2 shows a summary of 19 sMRI studies in CFS/ME
** Healthy controls ** Some studies provided average age and others provided a range. *** Not mentioned.*
M= Male F= Female CDC = Centre for Disease Control CFS = Chronic Fatigue Syndrome, HC = Healthy controls, FSL =FMRIB Software Library, SPM = Statistical Parametric Mapping, N/A= Not Applicable

Authors	CFS/ME definition	Sample size CFS/HC*	Age** ~CFS ~HC*	Sex M/F	Magnetic strength in Tesla (T)	Full MRI protocol Y= Yes N= Not mentioned	Corrected	Analysis method	Comparison groups
Barnden et al. (2011) [135]	Fukuda & Canadian	25/25	19-46	6/19	1.5T	Y	FWE+FDR	SPM5	CFS Vs. HC
Barnden et al. (2016) [142]	Canadian	25/25	19–46 *20–46	6/19	1.5T	Y	FWE+FDR	SPM5	CFS Vs. HC
Barnden et al. (2015) [147]	Fukuda &Canadian	25/25	19–46 *20–46	6/19	1.5 T	Y	FDR	SPM5	CFS Vs. HC
Shan et al. (2017) [139]	Canadian	38 / 14	34.8 *34.7	11/27 *4/10	1.5T	Y	FWE	SPM12	CFS Vs. HC
De Lange et al. (2005) [132]	CDC	28/28	19–37 *19–42	0/28	1.5T	N	FWE	SPM2	CFS Vs. HC
De Lange et al. (2008) [133]	CDC	22 / 22	~ 36 *~37	0/22	1.5 T	Y	FWE	SPM	CFS Vs. HC
Okada et al. (2004) [134]	CDC	16/49	24-46 *21-47	10/6 *27/22	1.5T	Y	FWE	SPM2	CFS Vs. HC
van der Schaaf et al. (2017) [143]	CDC	89/26	18-65 *19-55	0/89 0/26	3.0T	Y	FWE	SPM12	CFS Vs. HC
Finkelmeyer et al. (2018) [138]	Fukuda	42/28	45.2 *48.4	10/32 *9/19	3.0T	Y	FWE	SPM12	CFS Vs. HC
Puri et al. (2012) [136]	CDC	26 /26	~ 42.9 *~38.2	7/19	3.0T	Y	FWE	FSL	CFS Vs. HC
Shan et al. (2016) [137]	Fukuda & Canadian	15/10	~ 34.06 *~ 30.5 at first evaluation	4/11 2/8	1.5T	Y	FWE	SPM12	CFS Vs. HC
Barnden et al. (2018) [148]	Fukuda	43/27	N***	N***	3.0T	Y	FWE	SPM12	CFS Vs. HC
Natelson et al. (1993) [149]	CDC	52/52	16-56	6/46	0.35T, 0.5T, 1.0T, and 1.5T	Y	N/A	Visual inspection	CFS Vs. HC
Perrin et al. (2010) [141]	CDC	18 / 9	20-55 *22-53	10/8 *5/4	3.0T	Y	N/A	Visual inspection	CFS Vs. HC
Greco et al. (1997) [150]	Oxford & CDC	43/43	22–78	14/29	1.5T	Y	N/A	Visual inspection	CFS Vs. HC
Schwartz et al. (1993) [151]	CDC	16/15	24-61 *24-64	5/11 *5/10	1.5 T 0.5T	Y	N/A	Visual inspection	CFS Vs. HC
Lange et al (1999) [152]l	Fukuda	39/19	36- 40	20/40	1.0 T	Y	N/A	Visual inspection	CFS Vs. HC
Lange et al. (2001) [153]	CDC	28/15	~39.1 *~37.7	6/22 *2/13	1.0T	Y	N/A	Visual inspection	CFS Vs. HC
Zeineh et al. (2015) [140]	Fukuda	15/14	20-66	7/8 *6/8	3.0T	Y	FWE	FSL	CFS Vs. HC

2.7.3.1 General Findings

Of the 19 studies included in this systematic review, 16 showed some structural differences between CFS/ME and healthy controls. These included both grey matter volume and white matter volume reduction, ventricular enlargement, white matter hyper-intensities, lesions and

cortical thickening. In contrast, three studies did not reveal any differences between participants with CFS/ME and healthy controls and therefore questioned the ability of sMRI scans to detect brain changes in CFS/ME [141-143].

2.7.3.2 Radiological Reporting

To evaluate and compare the sMRI of CFS/ME and healthy controls, these studies used one reviewer [150], two neuroradiologists [141, 149, 152, 153] or three neuroradiologists [151] to visually inspect the images. Two MRI studies found ventricular enlargement [149, 153] While three studies reported white matter hyper-intensities or abnormalities, which were defined as lesions, identified by high signal intensity on T2 or proton density-weighted pulse sequences [149, 151, 152]. In these studies, age was only accounted for by using age-matched healthy controls. Lange et al. (1999) reported that 41% of the MRI scans showed abnormalities. Further changes or lesions were reported in the supratentorial periventricular white matter [150], periventricular white matter, subcortical white matter and in the centrum semiovale [151]. However, a longitudinal study, with one-year follow-up, failed to detect any differences between CFS/ME and healthy control groups at baseline and after a year in cerebrospinal fluid volume, white matter hyper-intensities, ventricular volume and failed to observe any abnormalities in the CFS/ME group [141]. These studies used manual segmentation of the cerebrospinal fluid. The resultant images were registered into a standard space, segmented into 12 segments, defined by mid-sagittal plane. Images were manually checked to exclude irrelevant areas, and finally, checked by an experienced neuroradiologist using Scheltens et al. (1993) method [141, 164].

2.7.3.3 White Matter Volume & Grey Matter Volume

White matter volume reduction was reported in five studies [136-140], and white matter changes or lesions were reported in 3 further studies [147, 150, 151]. In a recent study, Finkelmeyer et al. (2018) showed a substantial increase in grey matter volume and decrease in white matter volume in CFS/ME compared to healthy controls [138]. Moreover, they used an automated voxel-wise analysis, CAT12 in SPM12, which showed that the insula and amygdala had increased grey matter volume in the CFS/ME group, while the midbrain, pons and right temporal lobe, had decreased white matter volume [138]. Two studies, by the same research group, showed a marked reduction in the white matter volume in left inferior front-occipital fasciculus in participants with CFS/ME compared to healthy controls [137, 139]. Puri et al. (2012) found a reduction in white matter volume in the left occipital lobe as well as the

posterior part of the left parahippocampal gyrus in the CFS/ME group compared to the healthy control group [136]. Changes in white matter observed on T2-weighted images in right middle temporal lobe were related to cognition. Authors were able to demonstrate that white matter volume was negatively correlated with CFS/ME disease duration [135, 147]. Zeineh et al. (2015) showed bilateral white matter atrophy in supratentorial, a brain region located above the tentorium cerebelli, was present in CFS/ME [140].

Grey matter volume reduction was the main result in five studies [132-136]. There was reduced global grey matter volume in three studies [132, 133, 135] and regional grey matter volume difference in the two studies [134, 136]. The reduction in grey matter volume was observed in the occipital lobes, right angular gyrus, left parahippocampal gyrus and in the bilateral prefrontal cortex [134, 136]. Grey matter volume reduction has been associated with functional deficits, that may be influenced by pain [165, 166] illness or age factors, thereby having a detrimental impact on the quality of life for participants with CFS/ME [134, 136].

2.7.3.4 Longitudinal Studies

Three studies compared sMRI in participants with CFS/ME across two-time points [133, 137, 141]. De Lange et al. (2008) used MRI to look at the effects of cognitive behaviour therapy (CBT) on brain volume. At baseline, they described a decrease in grey matter volume in participants with CFS/ME compared to healthy controls. They also described an increase in grey matter volume between pre and post-treatment MRI in the lateral prefrontal cortex in participants with CFS/ME, but this region remained unchanged in healthy controls (with a *p*-value of 0.025). The between-group GMV difference decreased after CBT by 12%. The increased grey matter volume in the lateral prefrontal cortex was correlated with health status, processing speed and physical activity [133].

The CBT design was very comprehensive and accounted for a number of measures to cross-reference. In this study, participants were engaged in CBT in which fatigue-related cognitions were challenged in order to reduce somatic attributions, to develop a better sense of control over symptoms and to assess behavioural changes. Also, in parallel to these challenges, a planned physical activity program was implemented. In addition, a work rehabilitation schedule was designed to achieve a gradual work reentry. CBT final sessions were designed to deal with relapse prevention and further improvement of self-control.

Physical activity was measured using actometer measurements two weeks before the scan date for pre and post CBT. The cognitive speed was assessed using Wechsler Adult Intelligence Scale (WAIS-dst) (Wechsler, 1981) and the choice reaction time task (CRT). Also, they used

a simple reaction time task (SRT) in order to control for sensorimotor speed. They found that both, the WAIS-dst and CRT bore relation to GMV in participants with CFS/ME in which participants who completed fewer items on the WAIS-dst exhibited lower GMV ($r = 0.64$, $P = 0.001$) and those who had slower CRT exhibited lower GMV ($r = –0.40$, $P = 0.033$). However, the SRT showed no significant correlation with GMV ($r = –0.15$, $P = 0.26$). After CBT, patients with CFS/ME became faster on the CRT ($t21 = 2.30$, $P = 0.032$) which was significantly correlated with GMV CRT ($r = 0.42$, $P = 0.027$). The WAIS-dst showed no improvement after CBT but was significantly correlated with GMV (GMV (WAIS-dst: $r = 0.41$, $P = 0.028$). This means that significant improvement in cognitive speed was associated with a larger increase in GMV after CBT. There were no significant differences in the SRT ($t21 = 1.08$, $P = 0.30$), and no correlation between SRT and the GMV increase ($r = 0.24$, $P = 0.14$).

Shan et al. (2016) compared MRI images of participants with CFS/ME, and healthy controls acquired six years apart. They found a substantial decrease in white matter volume in the left inferior fronto-occipital fasciculus in the CFS/ME group compared to the healthy control group [137]. Perrin et al. (2010) conducted a one year follow up and demonstrated no significant abnormalities or differences between baseline and 12 months follow up MRI in CFS/ME compared to controls [141].

2.7.4 Functional MRI Results

Sixteen articles that employed fMRI were identified. Table 3 is a summary of these studies.

Table 3 shows a summary of 16 fMRI studies in CFS/ME.

Authors	CFS definition	Sample size CFS/HC*	Age** ~CFS ~HC	Sex M/F CFS HC	Magnetic strength in Tesla (T)	Full MRI protocol	Analysis method	Task for fMRI	Corrected	Comparison groups
Gay et al. (2016) [154]	Fukuda	19/ 17	~ 48.75	0/19 0/17	3.0 T	Y	SPM8	Resting-state	FWE	CFS Vs. HC
Kim et al. (2015) [155]	CDC	18/18	25-54	0/18 0/18	3.0 T	Y	SPM8	Resting-state	FDR	CFS Vs. HC
Wortinger et al. (2016a) [156]	Fukuda	15 / 24	12–18	1 / 14 8 / 16	1.5 T	Y	SPM8	Emotional conflict effect.	FWE	CFS only
Wortinger et al. (2017) [157]	Fukuda & NICE	18/18	12-18	2/16 2/16	3T	Y	SPM8	Resting-state	FDR	CFS Vs. HC
Cook et al. (2007) [158]	Fukuda	9/11	~43 ~ 42	3/6 3/8	3.0 T	Y	SPM2	Working memory	FDR	CFS Vs. HC
Mizuno et al. (2016) [131]	Fukuda	13/13	~ 13	4/9 7/6	3.0 T	Y	SPM8	Reward processing	FWE	CFS Vs. HC
Mizuno et al. (2015) [159]	Fukuda	15/13	11-14	9/6 4/9	3.0 T	Y	SPM5	Dual attention task	FWE	CFS Vs. HC
Lange et al. (2004) [160]	CDC & Fukuda	16/16	20 - 45	0/16 0/16	1.5T	Y	SPM99	Mental rotation task	FWE	CFS Vs. HC
De Lange et al. (2005) [108]	CDC & Fukuda	Study 1 6/7 Study 2 19 /15	Study 1 ~38.17 ~30.71 Study 2 ~37.53 ~30.80	Study 1 0/100 % 43/57 % Study 2 16/84 % 32/68 %	1.5 T	Y	SPM99	Simple attention & working memory	FDR	CFS Vs. HC
Tanaka et al. (2006) [161]	Fukuda	6/7	~30.4 ~26.1	6/0 7/0	3.0 T	Y	SPM99	Visual search	FWE	CFS Vs. HC
Caseras et al. (2006) [109]	CDC	17 /12	22–45	8/11	1.5 T	Y	XBAM software	Working memory	FWE	CFS Vs. HC

Caseras et al. (2008) [162]	CDC & Fukuda	12/11	22-45	34/66 %	1.5 T	Y	XBAM software	Fatigue and anxiety-provoking mimic real-life situation	FWE	CFS Vs. HC
Wortinger et al. (2016b) [163]	Fukuda & NICE	18 /18	12-18	2/16	3.0 T	Y	FSL	Resting-state	FEW+ Bonferroni	CFS Vs. HC
Miller et al. (2014) [129]	CDC	18/41	~47.2 ~44.2	2/16 33/8	3.0 T	Y	AFNI	Reward processing	FWE	CFS Vs. HC
Shan et al. (2017) [167]	Fukuda	45/ 27	47.12 (11.67) /43.10 (13.77)	12/33 9/18	3T	Y	SPM12	Stroop task & resting-state	FWE	CFS Vs. HC
Cook et al. (2017) [110]	CDC& Fukuda	15/15	42.7 (11.1) /43.2 (10.4)	0/15 0/15	3T	Y	AFNI	PASAT, simple number recognition & finger tapping.	FWE	CFS Vs. HC

2.7.4.1 General Findings

Sixteen fMRI studies were identified in this systematic review, five employed resting-state fMRI (rs-fMRI), and 11 used multiple tasks to investigate cognitive functioning in CFS/ME.

2.7.4.2 Resting-state fMRI & Functional Connectivity

Four out of the five rs-fMRI studies reported decreased functional connectivity in participants with CFS/ME compared to healthy controls [154, 157, 163, 167]. Among these studies, two used model-based approaches [157, 167] while two used data-driven approaches [155, 168] and Gay et al. (2016) used both approaches [169]. Two studies reported a decrease in functional connectivity between the salience network and the right posterior insula [157, 163]. Wortinger et al. (2016) reported a decrease in functional connectivity between the salience network and the right middle, posterior and anterior insula as well as between the salience network and superior temporal gyrus, precentral gyrus and thalamus, which are brain regions outside of the classic boundaries of the salience network [163]. Wortinger et al (2017) showed a reduction in functional connectivity between the right dorsal anterior insula and the right posterior parietal cortex of the central executive network [157]. Gay et al. (2016) found a disruption in the intrinsic connectivity within the left frontoparietal network. More specifically, they found reduced coupling of activity between the left superior frontal gyrus and the five networks they investigated. These five networks included the default mode network, salience network, sensory-motor network, and the left and right frontoparietal networks. Also, they found a

decrease in functional connectivity between the salience network and the left posterior cingulate cortex. The sensory-motor network showed decreased functional connectivity with the left anterior mid-cingulate cortex [154]. Shan et al. (2018) found decreased functional connectivity between the medial prefrontal cortex and both inferior parietal lobules [167]. On the other hand, Kim et al. (2015) reported an increase in functional connectivity between the posterior parietal cortex and the dorsal anterior cingulate cortex, rostral anterior cingulate cortex, middle temporal cortex and precuneus in participants with CFS/ME compared to healthy controls [155]. Collectively the findings from rs-fMRI studies suggest dysfunctional connectivity across a number of neural networks in CFS/ME.

2.7.4.3 fMRI & Cognition

Memory

Working memory was investigated in CFS/ME groups using a variety of tasks [108-110, 170]. Cook and colleagues in a recent study (2017) and an older study (2007) reported no differences between CFS/ME and healthy controls in simple non-fatiguing tasks like finger tapping or auditory monitoring [110, 170]. However, CFS/ME participants showed significantly widespread increased cortical and subcortical activation throughout the complex and fatiguing cognitive task [110, 170]. Caseras et al. (2006) showed that, during the performance of the n-back task, participants with CFS/ME exhibited increased activation compared to healthy controls in medial prefrontal regions, during the 1-back condition [109]. Conversely, in the more challenging conditions (2- and 3-back conditions) participants with CFS/ME showed decreased activation in dorsolateral prefrontal and parietal cortices, which are working memory-related brain regions [109]. Moreover, they found that the CFS/ME group activated a large cluster in the right inferior/medial temporal cortex while performing 2- and 3-back conditions not activated in healthy controls [109]. The analysis of the load of the task showed statistically significant differences in activation in the brain between the CFS/ME and the healthy controls groups as task demand increased [109].

Attention

Mizuno et al. (2015) reported that a dual attention task revealed activation in the left dorsal inferior frontal gyrus was greater in the dual-task condition than in the two single-task conditions in both healthy controls and adolescents with CFS/ME. In healthy controls, the level of activation was positively associated with the fatigue score and negatively correlated for the accuracy for story comprehension. In adolescents with CFS/ME, the activation of the dorsal

anterior cingulate cortex and left middle frontal gyrus was only observed in the dual-task condition. In addition, the levels of activation of the dorsal anterior cingulate cortex and left middle frontal gyrus were positively associated with both motivation and fatigue scores [159]. In a recent study, Shan et al. (2018) investigated CFS/ME using the Stroop task and could not find any differences between the groups [167]. However, when they examined the default mode network, they found lower functional connectivity between medial prefrontal cortex, left inferior parietal lobule, medial prefrontal cortex and posterior cingulate cortex in CFS/ME suggesting a more complex and less coordinated DMN network in this patient group [167].

Reward and Motivation

During a reward processing task/gambling task, the CFS/ME group showed significantly reduced activation in the right caudate and right globus pallidus compared to controls. Moreover, the decreased activation in the right globus pallidus was significantly associated with the elevation in mental fatigue and general fatigue, as evaluated by the multidimensional fatigue inventory [129]. Another study using a different gambling task showed activation of the bilateral caudate, putamen, and thalamus in both the healthy control group and the adolescents with CFS/ME group, when using high monetary reward condition [131]. In the low monetary reward condition activation of the bilateral caudate and thalamus was observed in both the healthy controls group and the adolescents CFS/ME group, but activation of the bilateral putamen was only observed in the healthy controls group [131].

Sensory Information Processing Tasks

A mental rotation task showed that participants with CFS/ME had stronger responses in visual structures. During error trials, the dorsal anterior cingulate cortex was activated in both groups. However, the ventral anterior cingulate cortex was activated only when healthy controls made an error and remained inactive when participants with CFS/ME made an error [160]. During the PASAT task, participants with CFS/ME demonstrated a significant increase in BOLD signal in bilateral premotor and left superior parietal regions [110, 160].

A visual search task was used by Tanaka et al. (2006) to examine the task-dependent brain regions. They showed a reduction in activation in bilateral visual cortices, left superior and inferior parietal lobules, and left precentral gyrus, for the fatigue-inducing task in both participants with CFS/ME and healthy controls. Furthermore, the amount of decrease in activation was the same in both groups. Conversely, the activation of auditory cortices throughout the fatigue-inducing period did not change in the healthy controls but was reduced

in the participants with CFS/ME. The amount of reduction was associated with fatigue which was measured immediately before the MRI session using a fatigue visual analogue scale [161]. When fatigue and anxiety were induced by mimicking real-life situations, the CFS/ME group reported fatigue and anxiety and exhibited an increase in activation in the occipitoparietal cortex, posterior cingulate gyrus and parahippocampal gyrus, as well as a reduction in functional connectivity between dorsolateral and dorsomedial prefrontal cortices compared to healthy controls. These results suggest a relationship between provocation of fatigue and activations in these brain areas [162].

Emotional Conflict

Emotional conflict tasks indicated that the CFS/ME group were less able to engage the left amygdala and left mid-posterior insula in response to conflict than the healthy group [25]. Moreover, there was an association between accuracy interference and conflict-related reactivity in the amygdala in adolescents with CFS/ME. A significant decrease was observed in the left amygdala of adolescents with CFS/ME when compared to healthy controls. No difference was measured in the right amygdala between the groups. A significant decrease in the activity of the left mid-posterior insula was observed [156]. No group differences between the two groups were reported in the right fronto-insular cortex, a key region of salience network responsible for integrating other salience network regions in the processing of emotional information [171], and dorsal anterior cingulate cortex [156].

2.7.5 Quality Assessment for Risk of Bias

We applied the criteria from Nichols et al. (2017) to assess study quality for risk of bias [146]. See table 4 and 5 for risk of bias assessment. The sMRI studies risk of bias assessment showed that study quality was highly variable. All fMRI studies were assessed to have a low risk of bias and therefore considered high quality.

Table 4 shows the risk of bias assessment for structural MRI studies.

Authors	**Research objectives**	**Recruitment procedure**	**Inclusion / exclusion**	**Population demographics**	**Imaging protocol**	**Comparison group**	**Quantitative / Narrative**	**Risk of bias**
Barnden et al. (2011) [135]	Y	Y	Y	Y	Y	CFS Vs. HC	Q	Low
Barnden et al. (2016) [142]	Y	Y	Y	Y	Y	CFS Vs. HC	Q	Low
Barnden et al. (2015) [147]	Y	Y	Y	Y	Y	CFS Vs. HC	Q	Low
Shan et al. (2017) [139]	Y	Y	Y	Y	Y	CFS Vs. HC	Q	Low
De Lange et al. (2005) [132]	Y	Y	Y	Y	N	CFS Vs. HC	Q	Medium
De Lange et al. (2008) [133]	Y	N	Y	Y	Y	CFS Vs. HC	Q	Medium
Okada et al. (2004) [134]	Y	Y	Y	Y	N	CFS Vs. HC	Q	Medium
van der Schaaf et al. (2017) [143]	Y	Y	Y	Y	Y	CFS Vs. HC	Q	Low
Finkelmeyer et al. (2018) [138]	Y	Y	Y	Y	Y	CFS Vs. HC	Q	Low
Puri et al. (2012) [136]	Y	N	Y	Y	Y	CFS Vs. HC	Q	Medium
Shan et al. (2016) [137]	Y	Y	N	Y	Y	CFS Vs. HC	Q	Medium
Barnden et al. (2018) [148]	Y	Y	Y	Y	Y	CFS Vs. HC	Q	Low
Natelson et al. (1993) [149]	Y	Y	Y	Y	Y	CFS Vs. HC	N	Medium
Perrin et al. (2010) [141]	Y	N	Y	Y	Y	CFS Vs. HC	N	High
Greco et al. (2010) [150]	Y	Y	Y	Y	Y	CFS Vs. HC	N	Medium
Schwartz et al. (1993) [151]	Y	Y	Y	Y	Y	CFS Vs. HC	N	Medium

Lange et al. (1999) [152]	Y	Y	Y	Y	Y	CFS Vs. HC	N	Medium
Lange et al. (2001) [153]	Y	Y	Y	Y	Y	CFS Vs. HC	N	Medium
Zeineh et al. (2015) [140]	Y	Y	Y	Y	Y	CFS Vs. HC	Q	Low

Table 5 shows the risk of bias assessment for functional MRI studies.

Authors	Research objectives	Recruitment procedure	Inclusion/ exclusion	Population demographics	Imaging protocol	Comparison group	Risk of Bias
Gay et al. (2016) [154]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Kim et al. (2015) [155]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Wortinger et al. (2016a) [156]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Wortinger et al. (2017) [157]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Cook et al. (2007) [158]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Mizuno et al. (2016) [131]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Mizuno et al. (2015) [159]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
De Lange et al. (2004) [160]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Lange et al. (2005) [108]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Tanaka et al. (2006)[161]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Caseras et al. (2006) [109]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Caseras et al. (2008) [162]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Wortinger et al. (2016b) [163]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Miller et al. (2014) [129]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Shan et al. (2017) [167]	Y	Y	Y	Y	Y	CFS Vs. HC	Low
Cook et al. (2017) [110]	Y	Y	Y	Y	Y	CFS Vs. HC	Low

2.8 General Discussion

2.8.1 Structural MRI

This is the first systematic review of structural MRI studies in CFS/ME. While authors were optimistic about finding a biomarker, the findings were inconsistent between studies which could be due to differences in methodology, sample sizes and underlying disease heterogeneity. Differences in methodology include using visual inspection, computational analysis, different sample sizes and CFS/ME patients with different duration of illness or symptom severity. The lack of automated analysis methods showed inconsistencies and found no differences between CFS/ME and healthy control groups in studies that used visual inspection [149, 150, 152, 153,

172]. Studies reporting white matter changes, such as white matter hyper-intensities and ventricular enlargement are not specific to CFS/ME. Illnesses such as multiple sclerosis also have a similar pattern of white matter alterations. As a result, the use of automated analysis method might be crucial in improving the ability to find differences between brain regions in these subgroups.

The results from quantitative studies using automated analysis of brain volume in the CFS/ME group compared to controls showed reductions in brain volume in the midbrain, pons and right temporal lobe [138], left inferior fronto-occipital fasciculus [137, 139] and left occipital lobe [136] while white matter abnormalities were reported in right middle temporal lobe [147]. White matter volume reduction and abnormalities were found to be related to cognition [173]. White matter facilitates information transfer in the brain to enable fast and effective neural systems which is essential for cognitive operations [173]. Any disturbance in these neural networks would affect aspects of cognition such as memory, visuospatial skills, language, attention, and executive function, which rely on structural connectivity delivered by the myelinated systems [173]. Barnden et al. (2016) concluded that although the brain regulatory nuclei are working, the signalling to and from the peripheral sensor might be affected due to impairment of the two-way communication [142]. Shan et al. (2016) found a reduction in inferior fronto-occipital fasciculus and an association with working memory deficits, impaired concentration, poor motor coordination and inability to focus vision. This region plays a role in connecting frontal lobe with the superior parietal lobe. In addition, the ventral subcomponent of inferior fronto-occipital fasciculus connects the frontal lobe with the inferior occipital lobe and temporo-basal area [137, 174].

Five studies showed a reduction in grey matter volume. Three of these showed a global reduction [132, 133, 135] and two showed a reduction in specific regions of the brain (occipital lobes, right angular gyrus and the posterior division left parahippocampal gyrus [136] and in bilateral prefrontal cortex [134]). Grey matter reduction is of particular interest because it provides a possible explanation for the memory problems seen in CFS/ME. Puri et al. (2012) found a reduction in grey matter volume in the posterior part of the left parahippocampal gyrus [136] which has been shown to be affected in other diseases like age-related memory decline [175]. Reduced grey matter volume was found among the CFS/ME group and was suggested to be a reason for neuronal down-regulation which might be caused by environmental impoverishment associated with the disease [133]. However, given the nature of these cross-sectional studies, further research is required to support this hypothesis.

Three studies investigated CFS/ME longitudinally with varying periods (6-9 months with CBT, one year and six years) [133, 137, 141]. Structural MRI showed evidence of CBT treatment effects on brain volume during a longitudinal study [133]. Moreover, the increase in grey matter volume indicates macroscopic cortical plasticity in the human brain and suggests that there is a dynamic relationship between cerebral anatomy and behavioural state [133]. Furthermore, the increase in grey matter volume after CBT might be age-dependant as younger CFS/ME participants showed more improvement compared to older participants [133]. Shan et al. (2016) showed a reduction in left inferior fronto-occipital fasciculus (white matter) during a six years longitudinal study [137] but this was not consistent with Perrin et al. (2010) who conducted a one year follow-up[141]. These differences in findings could be due to differences in follow-up time. CFS/ME may be a slow progressing illness, which is supported by studies which showed that neurodysfunction is related to the duration of illness [135, 147]. Alternatively, it could be due to patients in those studies having different secondary symptoms or subgroups (see Hickie et al. (1995) p 92 [176]), as none of the studies defined their CFS/ME population accounting for subgroups.

Pain is an important factor which can occur at multiple sites, from the cerebral cortex to the spinal cord and is believed to be caused by maladaptive functional or structural plasticity of the nociceptive system [177]. Pain is a common symptom in CFS/ME but not a primary symptom for diagnosis. Reduced GMV, on sMRI, has been reported in many types of pain disorders, including chronic back pain [178-180], chronic tension-type headache[181], fibromyalgia, migraine [182, 183], and somatoform pain disorder [184]. Thus, not unique to CFS/ME.

Moreover, sample sizes were usually small, less than 30 in most studies (12/19). CFS/ME is a heterogeneous condition, and therefore conflicting results could be caused by studying different phenotypes with different underlying disease mechanisms [185]. CFS/ME does not currently have any biomarkers or clinical signs; therefore, diagnosis is based upon self-reported symptoms and excluding alternative explanations for diagnosis. The use of self-reported symptoms leads to doubt about the validity of CFS/ME as an aetiologically homogeneous diagnosis [186, 187]. This, in turn, has produced research to empirically define cases and subgroups examining the heterogeneity of CFS/ME. Hickie et al. (1995) used symptoms and demographics to empirically define a core group and a smaller polysymptomatic subgroup [176]. A more recent study by Williams et al. (2017) used latent class analysis to empirically define subgroups in a sample of 541 CFS/ME patients and found 5 subgroups [188]. This

indicates that CFS/ME populations being studied may not have similar disease phenotypes, potentially resulting in the inconsistent findings.

2.8.2 Functional MRI

Most fMRI studies reported differences between CFS/ME participants and healthy controls in brain activity despite a lack of differences being detected in cognitive performance. Studies that investigated CFS/ME using rs-fMRI observed reduced functional connectivity of the salience network, which was interpreted as an altered or immature resting-state network [154, 155, 157, 163]. In addition, investigating the default mode network showed a more complex and less coordinated network in the CFS/ME group [167]. The authors suggested that brain network analysis could be a potential diagnostic biomarker for this disease. Kim et al. (2015) hypothesized that this abnormal connectivity might be a result of a cognitive and emotional deficit in this group [155]. The salience network plays a major role in the connection between other brain networks such as the detection and integration of salient sensory information [189, 190] and switching between default mode network and central executive network [191]. The altered functional connectivity may cause a disruption in the integration of important information, specifically for cognition [192]. However, disorders such as Alzheimer's disease and multiple sclerosis, with attentional disruptions, have been associated with the presence of abnormalities of the default mode network therefore not unique to CFS/ME. These diseases overlap with CFS/ME in clinical features such as attention and memory difficulties [154]. Kim et al. (2015) argued that the presence of default mode network deficit could be metabolically expensive thereby contributing to, or a causal factor of fatigue, cognitive symptoms and post-exertional malaise of CFS/ME [155].

Differences in task difficulty may play a major role in why some studies reported increased activation while others reported decreased activation. Bryer et al. (2013) conducted a meta-analysis of fMRI studies of memory function in traumatic brain injury patients and concluded that the primary reason for the discrepancy in activation patterns across studies is attributable to task classification. Where hyperactivation may be associated with continuous memory tasks and hypoactivation may be more prominent in discrete memory tasks [193]. There have been a wide variety of tasks used in fMRI studies to assess differences between participants with CFS/ME and healthy controls. When rs-fMRI or simple tasks, were employed, participants with CFS/ME showed decreased functional connectivity in various brain regions [129, 131, 154-157, 163, 167, 170]. However, when more challenging tasks are employed, participants with CFS/ME exhibit widespread difference in activation in task-related regions when

compared to healthy controls [108-110, 159, 162, 170]. Most of the CFS/ME participants performed at a similar level to healthy controls and it is not clear whether the difference in activation was due to the increase in the task difficulty or because CFS/ME participants were trying harder. This widespread activation may lead to an increase in demand on neural resources such as oxygen and glucose, which in turn would lead to fatigue [133]. Fatigue and lower performance have been associated with increased brain activity while performing a high-effort cognitive task [132, 158, 159]. It has been hypothesized that severe fatigue consumes a significant amount of attentional resources in term of recruiting additional brain regions for cognitive compensation to perform better in dual-task depending on the degree of mental effort [109, 159]. Caseras et al. (2006) suggested that the fear of being fatigued leads the CFS/ME group to avoid activity [109]. Impaired reward processing was suggested to decrease motivation to learn in adolescents with CFS/ME [131], while participants inability to engage the part of the brain (left amygdala and left mid posterior insula) that responds to conflict suggested an abnormal salience network functioning in term of effect and cognition [156]. The increased activity in task-related areas was hypothesized to be a result of cognitive and emotional deficits in participants with CFS/ME [109, 155] and impaired reward processing in adolescents [159]. Participants with CFS/ME failed to recruit working memory regions to the same level as the healthy controls, as evidenced by reduced activation when the task difficulty increased. [109].

The heterogeneity of tasks, behaviours and cognitive processes across the fMRI studies makes it difficult to discern how much of the increase or decrease in activation, reported in relation to task difficulty or demand, is associated with increasing cognitive fatigue. Understanding the impact of fatigue on brain function will be critical to our understanding of CFS/ME.

2.9Limitations

Our systematic review has highlighted a limitation of the fMRI studies in CFS/ME, which is the small sample sizes. Empirical and simulation studies conducted by Desmond & Glover 2002 [194] found that to achieve 80% power at the single voxel level for typical activations in fMRI studies with thresholds correcting for multiple comparisons a sample size of 24 is required. We found that 15 of the 16 fMRI studies had a patient sample size of less than 24. Studies with low power reduce the likelihood of detecting a true effect, increases the risk of false negatives and the likelihood of false positives by reducing the positive predictive value (PPV) of the test. However, this is not unique to neuroimaging studies in CFS/ME, fMRI

studies have been criticized for being underpowered due to small sample sizes resulting in overestimates of effect size and low reproducibility [195, 196].

All the studies in this systematic review did not report the signal to noise (SNR) in their MRI methods. The SNR compares the level of the signal of interest to the level of background noise. In MRI studies, SNR is important for comparison between different MRI scanners, imaging protocols and MR sequences [197]. Thus, limiting this systematic reviews' ability to do a comprehensive comparative analysis.

Structural MRI visual inspection methods are subjective and unable to detect quantitative differences between scans. Automated sMRI methods such as VBM are quantitative and therefore can detect differences even when no visual morphological changes are present. Furthermore, they can be used as an objective method for assessing brain volume changes associated with treatment and intervention. VBM will be the method used for the analysis of sMRI in this thesis. Task-based fMRI needs to carefully consider the paradigm being used as the systematic review has highlighted that differences between CFS/ME are more likely to be reported in memory and attention tasks with conditions that are more cognitively demanding. Resting-state fMRI is not influenced by task design and difficulty and it can be more easily implemented in clinical practice. Resting-state fMRI can be used in all patients and has a much higher compliance rate compared to task-based fMRI, as some patients find it hard to co-operate with task-based studies [198].

The automated computational methods for investigating structural anatomical differences may be superior to subjective visual inspection but does have some limitations. Voxel-based morphometric analysis has been criticized for being significantly biased toward group differences that are highly localized in space and of a linear nature. In addition, these techniques are poor at detecting group differences that are spatially complex and subtle [199]. The fMRI studies reported both increases and decreases in activations patterns in CFS/ME compared to controls, while this may be related to task demands, caution must also be taken when interpreting these results, bearing in mind fMRI is an indirect measure of neural activity. The fMRI signal is derived from the blood oxygen level dependant (BOLD) contrast mechanism, i.e. haemodynamics of the brain. Currently, we cannot easily estimate the cerebral metabolic rate of oxygen (CMRO2) from the BOLD signal. Furthermore, haemodynamic responses are sensitive to the size of the activated population, and less likely to detect cortical regions in which stimulus- or task-related perceptual or cognitive capacities have sparse neuronal representation. It is also not fully understood how neuromodulation might contribute to the spatiotemporal resolution of the fMRI signal [200]. In recent years there has been a shift

placing a greater emphasis on neural networks underlying behaviour and cognition. A functional connectivity approach considering the neural network difference between patients and healthy populations may lead to a better understanding of how the disease affects brain function.

The main limitation of the present systematic review is that there was insufficient data for meta-analysis. A meta-analysis of neuroimaging data can take two approaches, image-based or coordinate-based analysis. Image-based analysis requires the statistical images of the data, and this is not often available due to data sharing issues, e.g. data protection and other restrictions. Therefore, most neuroimaging meta-analyses are coordinated based as these are reported in the published research. Moreover, the spatial normalization of images into standardized coordinates as anatomical addresses within a reference space has been applied to human neuroimaging data for decades [201, 202]. In order to perform an appropriate co-ordinate based meta-analysis, some minimum criteria need to be met. Firstly, the power of the meta-analysis. For co-ordinate based meta-analysis the activation likelihood estimation (ALE) method is conventionally applied [203], or a revised ALE algorithm [204]. For sMRI, we have 19 studies, 6 of these are visual inspection, thus subjective reporting without quantitative coordinate data. Of the 13 quantitative studies, only six studies reported co-ordinates. A meta-analysis on six sMRI studies would be severely underpowered.

In recent years the issue of low power in neuroimaging studies has been highlighted and the impact of these underpowered studies on the reliability and reproducibility of scientific studies. Furthermore, the ethical problem of unreliable research with low power is inefficient research and wasteful [195]. Therefore, best practice and appropriate use of methodological guidelines should be strictly followed. Given the appropriate method of co-ordinate based meta-analysis for sMRI is activation likelihood estimation (ALE) and there is empirical evidence by Eickhoff et al. (2016), that shows a minimum requirement of 17–20 experiments in ALE meta-analyses for sufficient power to detect smaller effects and ensure results are not driven by single experiments it was in line with best scientific and statistical practice that we did not perform a wasteful and inefficient power analysis [204].

For fMRI we have 16 studies in total, five fMRI studies are rs-fMRI and 11 task-based fMRI. The methodological differences between them preclude us from combining the results to perform a coordinate-based meta-analysis; they did not all meet the two minimum criteria. Firstly, all studies must be whole-brain analyses, and secondly, they must use the same standardized coordinates system. Four rs-fMRI use ROI seed regions and 1 used a whole-brain approach for calculating connectivity. For task-based fMRI studies, the task selection criteria

are critical. In our systematic review, we identified 11 task-based fMRI studies. However, only two studies use the same PASAT task. During the PASAT task, participants with CFS/ME demonstrated a significant increase in BOLD signal in bilateral premotor and left superior parietal regions [110, 160]. We do not have the beta images of the results of fMRI studies to pool and perform an image-based meta-analysis. To perform a co-ordinate based meta-analysis on 2 studies would be underpowered and in violation of best scientific and statistical best practices. The remaining nine studies all use different tasks, and more importantly, each task is designed to examine a different cognitive, sensory or physical function. Therefore, the heterogeneity of task-based fMRI studies prohibits the creation of task selection criteria for meta-analysis.

2.10 Future Directions

The complexity of this illness and its related symptoms as well as using small sample sizes without controlling for population heterogeneity may explain the inconsistencies found in the literature. Future studies should use larger sample sizes with subgrouping according to the phenotypes and classification of participants according to CFS/ME severity and symptom patterns. Sub-phenotyping [185] could reduce the heterogeneity of the patient samples in case of control studies. Stratifying by symptoms, activity or sleep patterns may enable researchers to compare CFS/ME to other conditions (or healthy controls). Only one research group has matched controls for sleep pattern [139], which is altered in CFS/ME, and is known to have a strong association with BOLD signal measured by fMRI, grey matter and white matter volumes [205-208], suggesting that better matching between participants with CFS/ME and control group is required.

An additional important aspect is the use of longitudinal MRI data. Longitudinal studies enable us to examine the progression of structural changes in CFS/ME while controlling for age-related effects points [133, 137, 141]. In this systematic review, out of the thirteen quantitative studies, 11 corrected for age in their statistical analysis. It is also important to measure whether the use of a treatment method is effective. The findings from longitudinal studies [133, 137] demonstrate the importance of using two-time points to understand the impact that treatment, length of illness or symptom severity may have on MRI volumetric measures.

Brain white matter volume increases linearly with age in adolescence [209] and given the prevalence of CFS/ME in this age group [210], more research is required to determine if there are distinct neurobiological markers comparable to studies in adults with CFS/ME. For sMRI studies, as quantitative automated methods found differences [132-140], future research should

focus on using automated objective methods. By using voxel-based morphometry, De Lange et al. (2008) was able to show that improvement after CBT was associated with improving grey matter volume in the CFS/ME group [133]. Shan et al. (2016) were able to find progressive brain changes after six years follow up which therefore lead us to conclude that sMRI studies might not yet show evidence as a diagnostic tool, but can be used as an objective measure of treatment evaluation. Consistency in research might be achieved by using standardised MRI protocols which have been evaluated and compared with other illnesses such as the use of standard MRI protocols across multiple sites in Alzheimer disease [211]. Neuroimaging researchers can use the views of CFS/ME experts regarding different grouping strategies which can aid in finding CFS/ME biomarkers which may steer CFS/ME research in directions that hold promise and eventually help clinicians in the optimization of their practices.

Finally, we have limited this systematic review to include only neuroimaging studies that have used structural or functional MRI methods. However, other neuroimaging techniques have been used to investigate CFS/ME these include single-photon emission computerized tomography (SPECT), electroencephalogram (EEG), magnetic resonance spectroscopy and diffusion tensor imaging (DTI). These methods measure different neurophysiology from sMRI and fMRI. Future studies using multi-model imaging approaches could overcome some of the limitations of a single method alone.

2.11 Conclusion

In conclusion, there is no evidence to support the assertion that findings from neuroimaging studies have found any clear biomarkers of CSF/ME. However, MRI can be considered a powerful tool if rigorous procedures for the collection of data and analysis are employed, taking into account the limitations of the neurophysiology being measured by fMRI. There is a significant need for more research, given the sparsity of studies, as evident by our inability to conduct a meta-analysis. MRI studies in this systematic review have demonstrated the potential for significant insights into CFS/ME, which is not afforded by other techniques. For example, fMRI studies have provided objective measures of the impact of fatigue experienced by participants with CFS/ME on cognition, even in the absence of behavioural and cognitive deficits [108, 158]. Structural MRI has shown evidence of treatment effects on brain volume [133] while fMRI has demonstrated functional connectivity changes and altered patterns of activation. Future MRI studies could potentially, with proper study design, subgrouping and sample size lead to a breakthrough in our understanding of this illness.

This systematic review results provide a good understanding of fatigue in CFS/ME, which allow for better interpretation of the results in later chapters. Evaluating the strength and weaknesses illustrated that using a moderate difficulty working memory task would be the most appropriate for the aim of this thesis. Also, this systematic review highlighted the need for more investigation in this illness. In the past 25 years, only 16 fMRI studies were conducted, of which only four investigated working memory [108-111]. All these working memory studies used a sample size of <20, which illustrate the need for a larger sample size.

3 Chapter 3: Methods

3.1 Self report measures of fatigue

Fatigue is the lack of energy and motivation and as it is difficult to describe physical or mental symptom (or both) patients often use words like tired or exhausted. Fatigue is defined as “subjective lack of physical and/or mental energy which is perceived by the individual or caregiver to interfere with usual or desired activity” (Multiple Sclerosis Council for Clinical Practice Guidelines, 1998, page 2) [212]. The definition emphasized on the difference between physical (peripheral) fatigue and mental (central) fatigue. Clinicians take a complete and thorough history to help diagnose the cause of fatigue. Some causes of fatigue include heart and thyroid diseases, anaemia sleep disorders and CFS/ME. However, long-lasting complaints of fatigue do not necessarily equate to CFS/ME and specific criteria need to be met before declaring such a diagnosis of CFS/ME. Therefore, different well-designed measures have been designed to measure fatigue including the fatigue severity scale and the Chalder fatigue questionnaire. Those symptom-specific outcome measures deliver a standardised description of patients' experience in terms of health states. However, these subjective scales have both pros and cons which are the reason why these scales need to be used with caution. Advantages include quick and easy implementation and can be paper-based or computer-based. Also, it has been widely used in occupational research which is important to allow for straightforward comparisons between studies and populations [213]. These measures have been used in CFS/ME and are considered as a reliable measure [214-217]. However, relying on self report given by participants may bias the results. There are many biases and limitations that the self-report measures are subjected to which include honesty, introspective ability, interpretation of questions, and rating scales. Honesty bias happens when a participant chooses a more socially acceptable answer rather than choosing their own condition. Introspective ability bias is the inability of the participants to assess themselves accurately. Interpretation of questions bias happens as a result of misunderstanding the meaning of the words in the question or if the question has more than one meaning. Rating scales bias is a result of yes, no questions in too restrictive questions [218]. CFQ has been criticized for having ceiling effects [219]. Also, these fatigue measures had been criticised for their subjectivity, and being less valid and less reliable due to being brief and short [220].

3.2Neuroimaging

Magnetic Resonance Imaging (MRI) has many applications, such as structural MRI, resting-state functional MRI (rs-fMRI), and task-based functional MRI (fMRI). Structural MRI provides detailed images of the structure of the brain to detect any abnormalities. Rs-fMRI provides a measurement of brain functional connectivity when no goal-directed task is being performed. It helps in mapping brain networks as well as measuring brain regions temporal coherence. It can help in differentiate alterations in functional neural networks [221]. fMRI enables researchers to capture the brain while it functions during the performance of a task or at rest [222]. Therefore, the use of MRI in this thesis will allow the examination of the structure and function of the brain with high spatial resolution in patients with CFS/ME in comparison with healthy controls to investigate the neural substrates for cognitive dysfunction in CFS/ME.

3.2.1 Physics of Magnetic Resonance Imaging

MRI is an in vivo imaging technology whose functionality is based on the principle of nuclear magnetic resonance (NMR), which was discovered by Bloch (1946) [223] as detection of NMR absorption in paraffin and Purcell et al. (1946) as detection of nuclear induction signal in water [224]. When the nucleus is subjected to an applied magnetic field (B0), its protons produce net magnetisation by aligning with the applied magnetic field (B0) (see figure 5). Consequently, subjecting the nucleus to a rotating magnetic field (B1) and tips net magnetisation into a transverse plane, which is perpendicular to the applied magnetic field (B0). At this point, atoms in the nucleus precess towards the rotating magnetic field at the Larmor frequency, which is defined by a constant known as the gyromagnetic ratio (g-factor or γ). After that, external short radiofrequency waves, called RF pulses, are sent to disturb the protons' alignments in which low energy parallel protons flip to a high energy state resulting in decreasing longitudinal magnetisation and turning the net magnetisation vector toward the transverse plane. These disturbed protons will emit radio signals as they realign. The frequency magnitude and phase of nucleus precession reduce over time with the decrease and increase of the transverse relaxation (XY plane) and longitudinal relaxation (z plane) respectively. Consequently, the transverse relaxation (XY plane) produces a voltage signal that is recorded in NMR [225]. Transverse magnetisation and the direction of rotation of the nucleus is detected by pairs of receiver coils that are tuned to the Larmor frequency. Because human tissue has varied transverse and longitudinal relaxation times, MRI physicists can alter the pulse sequence to detect different tissues types [225]. All data is recorded in a k-space matrix, which derives respective data points from the MR signal detected by the receiver coils such that the matrix

axis aligns with the x and y axes of the image. A Fourier Transformation is used to define the relationship between the image data and the k-space data matrix based on phase encoding and frequency methods [225].

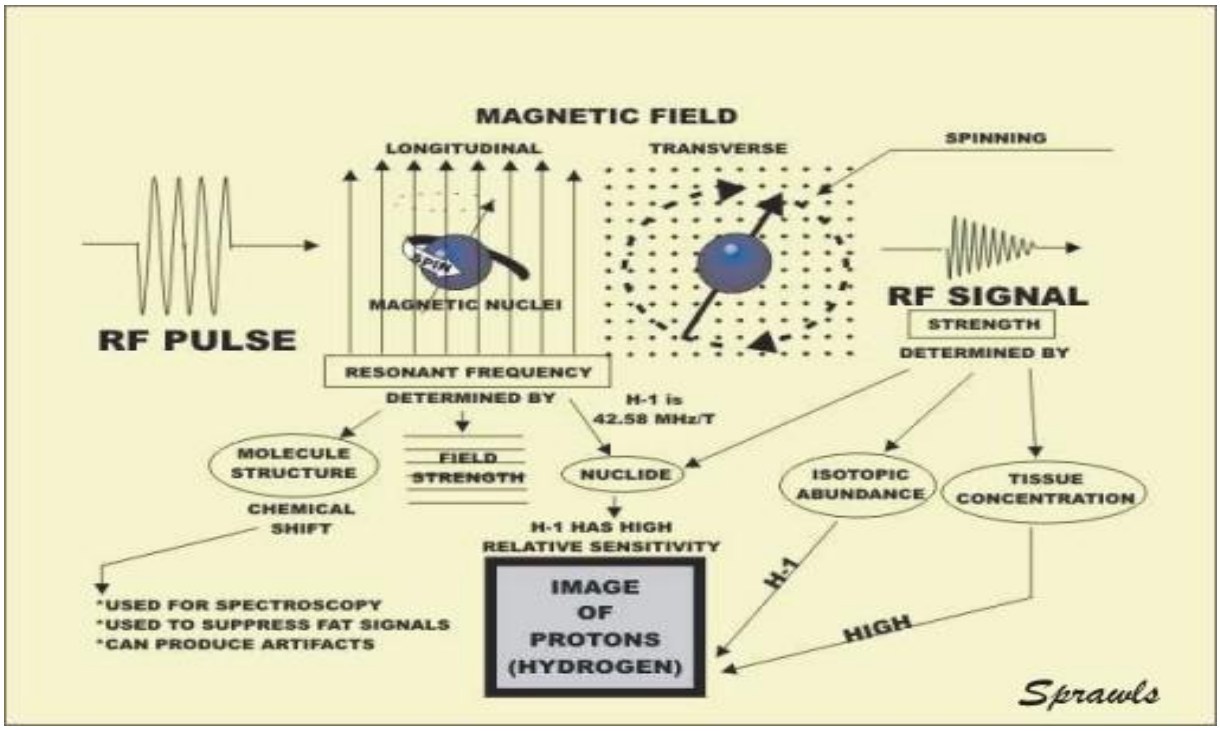


Figure 5 shows how protons align in the presence of a magnetic field and the production of MRI signal. Image is taken from Sprawls (2000) [226].

3.2.2 Spin and Gradient Echoes

Spin and gradient echoes are MRI pulse sequences introduced to resolve signal decay. Spin echo uses a pair of radiofrequency pulses (RF) while gradient-echo uses only one RF pulse and a combination of gradient reversal. Signal decay, or so-called free induction decay, which follows the initial RF pulse is a major issue in MRI, and it is caused by spin relaxation and inhomogeneities of the local magnetic field, thus resulting in different precession rate of the spins. As the transverse relaxation cannot be rephased, Hahn (1950) introduced a 90° RF pulse, which rephases the magnetic field to prevent the loss of MR signal through a spin-echo process [227]. A Spin Echo (SE) sequence established by Carr and Purcell (1954) functions such that a 180° RF pulse follows the initial 90° RF pulse given that the second 180° RF pulse is applied at echo time/2 thus increasing the MR signal and consequently rephasing the transverse relaxation. Gradient Echo (GE), which is an alternative to the SE rephases the relaxation by altering the resonance frequency using gradient echo. The main difference between GE and SE is that no 180° RF rephasing pulse is applied, and the flip angle used is lower than 90°. The reduced flip angle increases longitudinal relaxation, thus reducing the time taken during image

acquisition, thus making gradient echo sequences common in rapid MR imaging [228]. MRI has sequences that differ according to the parameter and techniques used in each sequence. In this thesis, two sequences were used: T2*-weighted gradient echo-planar imaging sequences for the rs-fMRI and the fMRI task-based scans and Magnetization-Prepared and Rapid Gradient-Echo (MPRAGE) for the structural images, [229]. T2* in principle results from the inhomogeneities in the main magnetic field, which might be due to the intrinsic defects in the magnet or susceptibility-induced field distortions [230]. This sequence uses gradient echoes and a relatively long time to echo (TE). The T2* sequence forms the basis for functional MRI (fMRI) using the BOLD (Blood Oxygen Level Dependent) technique and is used to show the effect of local magnetic homogeneity since blood is paramagnetic and this results in BOLD contrast being able to be measured. Three runs of the T2* sequences were used to acquire images for tasked fMRI examinations in this study and therefore capturing the brain while functioning using BOLD imaging contrast mechanism [231, 232]. On the other hand, T1 is the time needed for the recovery of the protons in which protons transfer energy from the nuclear spin system to its environment. This process is called the spin-lattice relaxation time. T1 weighted MPRAGE sequence, is a fast gradient echo pulse sequence that acquires 3D images by using a magnetization preparation pulse for high resolution structural imaging [228, 233].

3.2.3 Blood Oxygenation Level-Dependent Signal

Functional MRI (fMRI) uses the BOLD imaging contrast mechanism to image brain function non-invasively. This technique employs the difference in BOLD contrast within the brain to create images of cerebral activity [234]. As the brain does not store glucose (the primary source of energy), blood flow is required to transfer glucose which, during tasks, brings more oxygen through oxygenated haemoglobin molecules, using red blood cells [234]. BOLD imaging thereby images the oxygenated tissue areas since the oxygenated and deoxygenated haemoglobin exhibit differences in their paramagnetic capabilities. A local de-phasing of ions is observed in regions of low activity because of the high presence of deoxygenated haemoglobin which is paramagnetic as opposed to oxygenated haemoglobin which does not exhibit these properties. The high number of de-phased protons results in a reduced return signal, thereby mapping these regions of low oxygen to areas of low signal energy received [235].

The researcher in neuroscience faces many challenges. One of the important challenges in neuroscience is the so-called large-scale integration problem which represents how neural

activity distribution may lead to accurate cognitive moments [236]. This is because the recorded signals from the human brain can be either Local Field Potential (LFP) or spikes. LFP is a summation of the reflection of post-synaptic potentials over a long distance where Multi-unit Activity (MUA) reflects action potentials or so-called spiking. Mostly, LFPs represents events that reflect cooperative activity in neural populations. Also, LFP is the low-frequency range of mean extracellular field potential signals. It is typically computed using a low-pass filter to filter the mean extracellular field potential below 500 Hz. It was previously thought that these signals represent exclusively synaptic events [237-239], but recent studies showed that LFPs in EEG is independent of neuronal spiking [240]. Also, LFPs represent the slow change in voltages with a longer time course that is recorded from brain cells. Spikes, also known as action potentials, on the other hands, represents typically single-neuron action potentials which happen in frequencies of 0.5 kHz [241]. In term of transferring information in the brain, LFP is considered to be as accurate as action potentials or even more precise than those from action potentials (individual neurons) [242, 243]

Logothetis et al. (2001) used a combination of BOLD fMRI and electrophysiological recordings to investigate neural coupling with the BOLD signal. Logothetis et al. (2001) results showed that BOLD activity is closely coupled with Local Field potential LFPs but not single cell spiking. Their results demonstrated that BOLD fMRI is a reflection of input and intracortical processing for a specific area instead of its neuronal spiking output [244]. Logothetis et al. (2001) results were supported by two other studies in which they eliminated spiking in the visual cortex. Rauch et al. 2008, used pharmacological manipulation [245], while Viswanathan and Freeman 2007, used stimulus characteristics to reach the same result that BOLD fMRI is a reflection of input and intracortical processing [246].

These studies boosted and supported the notion that neuronal spiking occurs as a secondary result driven by the LFP [247]. A study on awake monkeys proposed that significant correlations between BOLD and LFPs might be found in the visual cortex [248]. Another study revealed that the reduction in LFPs and spiking is associated with decreases in the BOLD signal in the visual cortex [249]. Also, the reduction in LFPs (but not spiking) is associated with the reduction in blood oxygenation in the sensory cortex [250]. Mathiesen, in two studies (1998 and 2000), used a series of sophisticated experiments of simultaneous cerebral blood flow (CBF)/LFP/spiking measurements to investigate BOLD signals and neural firing in rat cerebellum. A strong correlation was found between the BOLD signal and LFPs and not spiking [251-253].

3.3 fMRI Paradigm

Memory is a domain of several brain regions and networks (see section 1.4) [58]. Therefore, the use of fMRI will allow capturing the whole network and system-level activation. Imaging the human brain while performing a task is essential for understanding how the brain works and may be conducted using several imaging techniques. fMRI, among all others, is a non-invasive technique that allows the scientist to acquire images of participants brains, showing patterns of brain activation as a result of conducting a specific task. It also allows the visualisation of subtle neurological differences between groups which cannot be measured by behavioural testing alone. However, fMRI has a disadvantage in that it cannot infer causal relationships as it can only establish correlations [254, 255]. Several tasks have been used to evoke these neuronal patterns depending on which behavioural or cognitive function is being investigated.

3.3.1 Block Design and Event-Related Design

fMRI uses three types of experimental designs: block design, event-related or a combination of both designs depending on how the stimuli are presented. In a block design, two or more conditions are alternated in a set sequence so the participant can distinguish between alternating blocks and controlled blocks. This design is usually used to study steady-state processes such as attention and to localise functional areas. It can be an alternating design where conditions A and B are alternating in separate blocks or a controlled block design where experimental conditions are separated by null blocks [256]. Block design might be a better choice to measure some cognitive functions as it is a simple and robust design. Higher statistical power can be obtained from blocked design as a BOLD signal from multiple repetitions is additive. Therefore, it ensures that signal variation from participants movement, scanner sensitivity, or attention shifts would have the same effect on the signal responses for each of the different states. It is also a good technique to detect small changes. However, it cannot be applied to all hypotheses and can result in habituation effects [257]. Habituation can be explained as the decrease in brain activation after repetitive or prolonged exposure to a stimulus [258, 259].

In an event-related design, the presented individual events are randomised, and time can vary between the stimuli. This design allows the use of multiple tasks and stimuli to provide the flexibility needed for complex neuropsychological experiments. This design tends to measure the BOLD signals that respond to neural events related to behavioural trials [256]. This design allows the detection of transient variations in hemodynamic responses, which in turns allow BOLD signal changes [260]. Advantages of event-related design include randomisations of

different type of events to prevent predictability and ensure that each event is not influenced by the other. The ability to categorise events after the experiment depends on the subject behaviour (post-hoc subjective classification of trials). The subject cannot define the occurrence of events. Also, the event-related design allows rare events to be measured [256]. It also has a few disadvantages as it is a more complex design and analysis. Because the MRI signal is small, the design needs to increase the number of trials. Whichever design, block or event-related, is being used, it can be analysed using Statistical Parametric Mapping.

3.4 Experimental designs and analysis methods

3.4.1 Statistical Parametric Mapping

Statistical Parametric Mapping (SPM) is free software that is written using MATLAB and used to help in the analysis of functional neuroimaging data. It was created by the Wellcome Department of Imaging Neuroscience at University College London [261-263]. SPM is a statistical technique used to examine brain activity differences in functional neuroimaging experiments. It can be used in many neuroimaging technologies such as fMRI, PET, SPECT, EEG and MEG. The idea behind it is to extend the use of statistical mapping processes that compare the activity between conditions to assess and examine the hypothesis about functional imaging data. Therefore, it has been designed to analyse brain imaging data, which can be a sequence of images from different patient groups or time-series obtained from the same subject (see figure 6). SPM applies a method for estimating multiple comparisons correction called the Random Field Theory. Random field theory, which defines the theoretical results for smooth statistical maps, creates inferences about topological features of statistical processes, which are continuous functions of time or space [264, 265]. Random field theory can be applied to find the height threshold for a smooth statistical map and provides the required family-wise error rate. SPM software produces statistical parametric maps (SPMs), which are images with voxel values that their allocation corresponds to a known probability density function such as the Student's T or F distributions. Those values are under the null hypothesis and distributed according to a known probability density function. Those distributions are the Student's t or F-distributions [264, 265]. Each voxel in this map is a representation of the activity of a specific coordinate in three-dimensional space. These images have values which are distributed according to a known probability density function, t or F-distributions, which are known generally as t- or F-maps. SPM is successful due to its simplicity of the idea as it analyses each voxel of the produced images using a standard statistical test. This is usually done based on a General Linear Model (GLM; see below) of the produced data. The result of this statistical

process is then assembled into an image. Random fields' is used to interpret SPM images as continuous statistical processes [266-268]. Random fields have the ability to model both the univariate probabilistic characteristics of SPM images and any other non-stationary spatial covariance structure. In summary, GLM is utilised to describe and explain continuous data such as images in just the same way as in conventional analyses of discrete data. Random field theory is then used to resolve problems arising from multiple comparisons when making inferences about the analysis of the volume. Random field theory offers a technique to adjust p-values in order to search volume and plays the same role for SPM images in the same way that Bonferroni correction is used for discrete statistical tests [265]. In fMRI literature, random field theory has been used widely to address the problem of multiple comparisons as it provides an analytical solution for calculating the p-value when meeting the assumptions [269-271]. The Bonferroni correction has been considered too stringent to be used in fMRI [272]. Random field theory is intended to work in continuous space whereas Bonferroni works in discrete space. Random field theory does not vary by image resampling. By contrast, Bonferroni thresholds change depending on the image resampling as it depends on the number of voxels [273]. Smoothing is important to meet the good lattice assumption and the majority of the current studies smooth the data by twice the voxel size. This smoothing, in conjunction with the Bonferroni assumption, would not be sufficient to meet the assumption of good lattice [273].

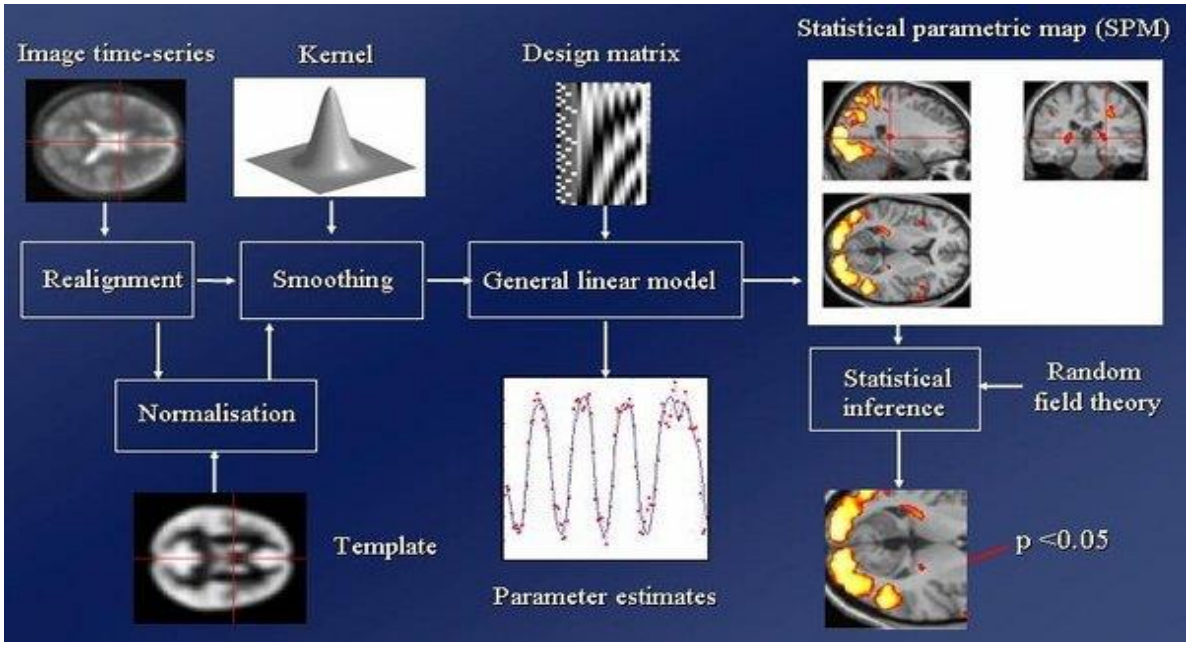


Figure 6 shows a schematic that shows the steps that it takes from obtaining imaging data sequence to SPM. The image is taken from Friston et al. (2003) [165].

3.4.2 General Linear Model (GLM)

GLM is one of the system-level approach techniques that are used to understand human brain activation by providing task performance information for different experimental conditions. As previously mentioned, GLM is used to explain continuous data, such as fMRI images. Most of the neuroimaging analysis methods use a variant of the general linear model with very few exceptions. The only way to differentiate between these methods is the design matrix encoding the experimental design or temporal model [261, 262, 265].

GLM is an equation $Y=X\beta+\epsilon$ which states the detected response variable as a linear combination of explanatory variables X and error term ϵ [262]. Analysis of covariance or multiple regression analysis, including simpler variants such as t-test to more complex linear convolution models like finite impulse response models, are other well-known terms for GLM. In our study, we used a canonical haemodynamic response function where columns in the design matrix represent an effect, which is known as explanatory variables, covariates or regressors, that being studied in the experiment or a confounder that interfere with the data and may lead to artefacts such as motion. These effects on the response variable are then presented as functions of the existing conditions and presented in the first four columns of the design matrix. Following that is a series of terms which are designed to eliminate or model low-frequency variations in the signal because of the presence of artefacts like aliased biorhythms and other drift terms. The last column among them is a whole-brain activity. Standard maximum likelihood is then used to assess the relative contribution of every single column.

After that, t or F-statistics are used to make inferences about the contribution of these columns. Those inferences depend on their linear combination, such as subtracting one condition from other confounders or all of them composed [261, 262, 265].

Data needs to be in the same anatomical position and space to perform voxel-based analysis. This can be done by realigning the data then performing non-linear warping and smoothing, to match the existed template to the standard anatomical space. These chosen test statistics, usually t or F-statistics, create the SPM which in the final stage make statistical inferences. Statistical inferences are based on the SPM and random field theory and describe the responses seen using the fitted responses or parameter estimates [262, 265].

3.4.3 Structural MRI (sMRI)

MRI produces detailed structural pictures (MRI images) of the human brain and allows the detection of the pathological changes in the brain without autopsy [274]. These images can be improved using several MRI techniques, such as increasing the number of excitations or using stronger magnetic fields [275]. Structural MRI findings are widely used in the diagnose of Alzheimer's disease [276, 277] and dementia with Lewy bodies [278]. MRI in the diagnosis of Multiple Sclerosis (MS) has an important role in assessing the brain damage in the first evaluation and then monitoring the disease progression or therapeutic efficacy by evaluating the magnitude of that damage over time [279]. However, other conditions such as CFS/ME might not have brain lesions as a biomarker. Therefore, this highlights the need to implement quantitative automated computational methods of looking at sMRI. One approach is Voxel-based morphometry (VBM).

3.4.4 Analysis Approach for Structural MRI Data (Voxel-Based Morphometry)

Morphometry is the evaluation of brain anatomy with respect to size, shape, and structure, and VBM evaluates the brain structure using anatomical MRI scans. VBM segments the brain into the cerebrospinal fluid, grey matter, and white matter to allow the examination of volumetric analysis of specific regions of interest, whole brain, and volumes in patient groups such as CFS/ME and MS, compared to healthy controls [280]. The normalisation of the T1-Weighted anatomical image of an individual to the standard SPM template is the first step of VBM to allow for voxel-by-voxel agreement across participants. This is achieved through non-linear registration that creates a deformation field by stretching or compressing regions on a template brain image to determine the extent to which the input image must be altered to match the template image. A segmented image is created by differentiating the deformed image into brain tissue groups based on the probability of finding tissue class at each voxel. The next step is to

apply voxel-wise statistical analysis in the spatially smoothed images and tissue concentration from different participants. The resulting brain volume structure can be used to predict various behavioural measures [280]. For example, according to Maguire et al. (2000), taxi drivers have increased brain volume in the regions that synthesize spatial navigation when compared to non-taxi drivers [281]. Moreover, studies on CFS/ME that have utilized VBM to examine changes in this patient group suggest that disease progression also affects the brain volume either by white matter volume reduction [136, 138, 139, 282-284], white matter changes or lesions [147, 150, 151], or grey matter volume reduction [136, 285-288].

3.4.5 Resting-state fMRI (rs-fMRI)

Resting-State fMRI investigates human brain functions while no goal-directed task is being performed. Functional connectivity has been used in neuroimaging to map large-scale brain networks, Resting-State Networks (RSNs), as it measures the temporal coherence among brain regions and distinguishes alterations in functional neural networks [221]. This is done by using statistical dependency between remote neurophysiological events. rsfMRI is easy to acquire and ideal for participants who cannot perform specific tasks. rsfMRI can be used for exploratory analyses, and one data set is enough to investigate different functional networks in the brain.

Functional connectivity analysis has many techniques, including graph theory analysis, seed-based analysis, and independent component analysis (ICA) to investigate rsfMRI connectivity. Graph theory enables the measurement of the topological properties of regions of interest within the human brain and if that network represents a particular function, while seed-based analysis only focuses on correlation strength between one region of interest to another [289]. The seed-based analysis is easy to interpret, but it ignores complex structure and noise by modelling seed-effect only. It is also influenced by small changes in the seed location, which results in seed-selection bias [290]. ICA is multivariate and decomposes a full dataset to test for shape and amplitude, but it is hard to interpret, and there is no control over decomposition [291]. Dynamic Causal Modelling (DMC) is a Bayesian generative model that analyses the brain activation mechanism through inferential coupling among brain regions under varying experimental conditions [292]. However, since DCM is specialized in hypothesis testing for neuronal mechanisms, it deals with a few regions of interest that underlie experimental brain activation [293]. The heterogeneous nature of CFS/ME implies the lack of a clear region of interests, thus making DMC an unsuitable functional connectivity approach. Therefore, the most suitable choice for analysing rs-fMRI data in this thesis would be the graph theory.

Although graph theory is a simple yet powerful model of the brain's functional connectome, the construction of a model goes through many steps such as making the basic assumption as well as different choices, the selection of links and nodes and making the decision about what topological parameters to be calculated. However, the inappropriate selection of nodes and edges in a network will lead to poor results and misleading conclusions [294]. Importantly, the anatomical definition used to identify the nodes used in graph theory may lead to bias or systematic errors due to functional inhomogeneity [295]. While many atlases have been used in graph theory, the different node definitions according to each atlas may lead to question the homogeneity within a node [295]. For example, regions such as the precentral and postcentral gyrus can be further segregated into subunits specialized for foot, hand, face, tongue, lip, etc [295]. Therefore, functional connectivity of such regions might be the mean functional connectivity of two regions causing incorrect functional connectivity due to intra-nodal inhomogeneity leading to erroneous construction of a brain graph [295].

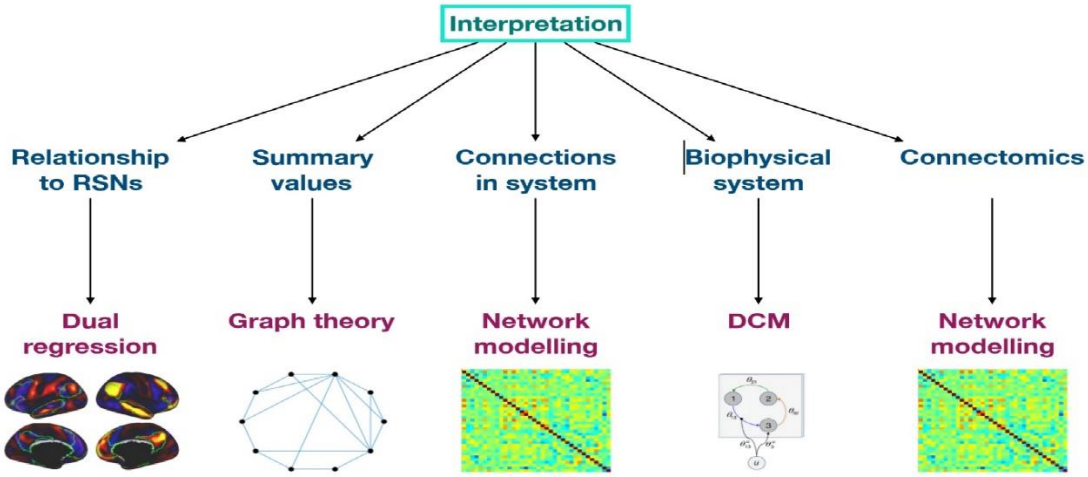


Figure 7 shows an illustration of different analysis methods used to analyze functional connectivity. The image is taken from Smith et al. (2013) [296].

3.4.6 Analysis Approach for rs-fMRI Data (Graph theory)

Graph theory is a mathematical technique for exploratory analysis that has been used for this thesis. It is concerned with the study of graphs to model relationships between nodes based on the interconnectivity of nodes and edges. Graphs are used to represent brain networks, while the regions of interest represent nodes, and the edges represent the connections between the respective region of interests. Graphs can be either weighted (with edges) or unweighted (without edges) in the connection between nodes. Consequently, nodes can be directed (one node has an influence on the other) or undirected, whereby nodes are connected without any

directionality. In the event that the number of nodes is equal to the number of rows and columns, a graph can be represented in the form of an adjacency matrix. The matrix is created using Pearson's correlation such that the matrix is symmetric in undirected graphs, unlike symmetric directed graphs. According to Kaiser (2011), matrix values are 0 when the edges are absent and 1 when edges are present in unweighted graphs as determined by a correlation threshold [297]. According to Garrison et al. (2015), Jalili (2017), and Zalesky et al. (2012), the threshold has a direct influence on the outcomes of graphical analysis [298-300]. In fMRI paradigms, it is complicated to determine the activation thresholds. Factors such as the influence of physiological rhythms (e.g., respiration), include the time-series nature of the data and the vacillations introduced by the experimental design (e.g., cueing) are needed to be included. Therefore, the authors argue that the approach to setting thresholds is absolutely arbitrary [291, 301, 302].

Graph theory is widely applied in cognitive neuroscience to study the structural and functional mechanisms of the brain. Network measures are used to statistically represent complex functional brain networks to quantify the global and local properties of the brain network according to key elements [291, 303]. For example, the path length is said to be the shortest distance between two nodes, while the clustering coefficient represents the fraction of triangles around individual nodes in a network [304]. Therefore, network measures can be used in the characterization of segregation, functional integration, centrality, and evaluation of functional connectivity differences between pathological and healthy brains.

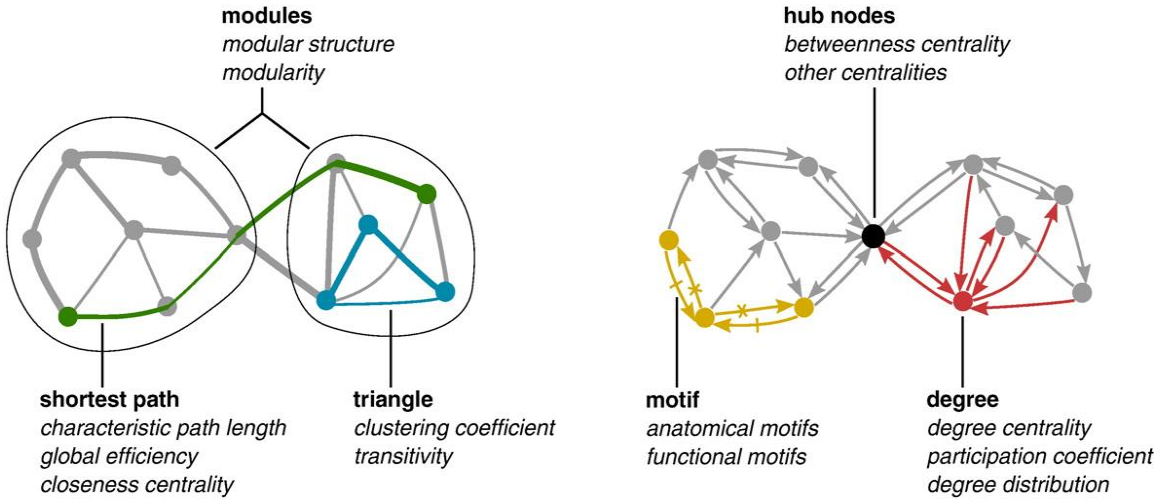


Figure 8 shows an illustration of key complex network measures. The image is taken from Rubinov and Sporns (2010) [291].

Integration measures (green) are based on the shortest paths, and segregation measures are based on the triangle counts (blue) as well as more complex decomposition into modules (ovals) (see figure 8). The hub nodes (black) are mainly found on the shortest paths while node

degree (red) is used as the basis of applying the measures of centrality and network motifs (yellow) quantify the connectivity patterns. For example, a three-node and four-link anatomical motif contain six possible functional motifs whereby one motif contains dashed links, and one motif contains crossed links as shown [291].

Definition of the region of interests through techniques such as the voxel-wise approach is an important aspect of the brain network [305]. This data-driven approach considers each voxel in the brain and grouping them to form regions of interests as functional units of fMRI data. This means that there about 140,000 nodes in the brain network. Detecting sharp transition in resting-state functional connectivity MRI patterns [306, 307], identifying functionally similar clusters [308-310], and regional growing methods [311] are the three main approaches that can be used. Automated Anatomical Labelling (AAL) is one of the atlases used for the region of interest definition according to Tzourio-Mazoyer et al. (2002) [312] because it allows for whole and partial brain network analysis based on pre-existing literature in Alzheimer disease [313]. Pre-existing functional activation is also a technique that can be used to determine the region of interests by establishing the coordinates of activation through the creation of 3-6mm radii spheres that are fixed on either centre of coordinates of a presumed functional area or the peak activity [314-316]. However, using a pre-existing functional activation approach means that only voxels that are contained in the sphere can be included in the analysis, thus creating a possibility of missing crucial information in poorly defined spheres. Several scholars have studied this technique to evaluate the human brain and concluded that it has a high potential for enhanced understanding of cognitive disorders that have not been explored using other methods [303, 317, 318].

3.4.6.1 Modularity:

Additional to the FC measure, there are several approaches that can be used in rs-fMRI to investigate the neural correlates of neurological and psychiatric disorders [319]. The individual differences in executive function processes have been shown to be related to changes in structural and functional connectivity between brain regions [320, 321]. To quantify these interactions, Newman and Gravian (2004) suggested conceptualizing the brain as a network comprised of sub-networks, or modules [322]. Therefore, modularity is used in resting-state fMRI to quantify the brain networks and connections between brain regions. It shows the connection strength between nodes in the brain. High modular networks (high modularity) mean that there are dense connections between nodes inside modules [316]. Therefore, the modularity of a given graph defines the possible formation of nodes in networks. Also, it shows

how strongly the nodes within the full network form relatively isolated sub-networks [318]. By detecting and characterising modular structures in the brain, specific anatomically, or functionally associated components that perform a particular function can be identified [323].

3.4.6.2 Global Efficiency:

The measurements of how easily two nodes are connected define the edge efficiency in a graph without passing through a third node. Latora and Marchiori (2001, 2003) described global efficiency and local efficiency as a measurement of the network's ability to transmit information at the global and local levels [324, 325]. Efficiency can be global when it represents the overall average of pairwise efficiency or local when it represents a specific node efficiency mean of neighbouring nodes subgraphs [326]. Global efficiency in resting-state fMRI describes brain networks information flow that can deal with either sparse or disconnected graphs or both (see figure 9) [324, 325]. As graphs are naturally sparse, in some applications, a priori is not given, and the application needs to learn from the data [327-330]. Due to not specifying a priori, a challenge arises from promoting sparsity which may make the graph disconnected [324].

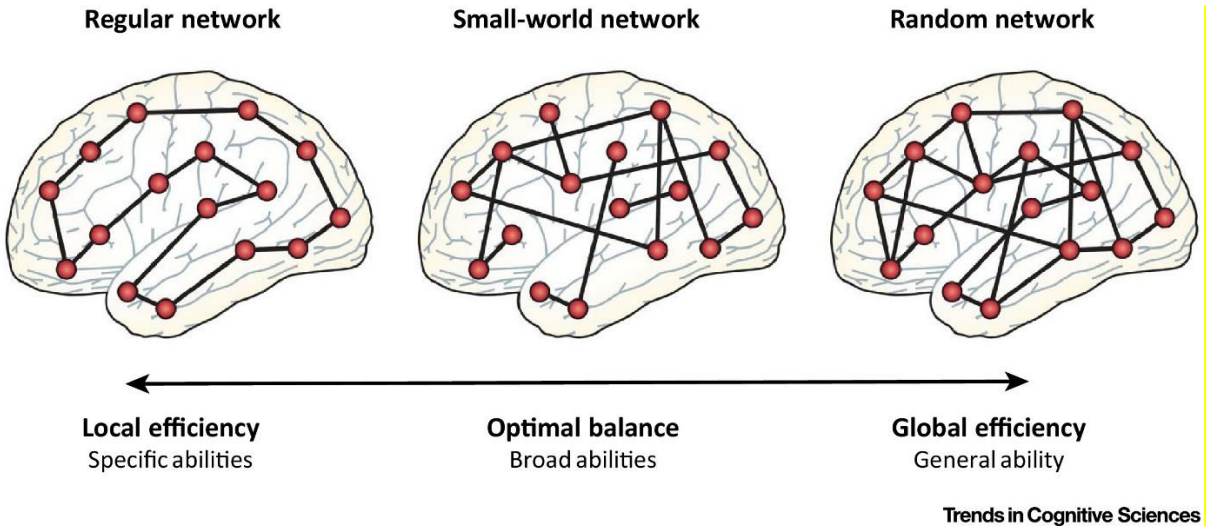


Figure 9 shows different global efficiency models of the brain network. image from Aron Barbey (2018).[331]

3.4.7 Multiple Comparisons and Family-Wise Error

Multiple comparisons are often considered when planning a study or to analyse data after an experiment has been accomplished. It refers to conditions in which a dataset is being used and subjected to statistical testing multiple times. The main types of statistical error are Type I and Type II. Type I errors (family-wise error) represent the probability within the statistical framework to make one or more false positives. This means that discovery is being made by

mistake and occurs when the null hypothesis is rejected by mistake at the significance level (α). Type II error occurs when a false null hypothesis is accepted as specified power (β) of a study. Multiple hypotheses increase the likelihood of Type I errors. For instance, in neuroimaging research with 100,000 voxels, there would be about 5000 false positives given a significance level of α = .05, or 100 false positives with α = 0.001 [332].

Multiple comparisons can be controlled using Bonferroni or Random field methods in which the false discovery rate (FDR) assumes the proportion of false positives in the rejected results [332]. The Bonferroni method uses a probability threshold (α) for n probability values. Thus, if the FWE rate is .05, .05/100 000 = .0000005. However, this approach is too stringent since it assumes no correlation between spatially correlated voxels. According to the random field theory, the spatial smoothness of data is used in the calculation of Euler characteristics, which is then applied in the calculation of threshold [333]. Flandin and Friston (2019) have briefly discussed the benefits of these parametric methods [264]. This method is used in SPM for multi-subject MRI [332].

3.4.8 Feasibility study

A feasibility study conducted by Dr Christelle Langley and led by Dr Thai in CRICBristol used the same modified Bayliss et al. (2003) tasks on healthy volunteers. Their aim was to investigate the residual variance in working memory performance which is not fully explained by the components of working memory (storage and processing) alone. Their sample size was 62 healthy participants, and they completed both verbal and spatial complex span working memory tasks. They found that the residual variance is supported by domain-general neural substrates; including the prefrontal cortex and anterior cingulate cortex which are brain regions associated with executive control of attention. Therefore, their finding shows that at least part of the residual variance is executive.

3.4.9 Effect Size Calculation

Recently, scientists started to use a simple way known as effect size that quantifies the difference between any two groups and mostly known as Cohen's d [334]. Cohen's d statistic explains the difference in means as the number of standard deviations that separates those means [334]. By using effect size calculation, scientists hope to emphasise the size of the effect instead of the statistical significance of the intervention. Also, it can help in promoting a scientific method for the accumulation of knowledge [335].

Cohen's d formula is: $$d = \frac{m_A - m_B}{SD_{pooled}}$$

Where:

- mA and mB represent the mean value of group A and B, respectively.
- SDpooled is an estimator of the pooled standard deviation of the two groups. It can be calculated as follow:

$$SD_{pooled} = \sqrt{\frac{\sum (x - m_A)^2 + \sum (x - m_B)^2}{n_A + n_B - 2}}$$

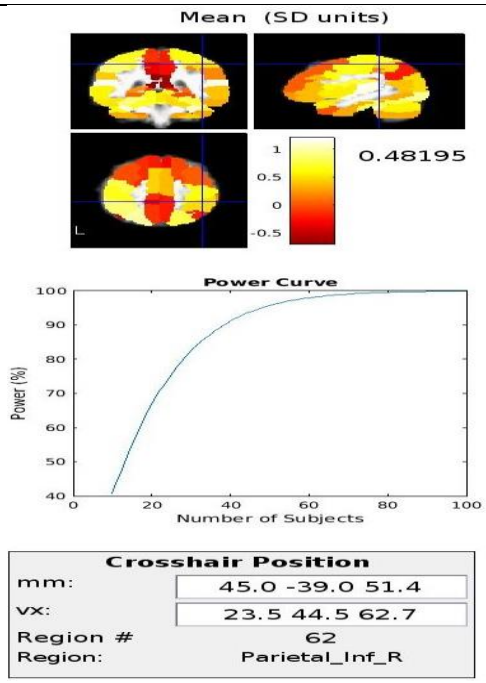
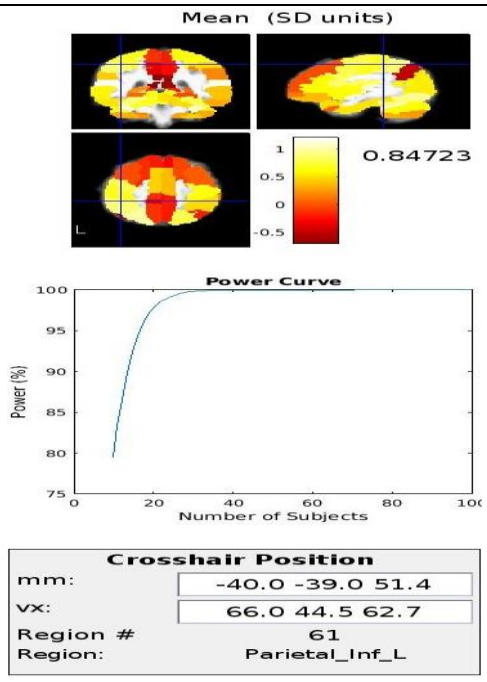
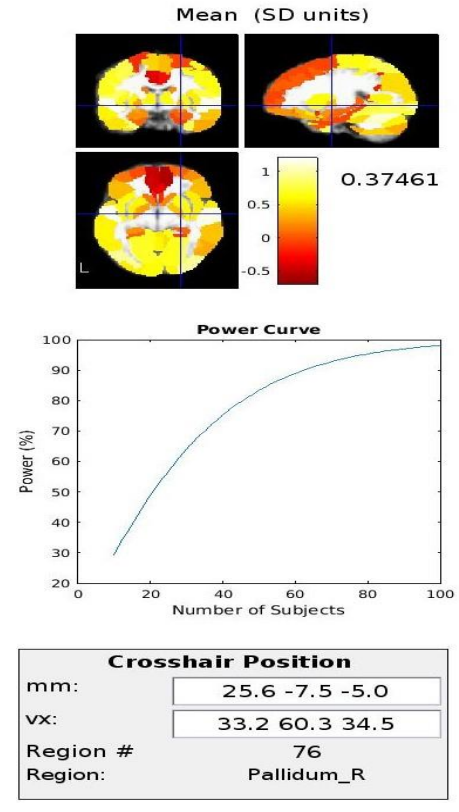
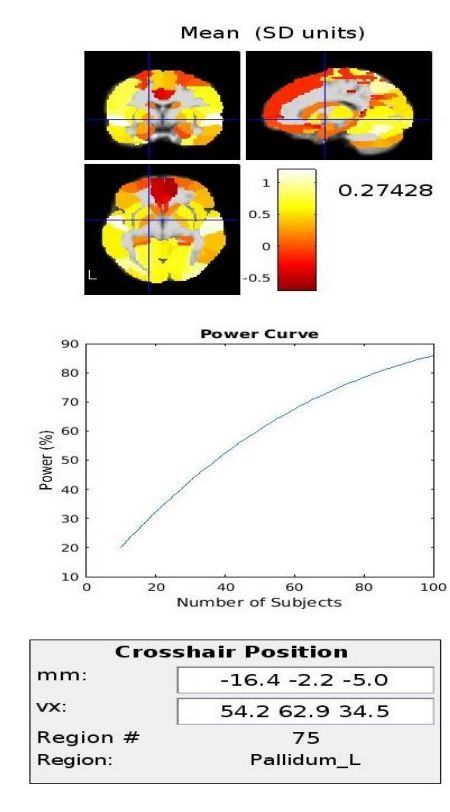
Cohen's d has a rough interpretation as 0.2 is considered as a small effect, 0.5 is considered as a moderate effect, and 0.8 is considered a large effect. This interpretation means that if Cohen's d between any two groups is 0.2 or less, the difference is small even if it was found to be statistically significant [334].

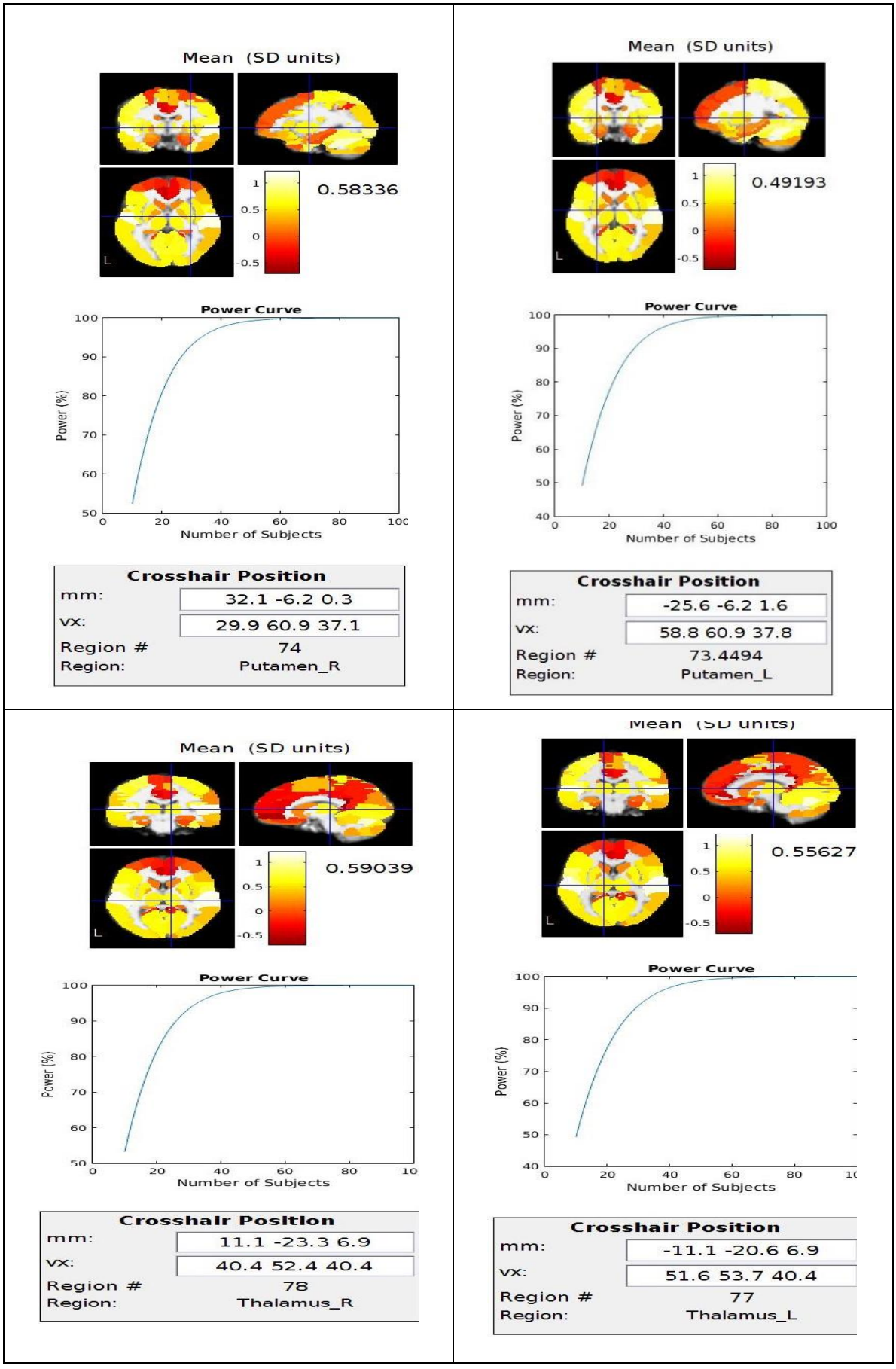
3.4.10 Sample Size Calculations

Mumford and Nichols (2008) have developed a novel method called fMRIpower (fmripower.org) to calculate the sample size needed for fMRI studies. This novel method estimates the power to detect substantial activations in specific regions of interest [336]. Using the previous feasibility study data, a power calculation was conducted using the fMRIpower software package. Because the plan was to use three different tasks, verbal processing, verbal storage, and complex verbal memory task from the previous study, an analysis of the previous data from these tasks only was conducted (for more details on the task, see section 7.3.3.1). Working memory brain regions in this analysis were taken from previous studies [49, 109, 110, 170, 337]. The maximum number of participants was set at 100, so if >100 participants were required, no power could be calculated.

The power analysis revealed that to obtain 80% of power depends on the brain region. These brain regions are known to be vital to working memory performance, including the dorsolateral prefrontal cortex, parahippocampal gyrus, anterior cingulate cortex, inferior parietal gyrus, caudate, putamen, thalamus, precuneus, and pallidum. Therefore, this thesis aimed to recruit 55 participants per group to allow for enough power to detect the regional difference in brain activation (see table 6).

Table 6: Illustrates the result of power calculations showing the sample size needed for 80% power.

Right hemisphere	Left hemisphere
	
	


Mean (SD units)
0.58336
Power Curve
Power (%)
Number of Subjects
Crosshair Position
mm: 32.1 -6.2 0.3
vx: 29.9 60.9 37.1
Region # 74
Region: Putamen_R
Mean (SD units)
0.49193
Power Curve
Power (%)
Number of Subjects
Crosshair Position
mm: -25.6 -6.2 1.6
vx: 58.8 60.9 37.8
Region # 73.4494
Region: Putamen_L
Mean (SD units)
0.59039
Power Curve
Power (%)
Number of Subjects
Crosshair Position
mm: 11.1 -23.3 6.9
vx: 40.4 52.4 40.4
Region # 78
Region: Thalamus_R
Mean (SD units)
0.55627
Power Curve
Power (%)
Number of Subjects
Crosshair Position
mm: -11.1 -20.6 6.9
vx: 51.6 53.7 40.4
Region # 77
Region: Thalamus_L

3.5 Recruitment

3.5.1 Controlling for confounding variables

Controlling for confounding variables is typically accounted for either in experimental design or statistical analysis. In research design, this is accounted for through Randomization, Restriction and Matching [338]. As the experimental design is a case-control study both Matching and Restriction was employed. We matched all patients and controls by age and gender. Because our clinical population under study are patients with CFS/ME and fatigue is the primary symptom we excluded controls with fatigue by screening them with the self-report measure of fatigue, Chalder Fatigue questionnaire, used in the clinical service from which we recruited our patient participants as part of their patient diagnosis and assessment. To investigate the relationship between fatigue and cognitive dysfunction in CFS/ME we performed statistical analysis between our working memory task performance measures, reaction time and accuracy with Chalder Fatigue questionnaire scores.

3.5.2 Participants

Ethics approval for this study was approved by the National Research Ethics Committee (NREC), Wales REC 6 committee (REC reference 17/WA/0401 IRAS project ID 236212). The samples consisted of English-speaking participants with no significant anxiety or depressive symptoms (using the HADS (appendix no 1)), scoring below 12 in each questionnaire, who were informed about the purpose of the study and asked to give informed consent before taking part in the study. All groups of participants were 18-60 years old. All participants completed a practice computer-based task prior to the MRI scan at CRICBristol.

3.5.1 Chalder Fatigue Questionnaire (CFQ)

In this thesis, the Chalder Fatigue Questionnaire (CFQ) is used to assess fatigue in both participants with CFS/ME and HC. The CFQ was created at King's College London by a research team led by Trudie Chalder in order to be used in fatiguing illnesses to measure the severity of tiredness [214]. It is considered as a measure of fatigue for adults with CFS/ME which is valid and reliable [214-217]. The CFQ is based on the individual's symptoms during the previous month. The questionnaire provides 11 questions with 5 rating options ranging from 1 (less than usual) to 5 (much more than usual). The result of this questionnaire is then reported as a sum of the 11 items on a 0±3 Likert scale, so it ranges from 0 (less severe fatigue) to 33 (more severe fatigue) [214]. CFQ has been used in numerous clinical randomized control

trials of CFS/ME patients as a primary outcome measure of behavioural interventions [339-341]. However, the CFQ has been criticized for having ceiling effects [219].

3.5.2 Length of Illness

Both the median and mean of the length of illness were calculated from the date participants with CFS/ME reported the first onset of the illness to the date they attended for MRI scans to take part in the study. This was done to illustrate that participants with CFS/ME live with this illness for a long time before they get diagnosed with CFS/ME. Therefore, the median and mean calculated length of illness in this study would roughly represent the exact date when the symptoms started. A UK study calculated the median length of illness in patients with CFS/ME who got access to the NHS service. Collins et al. (2011) reported a median length of illness of 3 years and a half in patients with CFS/ME who were employed at the time of the study [145]. Also, the median was four years among patients with CFS/ME who had ceased working [145]. In addition, Nisenbaum et al. (2000) reported that six years is the average length of illness [342]. Several studies have reported their participants' length of illness [131, 156, 159, 160, 285] and others did not [155, 343]. However, only one study reported that the number in the length of illness reflects the years the CFS/ME patient felt they had been affected by complaints of fatigue [285].

3.5.2.1 Recruitment of CFS/ME Participants

CFS/ME participants were recruited from the adult CFS/ME clinical service at the Cossham hospital in Bristol, UK using the NICE guidelines [6]. A study pack including the study information sheet, the MRI information sheet and how to contact the research team was given to eligible participants who were interested in taking part in the study at the end of their follow up appointment or assessment at the clinic. Participants were able to phone or email me for further information if required. Participants who were interested in participating were asked to return the completed contact details, the standard initial MRI screening form (Appendix no 2) and signed the study consent form (Appendix no 3a for participants with CFS/ME) in a stamped addressed envelope to me. All participants were asked to fill in several questionnaires (Appendix 4), including the Visual Analogue Pain Rating Scale to assess their pain, the SF-36 questionnaire to assess their quality-of-life, the EuroQol questionnaire (EQ-5D) to measure their health status and the Epworth Sleepiness Scale to assess their sleep pattern. Upon receipt of the consent form, a member of the CFS/ME team for the participant's existed data was contacted. If the participant met inclusion criteria after the screening, and there were no contraindications to having an MRI, then the participants were contacted to make an

appointment to participate in the study at CRICBristol. Each participant was consented when they attended their study appointment prior to the MRI scan taking place using MRI second consent form (Appendix 5).

3.5.2.2 Recruitment of Healthy Controls

Healthy controls were recruited via emails, websites and posters and a local newspaper advert. Participants who were interested in participating were checked for eligibility and sent a study pack. Participants who were interested in participating were asked to return the completed and signed study pack containing the contact details, the standard initial MRI screening form (Appendix no 2), all questionnaires and the study consent form (Appendix no 3b for healthy controls) in a stamped addressed envelope to me. If the participant met inclusion criteria after the screening, i.e., not having fatigue or MRI contraindications, participants were contacted to arrange an appointment for MRI and assessments at CRICBristol. When participants attended CRICBristol, participants were asked to sign the MRI second screening form prior to the MRI scan.

3.5.2.3 Eligibility Criteria

Participants with CFS/ME inclusion criteria:

- Participants with a diagnosis of CFS/ME made by the specialist CFS/ME service and aged 18– 60.

Healthy Control participant inclusion criteria:

- 55 Gender- and age-matched to CFS/ME group +/- 5 years

Participants' exclusion criteria:

- Participants with CFS/ME who are severely affected (mostly housebound and requiring help with activities of daily living)
- Participants with CFS/ME aged <18 or >60
- Participants who have another diagnosis as a cause of their fatigue
- Participants with a score greater than 12 on either the anxiety or depression component of the Hospital Anxiety and Depression scale (HADS)
- Participants who have excessive upper limb tremor
- Participants with eyesight inadequate for seeing the test display
- Participants showing contraindication for MRI assessed by MR screening forms
- Participants with contracture that interfere with comfortable positioning in the scanner

- Healthy control participants who have fatigue as assessed by the Chalder Fatigue questionnaire were excluded.
- Healthy control participants with a history of neurological condition or disease.
- Participants who may have difficulties in understanding English.

3.6 Image Acquisition

A 3 Tesla Siemens Skyra scanner with a 32 channel radiofrequency head coil was utilised to perform the MRI scans. Three 15 minutes long fMRI scans, one for each condition of the designed paradigm, using T2*-weighted multiband gradient echo-planar imaging were acquired. All fMRI sequences covered the whole brain using 39 axial slices orientated parallel to the anterior-posterior commissure plane with parameters: time to repetition (TR): 906 ms; time to echo (TE): 30 ms; the field of view (FoV): 192 mm; voxel size: 3x3x3 mm and a multiband acceleration factor of 3. No in-plane acceleration or slice-GRAPPA technique was used. Reducing slice aliasing was performed using the blipped-CAIPI method. Next, participants were asked to stay awake and fix their eyes on a fixed stationary cross for a 14 minutes rs-fMRI scan. The rs-fMRI multiband echo-planar imaging (EPI) pulse consists of 600 volumes (39 interleaved slices, multiband factor =3, TR = 906ms, TE = 30ms, flip angle = 60°, acquisition matrix = 195 × 100, voxel size 2mm × 2mm × 2mm) with 0 gap and scanned in a default interleaved sequence. Structural images were acquired in the sagittal plane using a T1-weighted inversion recovery magnetisation prepared rapid acquisition gradient-echo (MPRAGE). This sequence consists of 192 slices; TR: 1800 ms; TE: 2.25 ms; .9mm isotropic voxel; and FoV of 240 mm. Structural images were used to co-registration with functional scans as well as segmented to grey matter, white matter and cerebral-spinal fluid to employ VBM and to be used in brain connectivity analysis.

3.7 Procedure

Participants are asked to visit CRIC to participate in this study. On their arrival, a research member greeted the participant and explained the procedure of the whole experiment. Initial screening form (appendix no 2) was checked again to ensure no presence of any contraindication to MRI. Participants were guided to one of the clinical rooms at CRIC to complete a practice version of the main three neuropsychological tests presented in figures 10, 11 and 12 (for more details on the task, see section 2.7 below). Tests were explained and practised to make sure that participants understood what they needed to do and how to do it.

The whole memory paradigm was explained and discussed, ensuring a full understanding of the requirements of the study and tasks. Participants then were taken to the MRI changing room and asked to remove any metallic or electronic devices. Participants were asked to complete and sign the CRICBristol standard second MR screening form. Participants then were taken to the scanner room, where they were asked to lie on the MRI table and placing their head in the 32-channels coil. An alarm button was handed to each participant with an explanation on how to use it in case they needed the operator during the scan. Each participant was provided with a small headset to reduce the noise and to hear the instructions. The participant was given an MR safe response button box to make their responses during the cognitive task. The MRI operator closed the coil and attached a mirror so the participant can see the screen and receive the instructions during the functional tasks. Before they left, a research member or the MRI operator ensured that the participant was comfortable, and all questions were answered before moving the participant into the bore of the magnet. Before scanning, the MR operator (research team member) ensured that they and the participants could communicate through the intercom system. Also, the MRI operator made sure to check that the button box is working before starting the scan. The participants then performed three functional MRI scans during which they were asked to keep as still as possible and were presented with instructions on the screen. They were presented with the cognitive tasks and asked to make their responses using the button box provided. The full study MRI protocol includes structural, resting-state fMRI, 3 task-based fMRI sequences. The sample size of each chapter might differ as not all participants managed to complete all scans or tasks. Furthermore, some scan data would have to be excluded after pre-processing of the data due to quality issues e.g., excessive motion artefacts.

3.7.1.1 Processing Task

The processing task consisted of four coloured squares in each corner of the screen with a digit in the centre of each square shown to the study participants (see figure 10). A single word will be presented through the headphones, and participants need to think about the colour that is associated with that word then respond by pressing the button to select the colour square that represents that colour. For instance, if the word was "Banana" the accurate response was pressing the button that represents the yellow square. If the participant delayed for more than 4 seconds, a timed-out response was recorded, and the next trial would be presented.

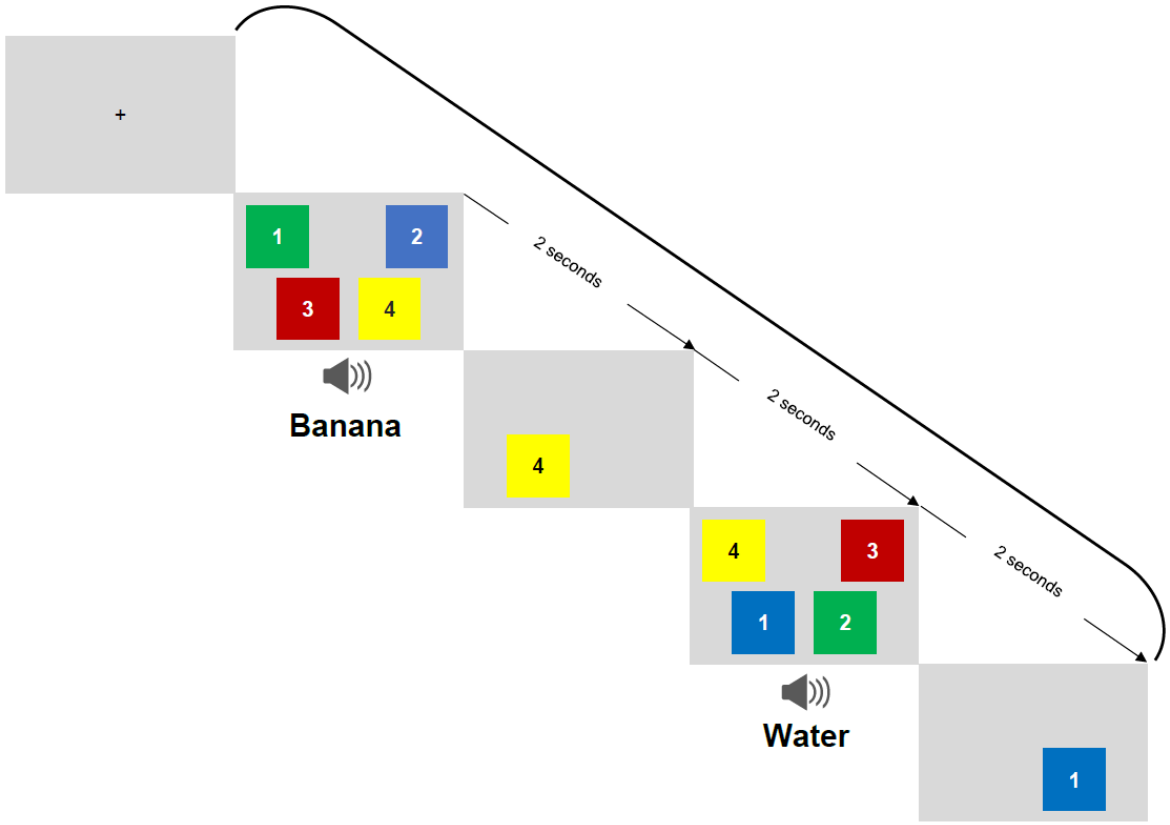


Figure 10 shows schematic of the processing task [26].

3.7.1.2 Verbal Storage Task

In this case, each square displayed had a figure number 1, 2, 3 or 4 (see figure 11). Only one square per presentation was displayed to make a sequence of digits with a time interval of 2 seconds. Participants were instructed to remember the sequence of the digits presented. In the second sequence, the participants were presented with a sequence of digits and asked to identify if the sequence was the same as the previous presentation. A total of twenty trials were presented. Each presentation took 2 seconds, and each recognition took 1 second. The participants were given 3 seconds to respond, resulting in a total time duration of 12-15 seconds, depending on the reaction time of the participant.

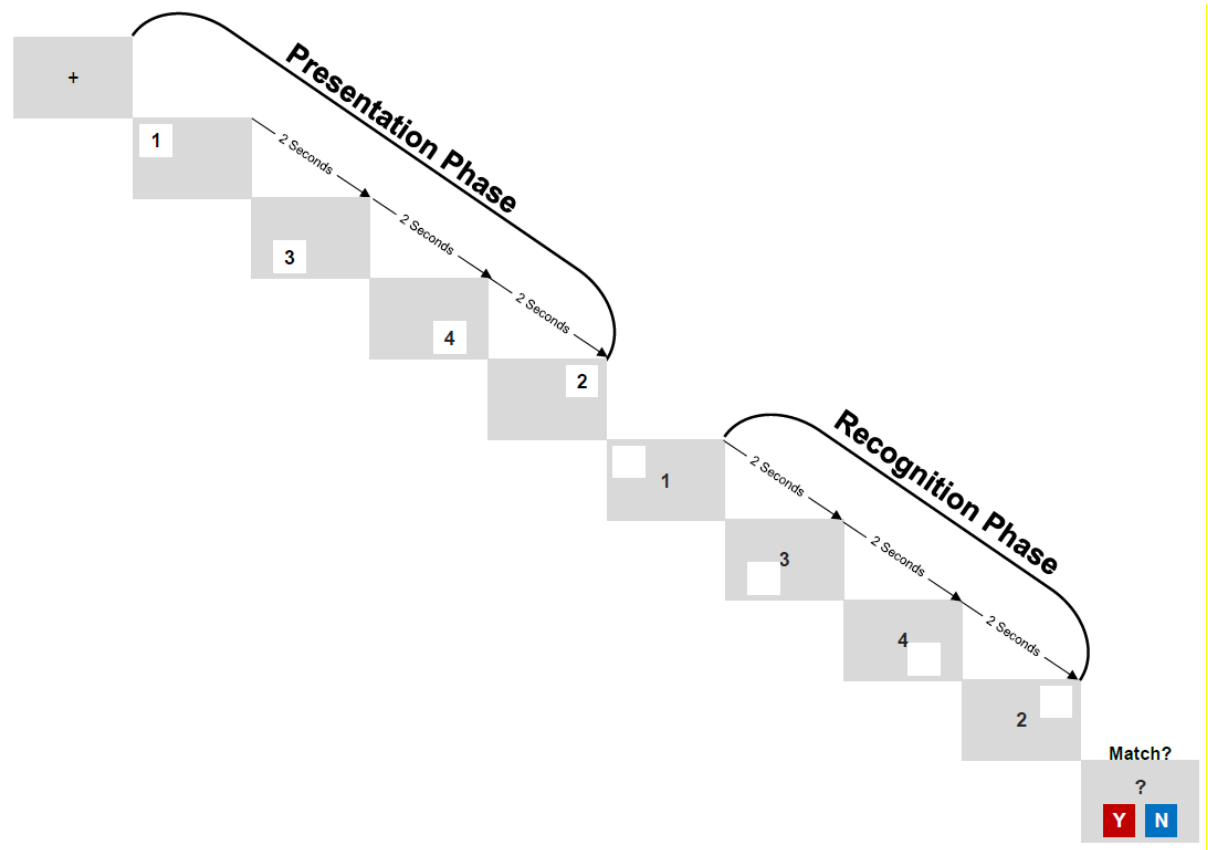


Figure 11 shows a schematic of the verbal storage task [28].

3.7.1.3 Complex Task

The complex task involves combining the verbal processing and the verbal storage task (see figure 12). It involves showing the participants four coloured squares located at the corners of the screen; however, in this case, the squares displayed had a digit assigned to each of them. The participants were asked to respond to the instructions presented via the headphones by pressing the button on the response box that corresponded to the coloured square, which represents the word mentioned in the headphones. Also, they were tasked to remember the sequence of numbers while appearing on the screen. The selection of the participant was then followed by displaying the correct target square even if their selection was wrong. As a result, the participants were still able to correctly respond to the recognition phase. The next sequence was done in the same manner as the previous one, and the participants were asked to recognise if it was the same as the previous one in terms of the sequence of digits displayed. There was a total of twenty trials presented. Individual trials took 20-23 seconds, and the presentation took a maximum of 4 seconds, which depended on the time the participant took to respond to the tasks. The recognition was timed for 1 second, and the participants were allowed 3 seconds to respond.

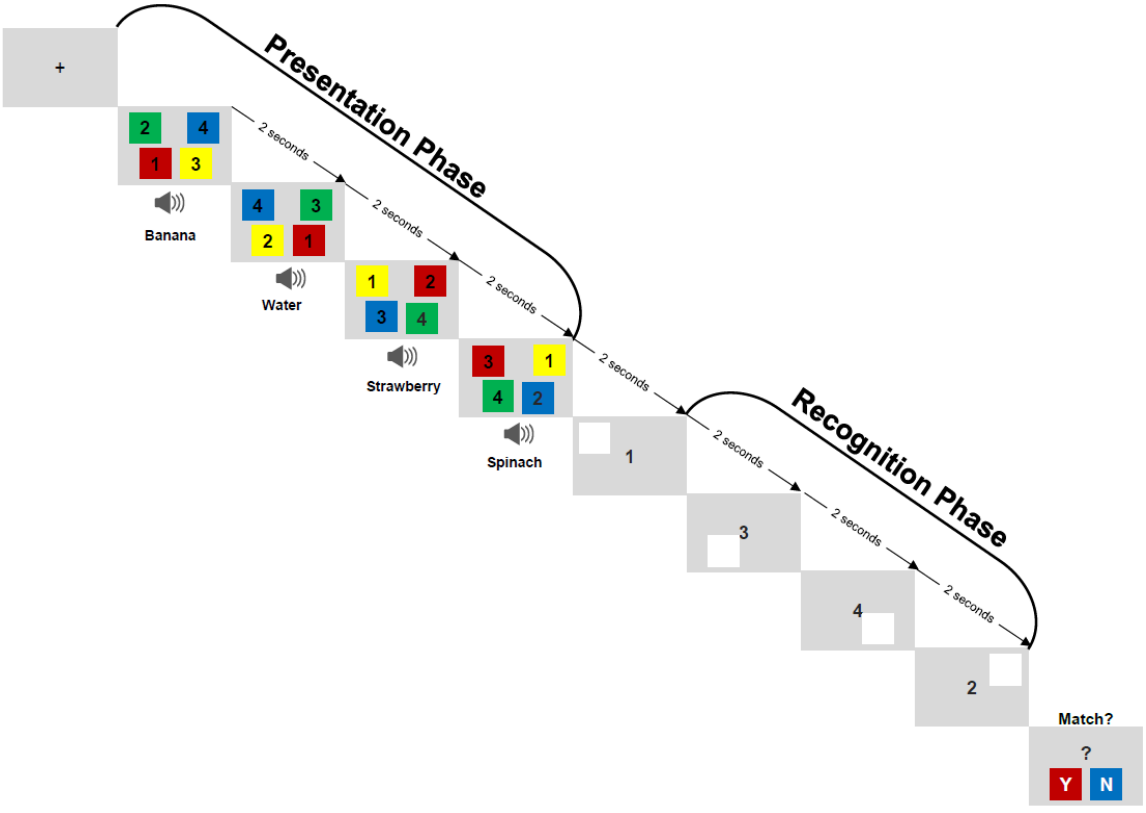


Figure 12 show a schematic of the complex verbal storage task [1].

4 Chapter 4: Investigating Brain Volume with Subjective Fatigue in CFS/ME: A Structural MRI study.

4.1 Overview of Chapter

Chapter 4 investigates the brain morphometry of CFS/ME using structural MRI. This includes the methods and the interpretation of the results, as well as a discussion of the results.

4.2 Introduction

Chronic Fatigue Syndrome (CFS), also known as Myalgic Encephalomyelitis /Myalgic Encephalomylopathy (ME), is an illness of unknown aetiology and pathophysiology which is characterised by continuous and persistent fatigue which lasts for at least 4 or 6 months [1, 5-7]. It is accompanied by a variety of symptoms such as post-exertion malaise which lasts for more than 24 hours, significant short-term memory impairment, un-refreshing sleep, headache, muscle pain, tender lymph nodes and frequent or recurrent sore throat [1, 5-7]. For decades, structural magnetic resonance imaging (MRI) has played a crucial role in aiding clinical diagnoses in many disorders such as brain tumours, multiple sclerosis, and stroke [344-346]. It has been used recently in research to investigate CFS/ME illness using different study designs and analysis methods. These methods include the use of visual inspection or automated analysis and longitudinal studies [141, 149-153].

Several studies conducted their image analysis relying on a visual inspection and accounted for age by using age-matched healthy Controls [141, 149-153]. Raters evaluated the images looking for abnormalities such as high signal intensity lesion which appears on T2 or proton density-weighted images and found ventricular enlargement [149, 153], white matter hyper-intensities or white matter abnormalities [149, 151, 152]. Abnormalities were found in 41% of the MRI scans in one study [152], and changes or lesions were reported in the two other studies [149, 151]. Changes were reported in white matter regions such as supratentorial periventricular [150], periventricular, subcortical and in the centrum semiovale [151]. However, a longitudinal study using visual inspection was conducted and found no significant differences at the baseline or after a year [141].

The use of the automated analysis technique shed light on the brain global and regional differences between the patients' group and healthy controls [347]. This technique is relatively easy to implement, time-efficient and can detect small differences with no prior decision on which structure to evaluate [348]. Voxel-based morphometry shows evidence of similar

accuracy to manual volumetry [349, 350]. Therefore, automated analysis is the most used method in other illnesses such as Alzheimer's disease, Multiple Sclerosis and Dementia to evaluate brain atrophy [348, 351, 352]. Analysing images using automated analysis methods in CFS/ME provided inconsistent findings such as differences in both grey matter volume [132-136], white matter volume reduction [136-140] or no differences at all between CFS/ME and healthy controls [141-143]. Studies reported global grey matter reduction [132, 133, 135] as well as regional differences in the occipital lobes, right angular gyrus, left parahippocampal gyrus and in the bilateral prefrontal cortex [134, 136]. This reduction was linked to functional deficits that might be influenced by pain [165, 166], illness or age factors [134, 136], thus having an impact on the quality of life for participants with CFS/ME [134, 136]. In contrast, Finkelmeyer et al. (2018) showed that CFS/ME had a significant increase in grey matter volume and decrease in white matter volume compared to healthy controls [138]. The increase in the grey matter was found in the insula and amygdala, while the white matter reduction was found in the midbrain, pons and right temporal lobe [138]. Other studies found a reduction in white matter volume in CFS/ME compared to healthy controls in the left inferior front-occipital fasciculus [137, 139] left occipital lobe as well as the posterior part of the left parahippocampal gyrus [136].

Longitudinal studies of this illness have used different time points and study settings. De Lange et al. (2008) investigated the effect of cognitive behaviour therapy (CBT) on brain volume and found an increase in grey matter volume after 6-9 months of CBT. They were able to link the increase in the grey matter to the improvement in health status, processing speed and physical activity [133]. By contrast, Shan et al. (2016) conducted a longitudinal study with six years of follow up and showed a significant reduction in white matter volume in the left inferior fronto-occipital fasciculus in the CFS/ME group [137]. Another longitudinal study, with one-year follow-up, reported no differences between CFS/ME and healthy control groups at baseline and after a year in cerebrospinal fluid, white matter hyper-intensities, ventricular volume and failed to observe any abnormalities in the CFS/ME group [141].

Fatigue has been associated with differences in brain volume in other illnesses such as multiple sclerosis. A positron emission tomography study that investigated MS patients with and without fatigue showed differences in the white and grey matter [353]. In CFS/ME, the decrease in white matter was found to be correlated with increasing fatigue duration [288]. Also, the loss of white matter volume has a rate of 1% per year [288]. Although that was evident, they were not able to exclude that this loss is not as a result of mental and physical inactivity [288]. Interestingly, they found no correlation between fatigue severity and white

matter loss, which would suggest that the reason for white matter loss is CFS/ME, not physical or mental inactivity [288]. In some studies, authors were able to demonstrate that increased illness duration has a negative correlation with the white matter volume in CFS/ME [135, 147]. Previous structural MRI studies on CFS/ME used a relatively small sample size. Most of these studies used a sample size of fewer than 50 participants (17/19). The use of small sample size in a heterogeneous illness such as CFS/ME might lead to different results due to the different phenotypes [176]. Therefore, this study aimed to investigate the brain volumes using structural MRI as well as investigating the correlation between brain volume and fatigue in CFS/ME in a larger sample size. The Chalder Fatigue Questionnaire (CFQ) was used as it is the most consistently applied questionnaire in CFS/ME research [354] and a reliable measure of fatigue severity in CFS/ME [215-217]. The aim was to implement automated analysis to investigate the differences between global tissue volumes as well as regional brain volumes in this illness. It is to ensure there was sufficient power by recruiting a considerably large sample size (>50). Due to the inconsistency in previous findings and the urge to perform more research, this study aims to add some value to previous studies. This study d to find global tissue volumes differences as seen in previous literature either in grey matter volume [132-136] or white matter volume [136-140]. Also, this study hypothesised to find regional brain volume differences in the multiple brain regions that were linked to fatigue, such as the basal ganglia, occipital lobes and bilateral prefrontal cortex [134, 136, 138]. These differences are hypothesised to correlate with the CFQ scores.

4.3 Method and Procedures

4.3.1 Participants

The original CFS/ME sample size was 69 participants. However, 16 declined attending the MRI scan due to fatigue and not feeling well. Therefore, fifty-three CFS/ME participants (43 females and ten males) met the NICE criteria for CFS/ME [6], and 45 healthy controls (37 females and eight males) were recruited for this study. Participants were well matched on age and sex with the control group. The CFS/ME group had a mean age of 37.94 years, SD= 11.20 and the healthy controls group had a mean age of 33.6, SD= 12.21 (see table 7). All participants gave informed consent before taking part (Appendix no 3a for CFS/ME and 3b for healthy controls). Ethics approval for this study was approved by the National Research Ethics Committee (NREC), Wales REC 6 committee (REC reference 17/WA/0401 IRAS project ID 236212). CFS/ME participants were recruited from the CFS/ME clinic at the Cossham hospital in Bristol, UK. All participants with neurological disorders from both groups were excluded.

The Hospital Anxiety and Depression Scale (HADS) was used to exclude those with anxiety or depression [355]. Participants who scored more than 12 in each scale were excluded. Also, participants filled in other questionnaires such as the visual analogue pain rating scale (for pain measures), the SF-36 (for activity measures), the EQ-5D (a measure of health outcome), and the Epworth sleepiness scale (to measure the average sleep propensity in daily life) (see table 7).

Table 7 shows the mean and standard deviation of participants demographic, the groups' age, sex, anxiety, depression, pain, physical activity, health outcome, sleep patterns scores and length of illness,

	HC	CFS/ME	t	df	P
CFQ	10.59 (3.92)	28.19 (19.37)	1.99	97	.000
Age	33.6 (12.34)	37.94 (11.30)	-1.81	96	.072
Gender	37 females, 8 males	43 females, 10 males			
Anxiety	3.31 (3.19)	8.25 (3.18)	7.39	97	.000
Depression	1.04 (2.39)	7.27 (3.31)	12.73	93	.000
Pain	0.51 (1.897)	51.06 (26.86)	-7.43	53	.000
SF36	97.55 (8.14)	49.62 (23.90)	-14.74	62	.000
EQ-5D	0.98 (0.049)	0.66 (0.076)	-13.70	61	.000
Epworth	5.11 (3.57)	11.11 (4.61)	5.89	97	.000
Length of illness (years)	0	Mean=6.04 (5.57) Median= 3.2			

4.3.2 Self-report Measures

Fatigue:

The Chalder Fatigue Questionnaire is considered a valid measure for adults with CFS/ME [214]. This questionnaire is based on the individual's symptoms during the previous month. The questionnaire provides 11 questions with 5 rating options ranging from 1 (less than usual) to 5 (much more than usual). The result of this questionnaire is then reported as a sum of the 11 items on a 0±3 Likert scale, so it ranges from 0 (less severe fatigue) to 33 (more severe fatigue).

4.3.3 Image Acquisition and VBM Analysis

Siemens Skyra 3T MRI scanner with a 32 channel radiofrequency head coil was used to acquire the MRI data for all participants. Structural images were acquired in the sagittal plane using a T1-weighted inversion recovery magnetisation prepared rapid acquisition gradient-echo (MPRAGE). This sequence consists of 192 slices; TR: 1800 ms; TE: 2.25 ms; .9mm isotropic voxel; and FoV of 240 mm. MATLAB (Mathworks Inc., Natick, MA, USA) and statistical parametric mapping package SPM12 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm) was used to conduct all pre-processing and analysis of the data. First, structural images were displayed and inspected visually and, if required,

repositioned to correspond with templates. Computational Anatomy Toolbox (CAT12) was used to segment and normalise the images into grey matter, white matter, and cerebrospinal fluid (CSF) with default settings. Then, the 'display one slice for all images' function was used to check the data quality. The sample homogeneity function was used to identify outliers. No images were discarded as all images had high correlation values. Images were then smoothed using an 8mm smoothing Gaussian kernel to increase the signal-to-noise ratio. The values of the segmented brain volumes such as the white and grey matters, CSF as well as the total intracranial volumes (TIV), were obtained from the segmentation report. SPSS was used to conduct a two-sample t-test to compare global tissue volumes between CFS/ME and healthy controls. Pearson's correlation was performed between TIV and CFQ. CAT12 was used to perform a two-sample t-test and multiple regression with a significance threshold of $p<.05$ to compare global tissue volumes as well as any regional brain volumes differences between the two groups. If specific coordinates were found, they were converted using GingerALE [356] from MNI space to Talairach space. After that, Talairach software [357] was used to convert these coordinates to brain regions for the comparison between CFS/ME and healthy controls. This is performed on a voxel-by-voxel basis to compare regional grey and white matter differences between the groups. To adjust for the effect of age and brain size in the analysis, age and TIV were included as covariates of no interest. Talairach has been criticised for being done on a single brain and having variations of slice thickness with low resolution [358].

4.4Results

4.4.1 Global Tissue Volume and Correlation

Figure 13 shows tissues volumes in both groups (see figure 13). The two-sample t-test showed no significant differences in global brain tissues between the groups in TIV, GM, WM, or CSF. (see Table 8).

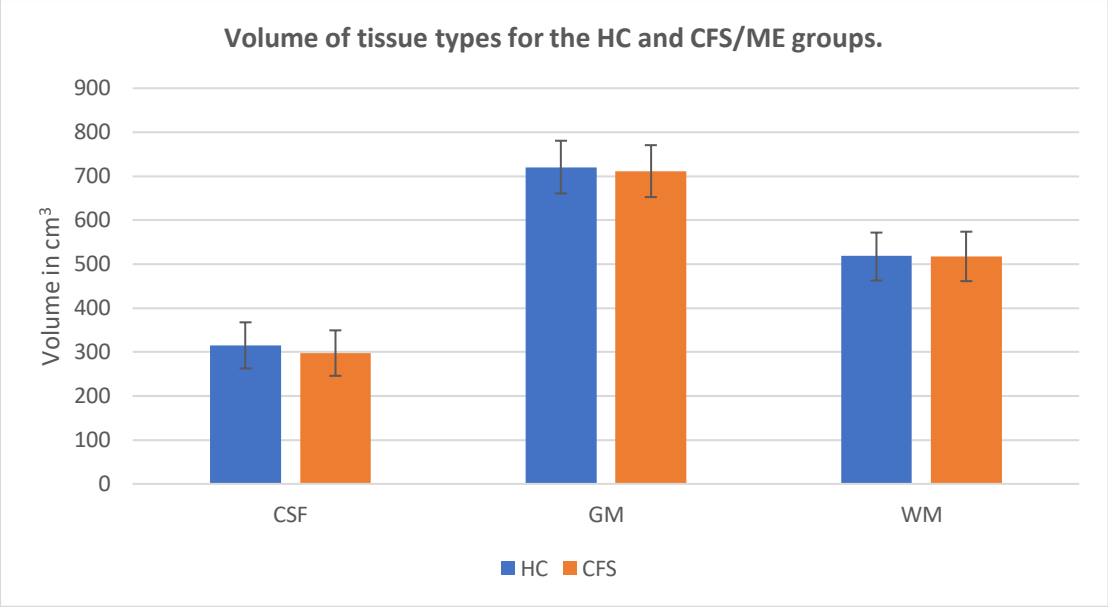


Figure 13 reveal the volume of tissue types for the HC and CFS/ME groups where the error bars represent one standard deviation from the mean.

Table 8 shows the mean and standard deviation of an independent two-sample uncorrected for multiple comparisons t-test for all brain volumes.

	HC	CFS/ME	t	df	p	Cohen's d
CSF	319.38 (56.46)	299.82 (48.27)	1.822	94	.072	.37
GM	718.35 (62.82)	713.21 (62.73)	.395	94	.694	.08
WM	521.10 (58.14)	515.61 (55.53)	.468	94	.641	0.09
TIV	1559.83 (137.10)	1531.77 (124.13)	1.045	94	.299	0.21

In the healthy controls group, a positive correlation was found between CFQ and the CSF (r= .297, *p*= .05) but not with grey matter (r= -.082, *p*= .593); white matter (r= .063, *p*=.682). No correlation was found between the CFQ in CFS/ME group for grey matter (r= -.194, *p*= .164); white matter (r= -.147, *p*= .294, CSF r= .032, *p*= .819). (see table 9).

Table 9 shows the uncorrected for multiple comparisons correlation between CFQ and all brain volumes in CFS/ME and healthy controls.

CFQ Correlations with		**HC**			**CFS/ME**		
		CSF	**GM**	**WM**	**CSF**	**GM**	**WM**
CFQ	**Pearson Correlation**	.297*	-.082	.063	.032	-.194	-.147
	Sig. (2-tailed)	.048	.593	.682	.819	.164	.294
	N	45	45	45	53	53	53
*. Correlation is significant at the 0.05 level (2-tailed).							

4.4.2 Regional Brain Volume and Correlation

A corrected two-sample t-test was conducted to examine regional brain differences between the groups and found no regional differences. Also, a two-sample t-test was conducted as TIV and age were used as covariates and showed no regional differences between the groups at corrected *p value of* <.05.

4.5Discussion

The aim of the present study was to implement an automated analysis method on structural MRI images to investigate brain volume differences between CFS/ME and healthy controls. Fatigue was measured and correlated to brain volumes. The main finding was that no global or regional differences were found when CFS/ME were compared to healthy controls. This same result was shown by a longitudinal study, which did not detect any differences between the groups at baseline and after a year [141] as well as two other studies [142, 143]. However, another longitudinal study found differences between the group in white matter after six years [282] which may give some evidence that CFS/ME is a slow progressing illness. Also, there was a positive correlation between CFQ and the CSF in HC only not the CFS/ME. Although there were no significant differences between the groups in the CSF global volume, and this correlation seems a relatively small correlation, in terms of effect size, it might be worth investigating this in future research.

Due to relying on self-reported symptoms and questionnaires, the validity of CFS/ME as an aetiologically homogenous diagnosis has been doubted [186, 187]. Therefore, many researchers aimed to examine the potential heterogeneity of CFS/ME as well as defining subgroups [188]. Hickie et al. (1995) evaluated the symptoms and demographics to characterise a core group and a smaller polysymptomatic subgroup. They found prolonged fatigue, musculoskeletal pain, impaired neurocognitive function, sleep disturbance, and symptoms suggestive of inflammation to be the five main domains for CFS/ME [176]. In a more recent study, Williams et al. (2017) utilised latent class analysis to describe subgroups in a large sample consisting of 541 CFS/ME patients and found five subgroups [188]. They grouped the patients in term of associated functional somatic syndromes, cognitive behavioural responses questionnaire scores, self-efficacy ratings, mood, and assessments of physical activity and sleep [188]. These differences in CFS/ME severity and disability show that subgrouping has some discriminative validation [188]. Also, it shows that the patient population in previous studies might not have similar disease phenotypes which could have eventually led to the inconsistencies in findings. Therefore, sub-phenotyping or sub-grouping, according to the CFS/ME symptoms, might be a solution to consistent results and a better understanding of this illness. In this current study, failure to find global or regional differences and correlations with fatigue scores suggest that it might be due to not sub-grouping the patients according to a specific phenotype. To date, too little attention has been paid to subgrouping in the MRI studies probably due to the difficulty in recruiting patients with such a complex illness. This was seen

in the current study, whilst despite recruiting the sample over a period of two and a half years, was still unable to recruit sufficient patients in each sub-phenotype (according to the Hickie et al (1995)) to perform such subgroup analyses [176].

Length of illness, physical inactivity and sleep disturbance in this patient group are confounders and might contribute to the brain volume decrease over time [207, 286, 359, 360]. CFS/ME symptoms and disability have been linked to the disease duration, suggesting that early stages might be different from later stages [361]. It has been reported that CFS/ME patients with long-duration (median =18 years) have significantly higher specific cognitive difficulties compared to participants with short-duration CFS/ME (median = 3 years). Also, they reported that the cognitive difficulties in this long-duration patient group were greater in severity [362]. Also, Kidd et al. (2016) investigated the illness duration effects in this illness and reported that younger patients with less illness duration (less than ten years) had greater vitality compared to the other group with same-age but longer illness duration [361]. Patients with a disease duration of more than two years show more fatigue, more significant concentration problems and more functional disability than those with shorter illness duration [362, 363]. In terms of MRI studies, a negative correlation was found between the CFS/ME length of illness and the activity of the left putamen [131]. Also, the increase in the length of illness led to a decrease in white and grey matter volumes in this patient group [135, 147, 285, 286, 288]. Physical inactivity is considered as another confounder in this illness. The sedentary lifestyle of CFS/ME patients might be responsible for brain volume loss. Physical activity and exercise improve brain volume in brain regions that are responsible for many cognitive functions such as attention, learning, and memory [359]. It has been suggested that physical activity in early life can preserve cognition in later life [359, 360]. Increasing physical activity through cognitive behavioural therapy improves brain volume in CFS/ME as seen in Lange et al. (2008) longitudinal study when they used cognitive behavioural therapy as a treatment method [286]. . People who achieve 10 thousand steps per day have been shown to have higher brain volume in comparison to those who achieve 5 thousand or less per day [364]. In the same study, it has been shown that every additional hour of physical activity was associated with higher brain volume. Also, grey matter volume has been positively correlated with physical activity [207]. Sleep disturbance has been investigated in other diseases and ageing. Sleep deprivation affects brain white matter by causing white matter microstructure changes [365]. Also, an association between brain abnormality and sleep disturbance has been reported [365-367]. In an MRI study investigating the relationship between brain volume and Pittsburgh Sleep Quality Index (PSQI) in CFS/ME, they found a negative correlation between PSQI and magnetisation transfer T1

weighted intensities in the medial prefrontal cortex. Also, in the same brain region, they found a negative correlation between PSQI and T1 weighted spin-echo signal intensities [139].
Therefore, the current findings suggest that CFS/ME might be an illness where the effect on brain volume might be due to reducing physical activity. Furthermore, this suggests that brain volume is not affected in the early stages and needs a long duration with severe symptoms that reduces physical activity to have an impact on brain volume. Importantly, if disease duration has a great impact on the brain volume, it further highlights the critical need for early detection in CFS/ME. Early detection aids the treatment programmes that can effectively counteract the detrimental effects of loss of physical activity and sleep disturbances in CFS/ME. These together may potentially be significant contributing factors to results of reduced or atrophied grey and white matter tissue in CFS/ME in previous studies.

4.6 Conclusion

The main finding in this study is that no global or regional structural differences were found between CFS/ME and healthy controls. Also, there was a significant positive correlation between CFQ and CSF in the healthy controls group only. Although there were no structural volume differences, the following chapters aimed to use rs-fMRI and task-based fMRI to investigate brain function and compare CFS/ME with HC.

5 Chapter 5: Investigating Functional Connectivity of Basal Ganglia in CFS/ME: an rs-fMRI Study.

5.1 Overview of Chapter

Chapter 5 was designed to investigate the hypothesis proposed by Chaudhuri & Behan (2000) [125] suggests that central/mental fatigue could potentially be the failure of integration between limbic input the basal ganglia which then impacts the striatal–thalamic–frontal cortical system. Chapter 5 investigates the regional and global connectivity of the Basal ganglia of CFS/ME using resting-state fMRI. This includes the methods and the interpretation of the results as well as a discussion of the results.

5.2 Introduction

Chronic Fatigue Syndrome/Myalgic encephalomyelitis (CFS/ME) is an illness that has many definition criteria in which all include the presence of one primary symptom (unexplained persistent fatigue). Depending on the definition criteria used, fatigue must be accompanied by secondary symptoms such as significant short-term memory impairment, un-refreshing sleep, headache, muscle pain, tender lymph nodes and frequent or recurrent sore throat [1]. Because fatigue is the primary symptom in CFS/ME illness, many neuroimaging researchers have conducted extensive research to understand the mechanism behind it. The results of older studies guided the more recent ones aiming to find biomarkers for such a complex illness [155, 168, 169, 368]. One of the neuroimaging applications is Functional Magnetic Resonance Imaging (fMRI). Scientists have used two fMRI approaches to investigate brain functional connectivity (FC), task-based and resting-state fMRI [255].

Basal ganglia have been reported to be involved in fatigue in CFS/ME [131] and other illnesses such as Multiple Sclerosis (MS) [129, 369, 370]. Basal ganglia comprise the caudate, putamen, pallidum, ventral striatum, substantia nigra, the nucleus accumbens and subthalamic nucleus (see figure 14). Each of the basal ganglia components has a complicated anatomical and functional organisation [113]. The striatum, which includes caudate, putamen and nucleus accumbens, is the largest component and known to receive information from various brain regions such as the prefrontal cortex and the orbital prefrontal cortex and transfer it to other BG components. Pallidum in its turn receives this information from caudate then sends inhibitory output to many motor-related areas. The substantia nigra performs an essential role

in BG function as it the source of the striatal input of the neurotransmitter dopamine. The subthalamic nucleus receives the input from not only the striatum but also cerebral cortex. The subthalamic nucleus then projects this input to the globus pallidus [113].

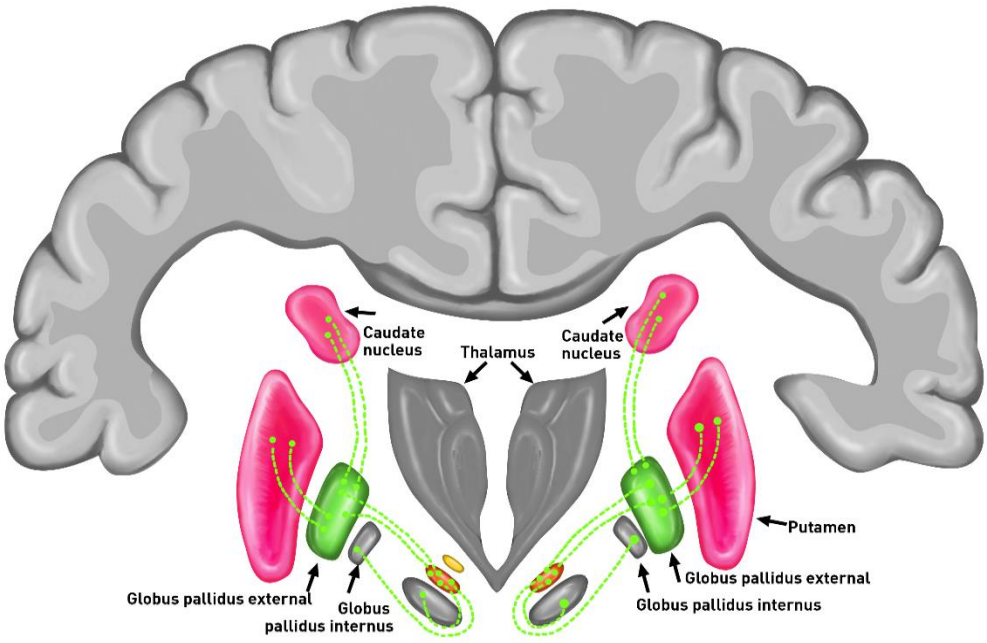


Figure 14 illustrates the connections within the BG regions. Created by Adobe software.

Studies using task-based fMRI to investigate CFS/ME showed the involvement of basal ganglia (BG) regions in fatigue while using different study designs and tasks [129, 131]. Also, these results were correlated to fatigue scores [129, 131]. Miller et al. (2014) employed a reward-related gambling task to investigate brain activation in BG and compare it to the Multidimensional Fatigue Inventory (MFI) scores. They found a significant reduction in activation in the right caudate and right globus pallidus in patients with CFS/ME compared to healthy controls. The reduction in these regions was found to be highly correlated with the fatigue scores [129]. Mizuno et al. (2016) investigated the high- and low-monetary-reward conditions in patients with CFS/ME compared to healthy controls. They found that adolescents with CFS/ME had a decrease in activation in the putamen in the low-monetary-reward condition compared to healthy controls. Also, they found a negative correlation between the putamen activation in the low-reward condition with the fatigue severity, meaning that the greater the fatigue, the lower the putamen activation. They linked altered dopaminergic

function to the decrease in putamen activity and suggested that this is the reason for lower reward sensitivity and motivation to learn [131].

Studies showed that BG is associated with fatigue, and disruption in the BG affects the connection between the prefrontal cortex and thalamus [125, 127]. Also, basal ganglia can affect cortically-driven voluntary activities by disrupting the limbic integration [371]. Parkinson's disease studies that involved treatment with bilateral pallidotomy showed that it is followed by profound fatigue as one of the main side effects [372]. Although BG dysfunction may not be the only reason for central fatigue, it is crucial to understand whether BG dysfunction is an essential pathogenic mechanism. Therefore, Chaudhuri and Behan (2000) proposed to use neuroimaging techniques to conduct more research to investigate the relationship between BG and fatigue [125].

Previous studies in other illnesses such as MS, where fatigue is one of the main symptoms, have shown an association between BG and fatigue [369, 370]. These studies acknowledge the involvement of BG in cognitive fatigue despite showing opposing effects and using different MRI applications. De Luca et al. (2008) used a task-based fMRI technique, and Finke et al. (2015) used rs-fMRI [369, 370]. De Luca et al. (2008) proposed that in healthy controls, brain activity reduces with continuous practice, what is known as the learning curve, due to the practice effect [373], a switch from controlled to automatic processing [374], priming [375] or habituation [376]. De Luca et al. (2008) were able to associate the fatigue in MS patients with increase activation in the basal ganglia, frontal areas including superior, medial, middle and inferior regions, parietal regions (precuneus and cuneus), thalamus and the occipital lobes. Therefore, they reported widespread increases in brain activation in MS patients, which may mean that when the effort increases, it may induce cognitive fatigue. They also suggested that the increased brain activation might be due to neural compensatory mechanism rather than cognitive fatigue itself [369]. Unfortunately, they were not able to distinguish whether it was a result of cognitive fatigue or a compensatory mechanism due to the lack of subjective fatigue measures [369].

On the other hand, Finke et al. (2015) utilised rs-fMRI and used self-report Fatigue Severity Scale (FSS) to investigate BG in MS. They found that BG showed decreased functional connectivity with other brain regions such as the medial prefrontal cortex, precuneus and posterior cingulate cortex with increase FSS scores. This negative correlation was assumed to contribute to fatigue pathophysiology in MS [370]. The reports from these studies, the task-based and rs-fMRI ones are contradicting but might be explained by the different methods that

they used. De Luca et al. (2008) was a task-based fMRI study and used performance to measure cognitive fatigue [369], while Finke et al. (2015) used rs-fMRI and FSS as a subjective measure of fatigue [370]. Therefore, these studies provide evidence that BG plays a crucial role in fatigue in MS.

Evidence from task-based fMRI studies in patients with CFS/ME provides inconsistent results depending on the nature of the task and what regions of the brain were involved. The inconsistency in the previous CFS/ME task-based fMRI studies might be due to the task differences which was designed to investigate certain cognitive behaviour. Also, the well-documented BG involvement in fatigue in other illnesses points towards the value of investigating it in CFS/ME. Resting state-fMRI studies show the involvement of brain regions, such as the medial prefrontal cortex, precuneus and posterior cingulate cortex, as well as brain networks which are both worth investigating to further understand the neural basis of fatigue or the differences in brain function due to CSF/ME. Therefore, this indicates a need to understand the involvement of BG in fatigue while the brain is not driven by any task first.

In this study, the aim was to implement a model-based approach to investigate the involvement of BG in CFS/ME and whether it has the same involvement as in previous MS studies. Chaudhuri & Behan (2000) [125]suggest that central/mental fatigue could potentially be the failure of integration between limbic input the basal ganglia which then impacts the striatal–thalamic–frontal cortical system. Therefore, the model-based approach requires investigation of BG regional connectivity and BG's global connectivity with whole brain cortex.

The self-report measure CFQ was used instead of FSS to measure fatigue in both groups as it is the most consistently applied questionnaire in UK CFS/ME research [354]. CFQ has been shown to be a reliable measure of fatigue severity in CFS/ME and MS [215-217]. The current approach hypothesised to find global connectivity differences between the two groups that manifest as an increase or decrease in brain connectivity. This dysfunctional connectivity would be associated with fatigue, as seen in previous task-based fMRI studies which were explained as a compensatory mechanism [109, 158, 377]. This study hypothesised to detect a decrease in connectivity within the basal ganglia as reported in MS patients in an rs-fMRI study [370]. Also, a correlation between the CFQ and FC within the BG regions would be found. However, if there were no correlation at all, then it might be specific to MS, and BG has no role in fatigue in CFS/ME illness.

5.3Methods

5.3.1 Participants

The original CFS/ME sample size was 48 participants. However, 16 declined to attend the MRI scan due to fatigue and not feeling well. Therefore, thirty-two patients with CFS/ME (26 females and six males) met the NICE criteria for CFS/ME [6], and 23 healthy controls (20 females and three males) were recruited for this study. Subjects were well matched on age and sex as CFS/ME group has a mean age of 37.80 years, SD= 11.09 and the healthy controls group has a mean age of 32.96, SD= 12.23 (see table 10). All participants gave informed consent before taking part (Appendix no 3a for CFS/ME and 3b for healthy controls). Ethics approval for this study was approved by the National Research Ethics Committee (NREC), Wales REC 6 committee (REC reference 17/WA/0401 IRAS project ID 236212). Patients with CFS/ME were recruited from the adult CFS/ME clinical service at the Cossham hospital in Bristol, UK. The Hospital Anxiety and Depression Scale (HADS) was used to exclude those with anxiety or depression [355]. Participants who scored more than 12 on each scale were excluded.

Table 10 shows the mean and s.d. of age, gender, and length of illness in each group.

	HC	**CFS/ME**	**t**	**df**	**P**
Age	32.95 (12.23)	37.80 (11.09)	1.48	47	.143
Gender	20 females, 3 males	26 females, 6 males			
Length of illness	NA	Mean= 5.69 (5.73) years Median= 3.5 years			

5.3.2 Chalder Fatigue Questionnaire

The Chalder Fatigue Questionnaire (CFQ) is considered a valid and reliable measure of fatigue for adults with CFS/ME [214-217]. The questionnaire is based on the individual's symptoms during the previous month. The questionnaire provides 11 questions with 5 rating options ranging from 1 (less than usual) to 5 (much more than usual). The result of this questionnaire is then reported as a sum of the 11 items on a 0±3 Likert scale, so it ranges from 0 (less severe fatigue) to 33 (more severe fatigue) [214]. Fatigue was assessed with the CFQ, and all scores were calculated for all participants.

5.3.3 Rs-fMRI Acquisition

3T Siemens Skyra MRI scanner with a 32 channel radiofrequency head coil was used to acquire the MRI data for all participants. Participants were asked to fix their eyes on a fixed stationary cross while staying awake for a scan time of 14 minutes. rsfMRI data were acquired using a multiband echo-planar imaging (EPI) pulse sequence developed at the CRICBristol. The

sequence consists of 600 volume (39 interleaved slices, multiband factor =3, TR = 906ms, TE = 30ms, flip angle = 60°, acquisition matrix = 195 × 100, voxel size 2mm × 2mm × 2mm) with 0 gap and scanned in a default interleaved sequence.

5.3.4 Resting-state fMRI pre-processing

SPM12 was used for spatial pre-processing as well as for statistical analysis of functional images (Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm). Pre-processing pipeline included: slice timing, realignment and unwarp, segmentation of the structural images, normalisation, smoothing and converting to 4D images. The segmentation of anatomical volume produced white matter, cerebrospinal fluid, and grey matter. The latter was then used to realign and unwarp the functional volume. After that, the automated anatomical labelling atlas (AAL) is used for the definition of ROIs. The AAL is a brain parcellation atlas that contains a total of 116 regions and based on anatomical brain regions. Preprocessed 4D images were taken into Matlab for further processing so, nuisance, white matter, CSF, and movement, were regressed from the 4D functional images using the function "b= nuisRegress_wholeBrain(a);". CONN-fMRI Functional Connectivity toolbox (www.nitrc.org/projects/conn) was then used to process rs-fMRI images to extract time-series from each of the 116 ROIs [378]. Brain activity is between 0.01~0.1 Hz, so a bandpass filter (0.01-0.08 Hz) was used to filter out any activity which is not associated with the brain (e.g. Respiratory is 0.2 Hz) [379]. Pearson correlation was then used to produce the adjacency matrix for each participant. Global functional connectivity as Small-world propensity (SWP) [380] and regional basal ganglia FC were calculated.

5.3.5 Small-world propensity (SWP):

By using SWP, scientists can model the human brain as a complex network of brain regions. Also, the non-invasive neuroimaging techniques, as well as graph-theoretical approaches, enables researchers to map human structural and functional connectivity patterns, known as connectome, at the macroscopic level [381]. The term, small world, in-network models represent a network of clusters of compactly connected nodes. Generally, structural and functional networks are believed to have small-world properties to sustain the best efficiency of the neural connectome. These connected nodes have maximised their efficiency compared to a random graph by minimizing energy consumption and costly processing [377], which can be explained as the probability of a connection between two nodes [382, 383]. Also, Watts and Strogatz (1998) reported that the small-world model is well suited for complex brain dynamics, such as a high rate of information transmission [384]. Importantly, the organisation between

these nodes experiences continuous changes throughout life and show alterations in neurological and psychiatric disorders [385, 386] providing an insight into the biological mechanisms in health and disease [381]. Also, the small-world model can support efficient information segregation and integration [381]. Friston (1994) defined functional connectivity as the interregional statistical coherence as in Pearson's correlation or the synchronisation likelihood between time-series recorded by fMRI [387].

5.3.6 Basal Ganglia Analysis

Previous studies have shown that basal ganglia components are topographically organized [388-390]. Therefore, a small-world network is an appropriate approach for investigating the functional connectivity of the BG. Global basal ganglia FC was calculated using the Conn toolbox and SWP function [380]. The regional BG FC was calculated by averaging the connectivity between the three main BG regions. These regions include the right and left caudate with the right and left putamen (Caudate-Putamen), the right and left caudate with the right and left pallidum (Caudate-Pallidum) and finally the right and left putamen with the right and left pallidum (Putamen-Pallidum).

5.3.7 Statistical Analysis

Global FC and regional BG FC was compared between patients with CFS/ME and healthy controls using an independent sample t-test. Pearson's correlation was used to correlate these connectivity measures with CFQ.

5.4Results

5.4.1 Questionnaire

The mean CFQ score in this study was 27.62 (±4.38) for patients with CFS/ME and 10.83 (±2.64) for healthy controls (see table 11).

Table 11 shows the mean and standard deviation of CFQ.

	HC	CFS/ME	t	df	P	Cohen's d
CFQ	10.83 (2.64)	27.62 (4.38)	-17.675	51.649	.000	4.642978.

5.4.2 Global Basal Ganglia FC Measures Results (SWP)

Two sample t-test shows that there was no difference between the two groups in global BG functional connectivity ($t(29.97) = 1.168$, $p = .252$). (see table 12).

5.4.3 Local Basal Ganglia Functional Connectivity

Two sample t-test shows that there were no differences between the two groups in any of the local BG regions FC with Caudate-Putamen ($t(53) = -.779$, $p = .439$), Caudate-Pallidum ($t(53) = -.645$, $p = .552$), and Putamen-Pallidum ($t(53) = -.657$, $p = .514$) (see table 12).

Table 12 shows the mean and standard deviation of an independent two-sample t-test for global FC and local BG FC.

	HC	CFS/ME	t	Df	p	Cohen's d
SWP (Global BG FC)	.32 (0.18)	.39 (0.27)	1.16	29.96	.252	0.31
Caudate-Putamen	.39 (.19)	.43 (.19)	-.77	53	.439	0.21
Caudate-Pallidum	.39 (.19)	.43 (.17)	-.64	53	.552	0.22
Putamen-Pallidum	.11 (.28)	.10(.20)	.65	53	.514	0.16

5.4.4 Local Basal Ganglia Functional Connectivity Correlation with Fatigue Scores (CFQ)

No correlation was found in functional connectivity between the BG regions and CFQ in both groups (see table 13). Also, there were no correlations between the BG regions and CFQ in each group separately (see table 13)

5.4.5 Global Basal Ganglia Functional Connectivity Correlation with CFQ

There was no correlation found between the global BG functional connectivity and the CFQ (see table 13). Also, there were no correlations between the SWP and CFQ in each group separately (see table 13).

Table 13 shows no correlation between CFQ, local BG FC or global FC in both groups together and separately.

		CFQ	CFS/ME	HC
SWP (Global BG FC)	Pearson Correlation	-.185	-.018	-.219
	Sig. (2-tailed)	.176	.920	.316
	N	55	32	23
Caudate-Putamen	Pearson Correlation	.098	.023	-.068
	Sig. (2-tailed)	.476	.900	.757
	N	55	32	23
Caudate-Pallidum	Pearson Correlation	.015	-.137	-.307
	Sig. (2-tailed)	.915	.455	.154
	N	55	32	23
Putamen-Pallidum	Pearson Correlation	-.137	-.176	-.117
	Sig. (2-tailed)	.319	.334	.596
	N	55	32	23

.

5.5 Discussion

The aim of the present study was to use rs-fMRI to investigate the global connectivity and the involvement of BG in CFS/ME illness. CFQ was measured to correlate fatigue to global brain connectivity and, specifically, BG functional connectivity. A decrease in connectivity within the basal ganglia, as shown in the previous fMRI study [370], was hypothesised as well as a finding a correlation between the CFQ and activity within the BG regions. However, the main finding in this study is that there were no significant differences in global or local basal ganglia connectivity between participants with CFS/ME and healthy controls. This might provide evidence that global connectivity might not be compromised in CFS/ME. This finding is not consistent with the previous task-based fMRI in CFS/ME, which showed the involvement of BG regions in fatigue while using different study designs and tasks [129, 131]. In this study, failure to find correlations between BG and fatigue scores suggest that such a relationship might be MS specific or due to other reasons, such as not using a task or a different analysis approach between this study and previous rs-fMRI studies.

A focus on brain networks, rather than connectivity with a single region of interest, might be the key for research in CFS/ME. Previous research in CFS/ME has investigated brain networks, including salience network (SN) [154, 155, 157, 163], default mode network (DMN) [154, 163, 167], central executive network (CEN) [163], sensory-motor network (SMN) as well as the left and right frontoparietal networks (LFPN, RFPN) [154]. CFS/ME studies that used rs-fMRI reported a decrease in FC within the SN and suggested the presence of an altered or immature resting-state network [155, 157, 168, 169]. This is valuable as the SN plays a crucial role in the connection and communication between other brain networks. It detects and integrates salient sensory information [189, 391] and provides the switch between DMN and CE network [191]. Therefore, it has been suggested that the altered FC in the SN might cause a disruption in the interaction of cognitively important information [192]. Also, it has been found that DMN is more complex and less coordinated in patients with CFS/ME and suggested to be a biomarker for this illness [167]. Other illnesses such as MS and Alzheimer's disease that been associated with the presence of abnormalities in the DMN. Since this overlaps with CFS/ME in terms of attentional disruptions and memory problems [169], it was argued that a deficit in this network might contribute or cause fatigue as it might be energy-expensive [155]. The conclusion from these studies suggests the existence of dysfunctional connectivity across many neural networks in patients with CFS/ME. Therefore, it might be valuable to investigate these networks in further details in this patient group (see chapter 6).

Investigating CFS/ME using a fatiguing task might also be an informative approach as most of the previous task-based fMRI studies in CFS/ME found differences between patients with CFS/ME and healthy controls. They used different tasks to induce fatigue and investigated different cognitive behavioural functions. Therefore, inducing fatigue might be the key to finding differences between patients with CFS/ME and healthy controls in the early phase of the disorder (see chapter 7). Miller et al. (2014), (see section 5.1) found a significant reduction in activation in the right caudate and right globus pallidus in patients with CFS/ME compared to healthy controls when using a reward processing task/gambling task. Also, several studies reported that although patients with CFS/ME performed at a similar level to healthy controls, they demonstrated widespread increased activation in task-related regions [108-110, 377, 392, 393]. Other studies had linked fatigue and lower performance to the increase in brain activity [158, 285, 377]. Mizuno et al. (2016) and Caseras et al. (2006) hypothesised that severe fatigue consumes a substantial amount of attentional resources by recruiting additional brain regions for cognitive compensation to perform better in dual-task depending on the degree of mental effort [109, 377]. Caseras et al. (2006) indicated that the patients with CFS/ME might have been driven by their fear to avoid activity [109]. Interestingly, a study found a decrease in activation in the putamen in the low-rewarding condition only but not in the high-reward condition [131]. Therefore, task-based fMRI can help to show how the brain manifests the main symptom of fatigue in this illness.

5.6 Conclusion

The result illustrates that global connectivity might not be compromised in CFS/ME. Since this study was not able to link the BG functional connectivity to fatigue in CFS/ME, further investigation of the brain networks in this patient group was performed. Therefore, the next chapter aimed to investigate these networks using the same available data.

6 Chapter 6: Investigating Brain Networks Associated with Subjective Fatigue in CFS/ME: an rs-fMRI study.

6.1 Overview of Chapter

Chapter 6 investigates the brain networks of CFS/ME using resting-state fMRI. This includes the methods and the interpretation of the results as well as a discussion of the results.

6.2 Brain Networks

Previous fMRI research has established that the brain is divided into functional networks that show connections between brain regions while performing a task or at rest [229, 394-396]. These networks include Default Mode Network (DMN) [154, 163, 167], Salience Network (SN) [154, 163], Central Executive Network (CEN) [163], Sensory Motor Network (SMN) as well as the left and right Frontoparietal Networks (LFPN, RFPN) [154]. The following sections will describe what is known to date about how these brain networks are affected by CFS/ME and where there are gaps in that evidence.

6.2.1 Default Mode Network (DMN) in CFS/ME

Whenever participants were asked to think freely in a neuroimaging modality, as in rs-fMRI, they activate a brain network system called the Default Mode Network (DMN), which is also deactivated during attentional tasks [221, 397]. This network includes brain regions that interact and activate spontaneously [394]. DMN is considered as one of the major networks in the human brain as it represents the baseline brain function [378]. This network consists of several brain regions including the posterior cingulate/precuneus cortex (PCC), the left inferior parietal cortex/angular gyrus (lIPC), the right inferior parietal cortex/angular gyrus (rIPC) and medial prefrontal/anterior cingulate cortex (mPFC) (see figure 15) [394, 395].

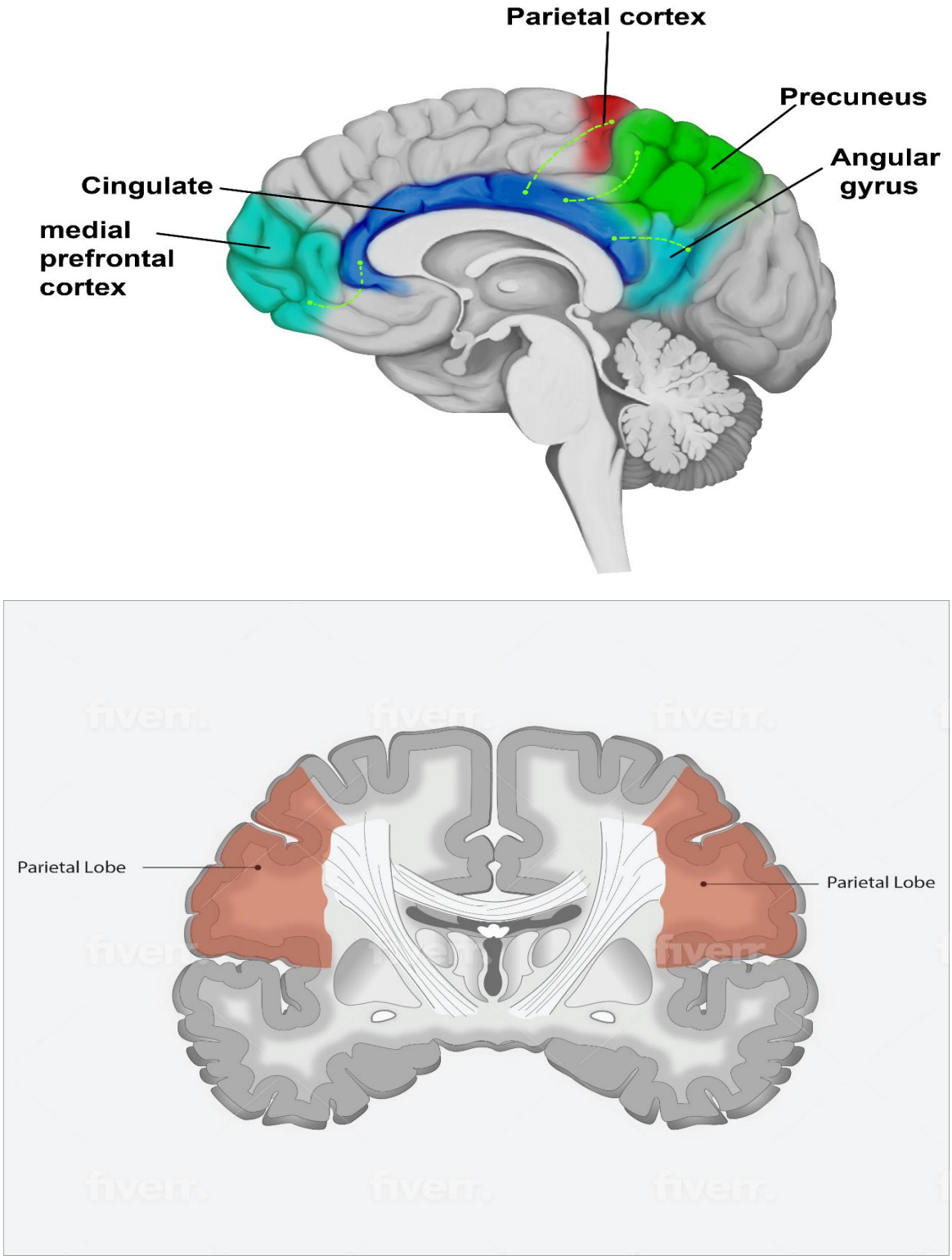


Figure 15 shows the brain regions involved in the DMN. Created by Adobe software.

6.2.2 Salience Network (SN)

Salience Network (SN) is another core intrinsic connectivity network of the brain, which involves the anterior insula (AI) and dorsal anterior cingulate cortex (dACC) (see figure 16). It is considered to be a large-scale brain network, and alteration to the insular anterior-posterior axis contribute to the alteration in interoceptive awareness and monitoring of the internal milieu [163, 398]. SN is involved in many brain functions, including audition, deception, interception, pain, and classical conditioning [399, 400]. It has been reported that it works as a transitional network to link cognition and emotion/ interoception [401, 402]. The SN has influences on other networks such as the DMN and frontoparietal networks (FPN) by mediating the switching between these networks and the executive control network. By doing so, it guides appropriate responses to salient stimuli [403].

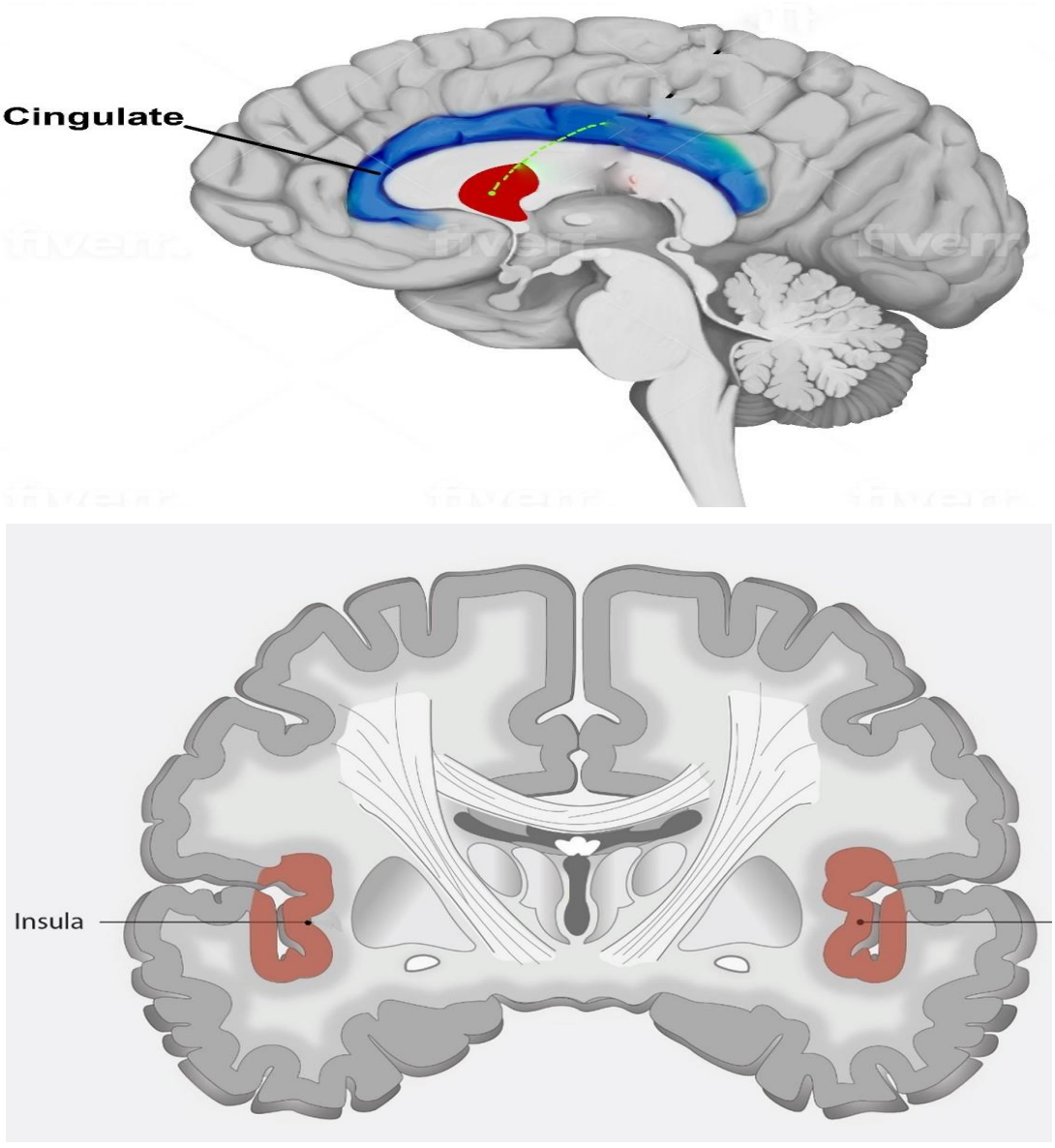


Figure 16 shows the brain regions involved in SN (Cingulate and Insula). Created by Adobe software.

6.2.3 Executive Control Network (ECN)

The Executive Control Network (ECN) plays a major role in extrinsic awareness as well as mediating cognitive processes. These cognitive processes include working memory, problem-solving, reasoning, flexibility and planning [404]. Earlier studies show that the ECN covers many brain regions, including the dorsolateral prefrontal cortex, anterior cingulate cortex, orbitofrontal cortex (see figure 17). Recently, brain regions, including subcortical and brainstem sites, were reported to have a role in this network functioning [404, 405].

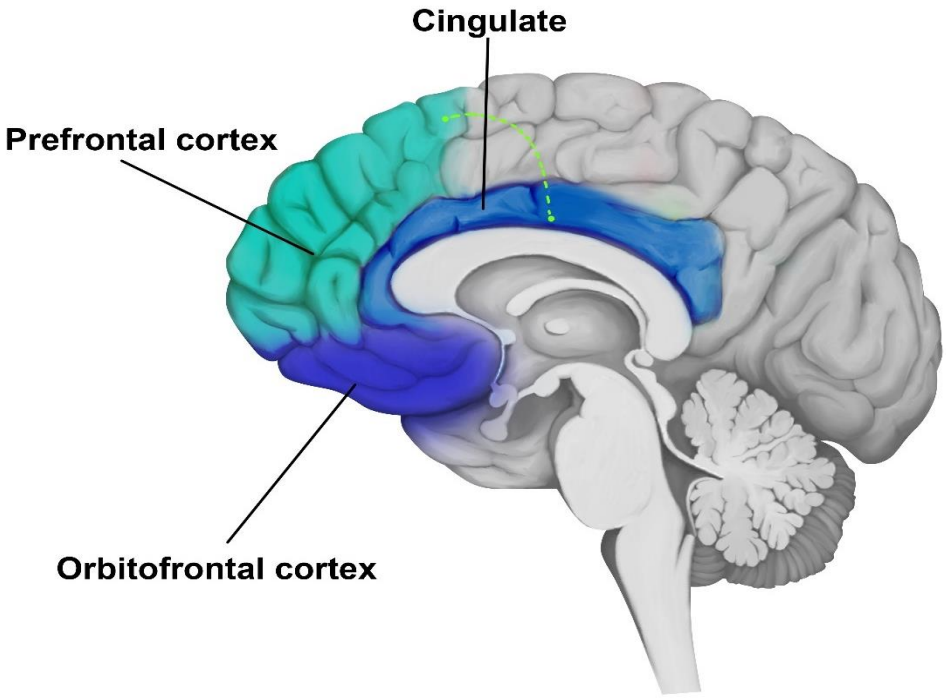


Figure 17 shows the brain regions involved in the ECN. Created by Adobe software.

6.2.4 Left frontoparietal networks (LFPN) and right frontoparietal networks (RFPN).

The LFPN consists of many brain regions, including the left middle frontal gyrus, left precuneus, right middle frontal gyrus, right superior parietal gyrus and posterior cingulate gyrus (see figure 18). Where the RFPN includes the right supramarginal gyrus and the right superior frontal gyrus, these networks were reported to support control initiation as well as providing flexibility. They use feedback to adjust control response [406].

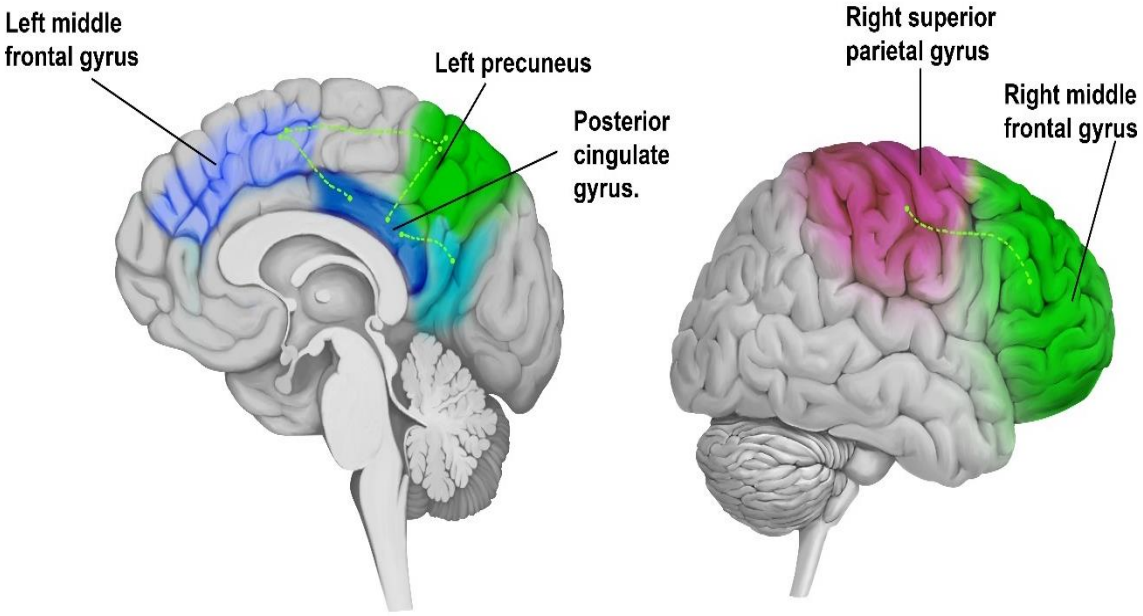


Figure 18 shows the brain regions involved in the FPN. Created by Adobe software.

6.2.5 Sensory Motor Network (SMN)

Lois et al. (2014) have demonstrated the role of SMN in action and somesthesis, a term used for all of our bodily sensations like sensing the functioning of internal organs, proprioception and skin senses, [402] and activated during tasks like finger tapping [229]. This network includes somatosensory and motor regions such as the post-central gyrus and precentral gyrus and encompasses supplementary motor areas (see figure 19) [154, 402].

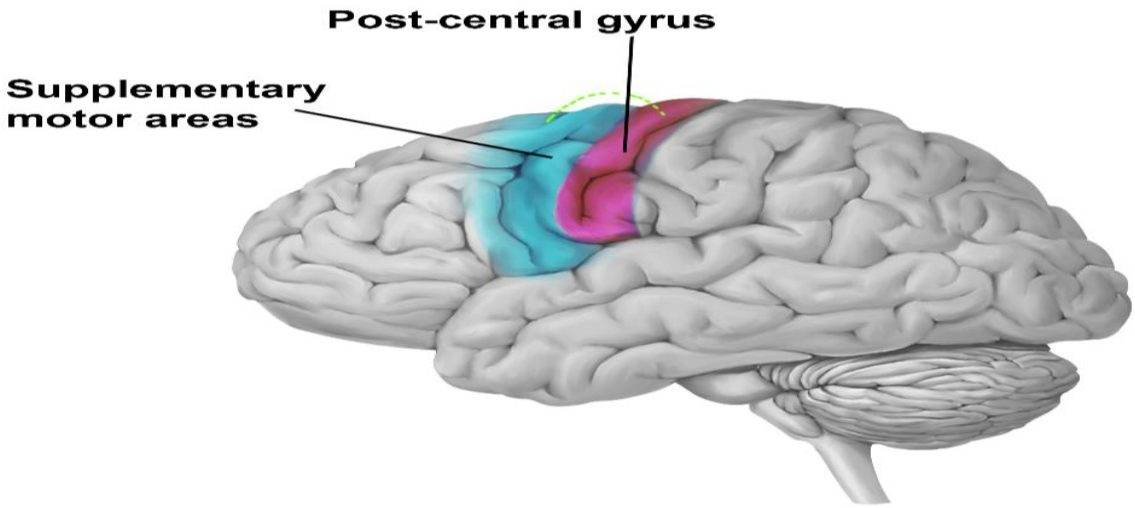


Figure 19 shows the brain regions involved in the SMN. Created by Adobe software.

6.2.6 Basal Ganglia (BG)

The basal ganglia have many components, of which each has a complicated anatomical and functional organisation [113]. Evidence suggests that this region is involved in fatigue in illnesses such as MS [129, 369, 370] and also in CFS/ME [131]. These brain regions include caudate, putamen, pallidum, ventral striatum, substantia nigra, the nucleus accumbens and subthalamic nucleus (see figure 20). It receives information from various brain regions and sends inhibitory output to many motor-related areas. Also, substantia nigra is the source of the striatal input of the neurotransmitter dopamine [113]. The analysis approach for functional connectivity used here is the same as the previous chapter using SWP analysis (see section 5.1). However, additional analysis, such as modularity and efficiency were conducted. Also, ut correlations with other networks were performed.

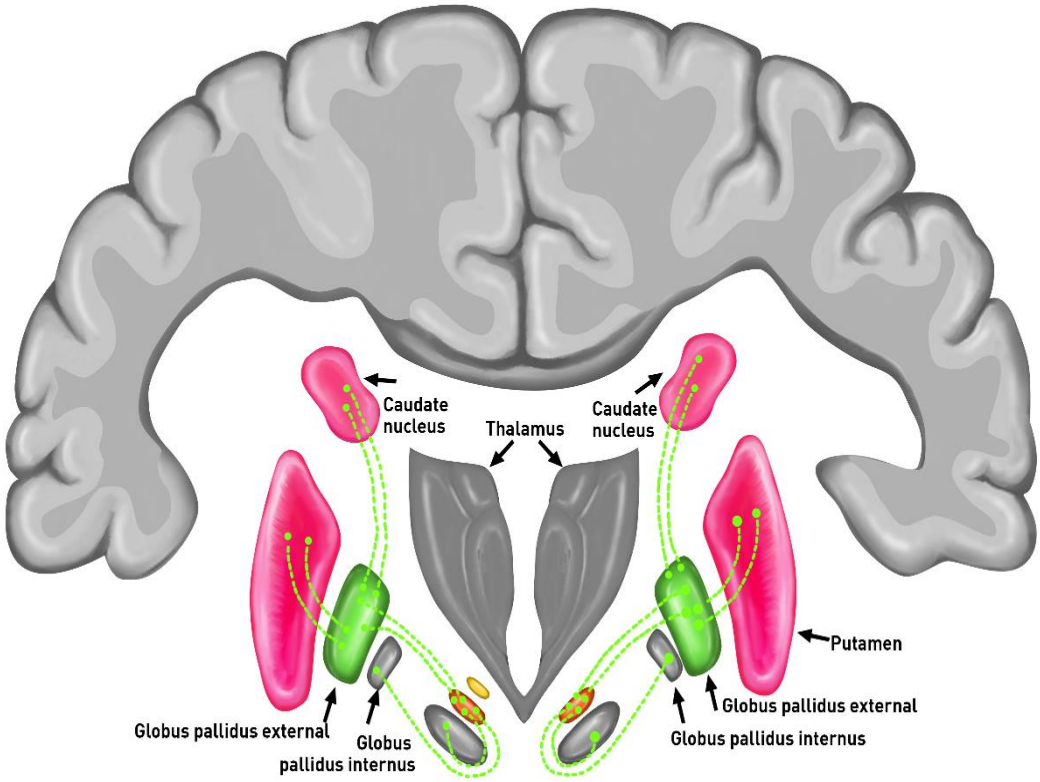


Figure 20 shows the brain regions involved in the BG network. Created by Adobe software.

6.2.7 Brain Networks in CFS/ME

Due to the complexity of CFS/ME and the high occurrence of cognitive dysfunction among this patient group, many studies have focused their research on investigating this illness using resting-state functional MRI (rs-fMRI). Previous rs-fMRI studies in this illness have investigated different brain networks, due to their importance in playing key roles for many brain functions, including DMN [154, 163, 167], SN [157, 168, 169], CEN [163], SMN as well as the left and right FPN (LFPN, RFPN) [154]. Four studies found that CFS/ME participants had decreased Functional Connectivity (FC) when compared to healthy controls [157, 168, 169], three of which reported a decrease in functional connectivity within the salience network [157, 168, 169]. Wortinger et al. (2016) showed a decrease in FC between the salience network and the right middle, posterior and anterior insula. Also, this decrease extended to areas outside the traditional boundaries of the SN, such as the superior temporal gyrus, precentral gyrus and thalamus [168]. Wortinger et al. (2017) reported a reduction in functional connectivity between the right dorsal anterior insula and the right posterior parietal cortex of the central executive network [157]. DMN is one of the three core intrinsic connectivity networks (ICN) of the brain, which also includes the CEN and the SN [407]. It has been shown that CFS/ME affects the DMN functions [408], such as mediating feelings and thoughts [409, 410] as well as being associated with cognitive performance [411, 412]. Gay et al. (2016) investigated five brain networks (DMN, SN, SMN, RFPN and LFPN) and reported a decrease in FC between the salience network and the left posterior cingulate cortex as well as a disturbance in the intrinsic connectivity within the left FPN. Also, they reported that the SMN showed a decreased FC with the left anterior mid-cingulate cortex [169]. While Shan et al. (2018) reported a decrease in FC between the medial prefrontal cortex and both inferior parietal lobules in CFS/ME compared to healthy controls [167], Kim et al. (2015) was the only study to report an increase in FC in the posterior parietal cortex and the dorsal anterior cingulate cortex, rostral anterior cingulate cortex, middle temporal cortex and precuneus [155]. The sum of the results from these studies possibly suggests the presence of dysfunctional connectivity across many neural networks in CFS/ME.

Basal ganglia have been associated with fatigue in CFS/ME and other illnesses such as Multiple Sclerosis (MS) and Parkinson's disease [125, 127] (see section 5.1). Treating Parkinson's disease patients with bilateral pallidotomy resulted in profound fatigue as one of the main side effects [372]. Knowing that it might not be the only reason for central fatigue, it is essential to recognise that basal ganglia dysfunction is a vital pathogenic mechanism. Therefore, scientists

utilised neuroimaging techniques such as fMRI to investigate the relationship between BG and fatigue (see 5.1) [125]. Task-based fMRI studies showed evidence of inconsistent results due to the nature of the task and the regions under investigation [129, 131, 159]. On the other hand, rs-fMRI studies showed the involvement of different brain regions as well as brain networks [155, 167]. These results combined illustrate the importance of investigating brain regions and networks that are known to be involved in fatigue.

Data analysis in rs-fMRI can take three approaches, graph theory (see p70), model-based or data-driven. The model-based approach is used to show the validity of earlier hypotheses [387, 413] where the data driven approach uses Independent Component Analysis (ICA) which examines the whole brain and is best used in the absence of a good model [414]. The problem of ICA is that it is hard to interpret and there is no control over decomposition (for further details on analysis methods see section 3.4.1 Resting-state fMRI (rs-fMRI) p69). The study hypothesised to find an association between CFQ and altered FC within these networks. Specifically, to find decreased FC in the SN, DMN, FPN and SMN as seen in previous studies [154, 163, 167]. Also, to find a negative correlation between the decrease in these networks and the CFQ, meaning that whenever fatigue score increases, the FC in these networks decreases.

6.3Method and Procedures

6.3.1 Participants

The same data and sample size that were included in the previous chapter (chapter 5) were analysed again for brain networks connectivity (see section 5.2.1 for more details).

6.3.2 Self-reporting Measures

Fatigue:

The Chalder Fatigue Questionnaire is considered a valid measure for adults with CFS/ME [214] (see section 5.2.2).

6.3.3 MRI Acquisition:

The same rs-fMRI sequence and acquisition parameters from the previous chapter (chapter 5) were used.

6.3.4 Resting-state fMRI pre-processing

SPM12 was used for spatial pre-processing as well as for statistical analysis of functional images (Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm). Pre-processing pipeline included: slice timing, realignment and unwarp, segmentation of the

structural images, normalisation, smoothing and converting to 4D images. The segmentation of anatomical volume produced white matter, cerebrospinal fluid and grey matter. The latter was then used to realign and unwarp the functional volume. After that, functional volumes were resampled to voxels of 2x2x2 and smoothed with a Gaussian kernel (FWHM =8mm). This is done in order to match the data with the automated anatomical labelling atlas (AAL) that is used for the definition of ROIs. The AAL is a brain parcellation atlas that contains a total of 116 regions and based on anatomical brain regions. Nuisance variables, i.e., white matter, cerebrospinal fluid and movement, were regressed from the functional images. CONN-fMRI Functional Connectivity toolbox (www.nitrc.org/projects/conn) was then used to process rs-fMRI images to extract time-series [378]. Brain activity is between 0.01~0.1 Hz, so a bandpass filter was used to filter out any activity which is not associated with the brain (e.g., Respiratory is 0.2 Hz). A weighted connectivity matrix of 116x116 was produced for each participant using Pearson's correlation by measuring the FC between the regions of interest. Fisher z-transform was conducted to increase the normality and standardise the data for group comparison. The absolute function was used to disregard the sign of negative values. Finally, MATLAB 2019a (Mathworks Inc., Natick, MA, USA) was utilised to analyse the networks using standardised weighted connectivity matrices.

6.3.5 Graph Theory

Graph theory is used extensively in cognitive neuroscience to investigate the structural and functional mechanisms of the brain. It is used to show the topological relationship among brain nodes which consequently allow comparing rs-fMRI functional connectivity between groups. In this method, the graph theory was used to represent brain networks by using regions of interest, those representing nodes, and edges, which represent the connections between these nodes (see section 5.2.5) [291, 303]. Therefore, brain regions that represent the network was identified as nodes using AAL then used for connectivity calculation [379].

6.3.5.1 Global FC Measures

The standardised weighted connectivity matrices were used to calculate modularity [291, 415], global efficiency [291, 324] by using the Conn toolbox. Also, the Small World Propensity (SWP) was calculated by using the SWP function (see section 5.3.5.) [380].

6.3.6 Model-based Approaches to RS Network Analysis

For global networks FC, the SWP for all networks was calculated, including DMN regions, SN regions, L&R LFP regions, ECN regions, and BG regions. For regional FC, the average time-series of the pre-and post-central gyrus with the whole brain regions were used to calculate the

SMN FC. For in-between networks FC, the average FC of the regions involved for two networks at a time was calculated. Therefore, the in-between networks for all possible 15 in-between network connections were calculated in the same way.

6.3.7 Statistical Analysis

An Independent t-test between the two groups was performed to compare modularity, global efficiency, global SWP and the SWP of the six chosen networks. Pearson's correlation was used to correlate these connectivity measures with CFQ.

6.4Results

6.4.1 Demographic and Clinical Variables of Participants

Self-reported psychometric scales t-test showed that the CFS/ME group had significantly higher levels of fatigue (see figure 21).

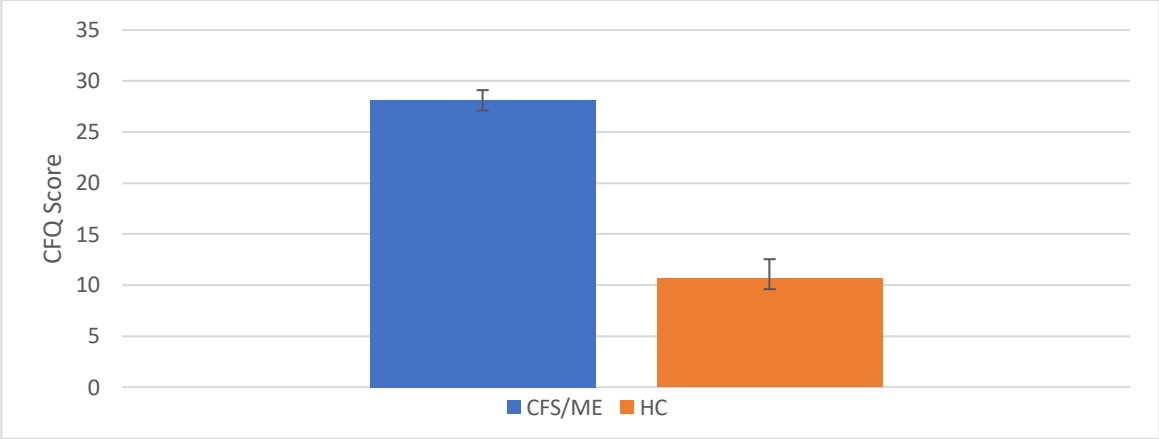


Figure 21 shows the CFQ results of CFS/ME and healthy controls.

6.4.2 Global FC Measures Results (Modularity, efficiency and SWP)

Two sample t-test shows that there were no significant differences between CFS/ME and healthy controls in modularity, efficiency and whole-brain SWP (see table 14).

Table 14 shows the mean and standard deviation of pearson's correlation in SWP, Global modularity, global efficiency, and Cohen's d for all participants.

	HC	CFS/ME	t	df	p	Cohen's d
SWP (Whole Brain)	0.682 (0.076)	0.668 (0.085)	0.629	53	.532	0.116505.
Global Modularity	0.157 (0.0178)	0.152 (0.0194)	1.112	53	.278	0.081423.
Global Efficiency	0.350 (0.029)	0.342 (0.021)	1.180	53	.243	0.315981.

6.4.3 Networks

Two sample t-test shows that there was a significant difference in regional FC between the two groups in SN ($t(48.433) = -2.463$, $p = .017$). There was no difference between the two groups

in DMN, R&L FPN, ECN, SMN, or BG. Also, in the SN, Cohen's d calculation indicates the presence of a very large effect, meaning that there is evidence for a meaningful difference between groups (see table 15).

Table 15 shows the mean and standard deviation of independent two-sample t-test for all networks.

	HC	CFS/ME	t	df	p	Cohen's d
Salience network	.3525 (.16)	.2053 (.12)	-2.232	48.433	.017	1.04
Default Mode Network	.2493 (.10)	.2490 (.10)	.010	53	.992	0.003
R&L frontoparietal networks	.2174 (.15)	.1743 (.10)	1.284	53	.205	0.33
Executive Control Network	.1769 (.15)	.2016 (.12)	-.660	53	.512	0.18
Sensory Motor network	.3089 (.22)	.2491 (.05)	1.339	23.696	.193	0.37
Basal ganglia	.39 (0.27)	0.32 (0.14)	1.168	29.969	.252	0.37

6.4.4 Between Networks Analysis

Two sample t-test for between networks analysis showed a significant decrease in FC in the SN to the BG in the CFS/ME compared to healthy controls ($t(53) = 2.22$, $p = .031$), and between the BG to the SMN ($t(53) = 2.34$, $p = .023$). Also, in the SN/BG and BG/SMN, Cohen's d calculation indicates the presence of a medium effect in SN/BG, BG/SMN and DMN/SN (see table 16). No other in-between networks differences were found.

Table 16 shows the mean and standard deviation of an independent two-sample t-test for FC of in-between networks.

Networks	HC	CFS/ME	T	df	p	Cohen's d
SN / BG	.39237 (.100)	.33150 (.100)	2.220	53	.031	.6
BG / SMN	.2384 (.127)	.1725 (.080)	2.340	53	.023	.54
DMN / FPN	.3111 (.041)	.3189 (.045)	-.640	53	.525	.02
DMN / SN	.3194 (.060)	.3457 (.062)	-1.555	53	.126	.58
DMN / ECN	.3027 (.041)	.3142 (.047)	-.934	53	.355	.26
DMN / BG	.2088 (.042)	.1955 (.057)	.944	53	.349	.18
DMN / SMN	.2585 (.074)	.2675 (.082)	-.418	53	.677	.11
FPN / SN	.2907 (.045)	.2960 (.057)	-.365	53	.717	.10
FPN / ECN	.2673 (.042)	.2564 (.047)	.882	53	.382	.24
FPN / BG	.2056 (.048)	.1938 (.065)	.731	53	.468	.20
FPN / SMN	.2247 (.068)	.2393 (.073)	-.747	53	.459	.20
SN / ECN	.2793 (.039)	.2691 (.057)	.735	53	.465	.20
SN / SMN	.2477 (.081)	.2310 (.096)	.677	53	.501	.18
ECN / BG	.2131 (.053)	.2064 (.055)	.448	53	.656	.12
ECN / SMN	.2246 (.061)	.2320 (.055)	-.470	53	.640	.12

6.4.5 Correlation Between CFQ and the Networks

A significant negative correlation was found between CFQ and FC in the SN (r= -.659, p=.01) in healthy controls only. Also, a significant positive correlation was found between CFQ and FC in the DMN (r= .528, p= .01) in healthy controls only. No other correlations were found in FC of other networks in both groups (see table 17 and 18).

Table 17 shows the correlation between the CFQ and FC of the networks in HC as well as the Fisher-Z-Transformation.

CFQ Correlations with HC brain networks

		DMN	FPN	SN	ECN	SMN	BG
CFQ	**Pearson Correlation**	.528**	.061	-.659**	.040	-.099	-.069
	Sig. (2-tailed)	.010	.782	.001	.855	.652	.754
	Fisher-Z-Transformation	.587	.061	-.791	.04	-.099	.069
**. Correlation is significant at the 0.01 level (2-tailed).							

Table 18 shows the correlation between the CFQ and FC of the networks in CFS/ME as well as the Fisher-Z-Transformation.

CFQ Correlations with CFS/ME brain networks							
		DMN	**FPN**	**SN**	**ECN**	**SMN**	**BG**
CFQ	**Pearson Correlation**	.098	-.048	-.090	-.142	.058	-.085
	Sig. (2-tailed)	.593	.792	.626	.438	.752	.644
	Fisher-Z-Transformation	.098	.048	.090	.143	.058	.085
*. Correlation is significant at the 0.05 level (2-tailed).							

6.4.6 Correlation Between Task Performance and Networks

When both groups, CFS/ME and HC, were combined, there was no significant correlation between CFQ and the FC of all investigated networks. Therefore, an investigation of the correlation of each group separately between CFQ and the FC of all networks was conducted.

Using the correlation between task accuracy and networks resulted in a significant negative correlation between the SN functional connectivity and the verbal storage task in CFS/ME only (see table 19) (see section 7.3.3. for tasks design). Also, a positive correlation was found between the DMN functional connectivity and the complex verbal task in the presentation phase in participants with CFS/ME (see tables 19). In addition, a negative correlation between the SMN functional connectivity and the complex verbal task in the recognition phase (see table 19). A significant positive correlation was found in healthy controls between the accuracy in the processing task and the DMN functional connectivity (see table 20).

Table 19 shows the correlation between FC and task accuracy in CFS/ME.

		DMN	FPN	SN	ECN	SMN
Processing Accuracy	**Pearson Correlation**	-.091	-.338	-.281	-.084	.270
	Sig. (2-tailed)	.621	.058	.119	.646	.135
	N	32	32	32	32	32
Verbal storage Accuracy	**Pearson Correlation**	-.232	-.082	-.404*	.071	-.077
	Sig. (2-tailed)	.202	.654	.022	.701	.675
	N	32	32	32	32	32
Complex Verbal Accuracy (Presentation)	**Pearson Correlation**	.427*	.236	.185	-.161	-.131
	Sig. (2-tailed)	.015	.193	.311	.379	.474
	N	32	32	32	32	32
Complex Verbal Accuracy (Recognition)	**Pearson Correlation**	.231	.194	.316	-.215	-.393*
	Sig. (2-tailed)	.204	.286	.078	.238	.026
	N	32	32	32	32	32

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

Table 20 shows the correlation between FC and task accuracy in healthy controls.

		DMN	FPN	SN	ECN	SMN
Processing Accuracy	**Pearson Correlation**	.592**	-.126	-.022	.176	.000
	Sig. (2-tailed)	.003	.566	.920	.422	1.000
	N	23	23	23	23	23
Verbal storage Accuracy	**Pearson Correlation**	.053	.042	.112	.150	.040
	Sig. (2-tailed)	.810	.848	.611	.494	.857
	N	23	23	23	23	23
Complex Verbal Accuracy (Presentation)	**Pearson Correlation**	.183	.153	.004	.034	.209
	Sig. (2-tailed)	.403	.486	.987	.878	.337
	N	23	23	23	23	23
Complex Verbal Accuracy (Recognition)	**Pearson Correlation**	-.065	-.167	.281	.044	.067
	Sig. (2-tailed)	.770	.446	.194	.844	.762
	N	23	23	23	23	23

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

6.5 Discussion

In order to identify the neural correlates of self-reported fatigue, rs-fMRI was used to compare the five main neural networks (DMN, SN, ECN, FPN and SMN) in CFS/ME with age–sex-matched healthy controls. This chapter hypothesised to find differences between groups in networks connectivity. Also, fatigue was measured using CFQ to correlate fatigue to these networks as this chapter hypothesised to find a negative relationship between CFQ and FC in these networks. The main finding in this chapter is a significant decrease in intrinsic functional connectivity of the SN in CFS/ME compared to healthy controls. In addition, decreased FC was found between the BG to the SMN and between the SN to the BG in CFS/ME compared to healthy controls. Also, this study found a negative correlation between CFQ and SN and a positive correlation between CFQ and DMN in healthy controls only. In addition, it found a negative correlation between the accuracy of the verbal storage task and the SN functional connectivity and between the SMN and the complex verbal task in the recognition phase in the CFS/ME group only. Also, a positive correlation between the DMN functional connectivity and the complex verbal task in the presentation phase in the CFS/ME group. However, in healthy controls, a positive correlation was found between the processing task and the DMN functional connectivity.

The differences found in the SN is supported by previous fMRI studies, which found altered resting-state functional connectivity in SN in participants with CFS/ME [154, 168]. Salience

network alteration, also reported in previous studies [154, 157, 163], may imply the involvement of SN in the pathophysiology of CFS/ME. Studies have reported that the decrease in affected regions inside the SN such as right middle, posterior and anterior insula, and outside the SN such as superior temporal gyrus, precentral gyrus and thalamus were associated with fatigue [157, 168]. Knowing the SN function (see 6.2.2.) shows that it plays a critical role in connecting brain regions and networks. Alterations or immature network had been suggested to interfere with the interaction of cognitively important information [192], which adds to the brain energy cost [155, 157, 168, 169, 192]. This may explain the fatigue and some CFS/ME symptoms, such as impaired memory [368, 416]. Also, alteration of the SN may cause an influence on the other networks such as the DMN, ECN and FPN as this network steers appropriate responses to salient stimuli [403].

Salience network comprised of cingulate and insula, which mostly activated among a varied range of tasks [417]. These regions are known to have a role in high-level neurocognitive control and attention [398, 418]. BG regions (caudate, putamen, pallidum) are previously linked to fatigue in CFS/ME [131] and other illnesses such as MS [129, 369, 370] and has a significant role in sending inhibitory output to many motor-related areas [113]. Disorders such as post-traumatic stress disorder, depression and pain showed that aberrations along the insular pathway (a part of the SN) appear to be common when there is a disturbance in the interpretation of salient biological and cognitive information [157, 419]. Functional connectivity efficiency in the insula was associated with physical activity [207, 420, 421], cognition [422], and pain [423, 424] in healthy controls studies. Previous fMRI studies in CFS/ME showed the involvement of BG regions in fatigue when using different study settings and correlated their results to fatigue scores [129, 131]. Therefore, alteration in these networks and regions might be associated with the abnormal signalling assessment of interoceptive fatigue in the body leading to increased sensitive fatigue awareness in CFS/ME [168]. A decreased FC was found between the BG to the SMN and between the SN to the BG in CFS/ME compared to healthy controls. SMN has been reported to have decreased FC with the left anterior mid-cingulate cortex [169]. Patients with CFS/ME suffer from physical impairment as well as cognitive function impairment. SMN is corresponding to action and somesthesis, all of our bodily sensations [402]. It could be argued, therefore, that FC patterns either within or between these networks may be impaired in CFS/ME [169, 425]. Future research is necessary to understand the altered relationship of the SMN better.

In this chapter, healthy controls' brain had decreases in SN connectivity when fatigue increases, which may imply that less fatigue means better connectivity. Also, a positive

correlation between CFQ and the DMN was found, which means that FC increases in this network with increased fatigue level in healthy controls. Shan et al. (2018) reported that the DMN was more complex and less coordinated in the CFS/ME group and suggested that it can be used as a biomarker for CFS/ME [167]. However, this was not found in this thesis which may question the validity of using DMN functional connectivity as a biomarker for CFS/ME. Other illnesses such as Alzheimer's disease and multiple sclerosis have similar clinical features such as attentional disruptions and memory difficulties, as seen in CFS/ME [169]. In these illnesses, DMN abnormalities have been argued to be energy expensive and might have a role in or be the cause of fatigue, cognitive symptoms and post-exertional malaise of CFS/ME [155]. Kim et al. (2015), on the other hand, found that CFS/ME participants had an increase in FC between the posterior parietal cortex and the dorsal anterior cingulate cortex, rostral anterior cingulate cortex, middle temporal cortex and precuneus, which was significantly associated with fatigue [155]. Therefore, these discrepancies in detecting differences in the different networks might be due to years of chronicity with the disease or using different analysis method [163].

Correlating networks' functional connectivity with tasks accuracy showed some differences between the CFS/ME group and the healthy controls. In the healthy control group, they showed a positive correlation between the accuracy in the processing task and the DMN functional connectivity. While in the CFS/ME group, a negative correlation was found between the accuracy in the verbal storage task and the SN functional connectivity and between the SMN and the complex verbal task in the recognition phase. Also, a positive correlation between the DMN and the complex verbal task in the presentation phase. Although DMN and working memory were thought to be inversely correlated, Piccoli et al. (2015) showed that this is not completely true during all working memory phases [426]. Also, their finding support that the DMN has direct dynamic involvement in cognitive function rather than a static interaction with specific task-positive networks [426]. They also reported that when the stimulus is present during the encoding and retrieval phases, the DMN and the working memory network are positively coupled [426]. Their finding suggests the presence of dynamic functional connectivity switching between the DMN and working memory network [426]. Eichele et al. (2008) reported that in cognitive tasks, the failure in suppressing the DMN activity would increase the error rate as well as interfering with goal-directed behaviours [427]. Therefore, alteration in such an important network would expect to impact other networks. Also, it would affect the SN impacting its function in the audition, which would explain the increase in error

rate. The increased functional connectivity with the increased error rate would suggest that there is more energy expenditure with no gain, which might explain the fatigue in this illness.

Functional connectivity did not differ in the DMN, ECN, FPN or SMN between CFS/ME and healthy controls, which might mean that these networks are not compromised in patients. This is different from previous studies and might be due to the use of different analysis approaches. This can be explained by illustrating Wortinger and colleague's two studies findings. In rs-fMRI, data can be analysed using either a data-driven [414] or a model-based [387] approach. Wortinger and colleagues used these two different approaches to analyse their data in adolescents with CFS/ME. The first approach was a data-driven one using Independent Component Analysis (ICA) [414]. This approach is multivariate and helps in examining the full spatial structure of the brain. They analysed the same sample using this method and found an FC decrease between a right dorsal anterior insula hub and the posterior parietal cortex in adolescents with CFS/ME, which was not associated with fatigue [168]. The other approach that they used was the model-based approach which focuses on validating an earlier hypothesis. This approach shows how well a pre-defined model fits the fMRI data [387, 413]. So, when Wortinger et al. (2017) used a model-based approach, they found decreased FC in the SN in CFS/ME compared to healthy controls, which was associated with the increase in fatigue [157]. The latter was the same approach used in this chapter and supported their findings on adults with CFS/ME and suggested SN's involvement in fatigue [157]. Therefore, different analysis methods are complementary to each other and need to be applied to the same data set to produce conclusive and robust results. Also, each method on its own provides valuable information regarding the common and divergent findings, which have a different and helpful interpretation for the literature [428].

A proposed model of CFS/ME is the sustained arousal model, which can explain documented variations by establishing vicious circles in many types of research, including cognition (impaired memory and information processing), and skeletal muscle function (increased oxidative stress, attenuated cortical activation) [429]. Sustained arousal model can originate from different factors and can interact with predisposing factors (genetic traits, personality), precipitating factors (infections, psychosocial challenges) and learned expectancies (classical and operant conditioning) [429]. This model supported some of the CFS/ME main findings in one theoretical framework and proposed a causal link between sustained arousal and the experience of fatigue [429]. The insular cortex function is influenced by persistent stress

activation, which affects its ability to communicate with other systems that are involved in the descending pain inhibition pathways [430, 431]. The decrease in insula activity might be related to the abnormal assessment of fatigue signalling in the body, which leads to enhanced fatigue awareness in CFS/ME [162]. The results presented in this chapter add to the literature that SN dysfunction identification shows evidence of stress abnormalities in facilitatory and inhibitory neural pathways [432] and autonomic nervous system activity [433, 434]. Therefore, SN dysfunction might be explained by sustained arousal, which manifests as a hypoconnectivity in this network. A recent study showed that SN represents the homeostatic system that engages with the task, such as maintaining the relevant task as long as the stimulus is presented and orchestrating the switching to a new task set [435]. In living systems, homeostasis represents steady internal physical and chemical conditions [436].

6.6Conclusion

Findings in this chapter have provided additional evidence with respect to brain network alteration in CFS/ME. Finding a significant decrease in intrinsic functional connectivity of the SN in CFS/ME compared to healthy controls illustrates the importance of using fMRI. Also, investigating CFS/ME using a fatiguing task might be useful in the future as most of the previous task-based fMRI studies in CFS/ME found differences between CFS/ME and healthy controls [108-110, 392]. Previous studies used different tasks to induce fatigue and investigated different cognitive behavioural functions [108-110, 392]. Therefore, using a task might be the key to find differences between CFS/ME and healthy controls. The next chapter explores this theory in further details.

7 Chapter 7: Investigating CFS/ME Using a Verbal Working Memory Task: an fMRI Study.

7.1 Overview of Chapter

Chapter 7 investigates the brain activity in CFS/ME during a verbal working memory task using functional MRI. This includes the methods and the interpretation of the results as well as a discussion of the results.

7.2 Introduction

Most patients with CFS/ME, around 90%, report cognitive impairments such as memory loss, poor concentration, reduction in cognitive ability, and lack of ability to take in information [26, 27, 437-440]. Cockshell et al. (2010), in their meta-analysis, reported patients with CFS/ME experience cognitive difficulties, especially in memory, attention, and motor speed [441]. More recently, Cvejic et al. (2016) showed that patients with CFS/ME often reported difficulties with memory, concentration and attention as well as problems in higher-order cognitive domains. In addition, they reported that there is an indication of a more global and non-specific deficit which manifest as a generalised slowing of response speed with tasks that require simple and complex information processing and sustained attention [442]. Cvejic et al. (2016) argued the literature demonstrated numerous inconsistencies, and this might arise from the differences in the measures used either to measure the cognitive performance or individual complaints. Furthermore, some tasks might be better than others in detecting cognitive deficits in this illness, as seen in other illnesses such as traumatic brain injury or dementia [443]. They proposed that the traditional laboratory-based cognitive tasks might not be a good representation of what a participant with CFS/ME would experience in term of difficulties in cognitive processes in real life [442].

Previous studies using speeded button-press responses to simple visual stimuli showed that participants with CFS/ME tend to take longer to respond compared to the healthy controls [444-449]. In more demanding complex information-processing tasks, participants with CFS/ME were slower than the healthy controls [445-447]. However, when finger tapping was used to assess motor functioning, no differences between the groups were found [444, 450], which suggest that the delay is not a result of slow motor functioning [442]. Although participants with CFS/ME were slower in response, they performed at the same accuracy level

as the healthy controls [444, 446-450] even in more specific cognitive domains such as working memory [444, 445, 447-449], logical thinking [444, 451] and verbal ability [444, 451].

Functional MRI (fMRI) has been used to investigate cognitive impairment in CFS/ME to investigate attention, rewards and motivation, sensory information processing, emotional conflict, and working memory. Previous studies used a variety tasks including: PASAT [108, 110, 392], auditory monitoring task (simple attention) [108], non-fatiguing motor task (finger tapping) [110, 392], non-fatiguing cognitive task (simple auditory) [110, 392] and n-back [109]. The findings from these studies vary with the type of task used. When simple and non-fatiguing tasks are employed, no differences were found between the CFS/ME group and the healthy control group [110, 392]. Conversely, studies using complex and fatiguing cognitive tasks found that participants with CFS/ME showed significantly increased and decreased activation in several cortical and subcortical regions [108, 110, 392]. There is a sparsity of fMRI studies that have investigated working memory impairment in CFS/ME.

Caseras et al. (2006) used an n-back task and reported both increases and decreases in BOLD activations of task-specific brain regions in CFS/ME compared to the healthy controls. During the complex and more challenging conditions, participants with CFS/ME showed a decrease in activation in dorsolateral prefrontal and parietal cortices. This reduction in activation was interpreted as when the task gets more difficult; participants with CFS/ME failed to recruit working memory regions, dorsolateral prefrontal and parietal cortices, to the same level as the healthy controls [109]. They reported that in the 2- and 3-back conditions, participants with CFS/ME had increased activation in the right inferior/medial temporal cortex [109]. They suggested this increase in activation might be a compensatory strategy of the working memory neural network when impaired or saturated [108-110, 392]. During the PASAT task, participants with CFS/ME demonstrated a significant increase in BOLD signal in bilateral premotor and left superior parietal regions [452]. The differences in results from these studies might be due to the task used. According to Caseras et al. (2006), they suggested that the 2- and 3-back conditions might not be demanding enough to show differences between the groups in term of behavioural level but still enough to show differences at neurophysiological level [109]. The N-back task has been argued not to be a pure measurement of working memory but might be able to detect differences in cognitive functioning [76]. PASAT, on the other hand, is highly susceptible to practice effects. Tombaugh (2006) discussed PASAT intensively and showed that it is negatively affected by several factors, including ageing, low math ability, and

decreased IQ. Tombaugh (2006) also illustrated that administration of this task puts the participant under an enormous amount of anxiety and frustration, which affects their performance on PASAT itself and other neuropsychological tests. Tombaugh (2006) argued that PASAT is a non-specific highly sensitive test in which caution must be taken in identifying the reason underlying any low scores before considering it as clinically significant [453].

According to the Baddeley model, complex span tasks' cognitive demands would be maintained by different working memory components [40]. Each component has separate constraints linked with the specific functions they provide. For instance, domain-specific storage would be supported by the visuospatial sketchpad and the phonological loop. On the other hand, processing would depend on domain-general resources. In this model, the coordination between processing and storage operations is supported by the central executive component [40, 454]. Bayliss et al. (2003) proposed a task to measure the performance of working memory in terms of processing and storage components separately and combined [33]. Bayliss et al. (2003) result supports the notion that there are two constraints of working memory performance. These two constrains are short-term memory capacity and processing efficiency. They discovered a residual variance in working memory function, which was not explained by the two constraints. The importance of this residual variance is that it can predict the measures of intelligence and educational attainment even after accounting for storage capacity and processing efficiency. According to Bayliss et al. (2003), the residual variance represents an executive component and comes from the need to combine storage and processing [33]. It has been suggested that the residual variance represents the information forgotten rate [77]. This argument opens the debate over the contribution of the residual variance and whether the residual variance is represented by an executive component or other contributing factors. Therefore, neuroimaging may be a suitable technique to explore the brain regions that underlie the short-term memory capacity, processing efficiency and residual variance. It would allow for the examination of whether brain regions such as dorsolateral prefrontal and parietal cortices, are executive or not and whether the residual variance is domain-general.

The use of the Bayliss et al. (2003) task [33] in investigating CFS/ME has the advantage that it is distinct from other tasks, which only measure processing or storage separately. This is because it is important to understand how the brain of participants with CFS/ME work during each task and when they are combined [33]. It can be argued that participants with CFS/ME will perform at the same level as the healthy controls when doing a task which measures

processing or storage alone. However, when they need to do both tasks together, the differences will appear, causing the activation of additional brain regions such as bilateral supplementary and premotor and left superior parietal regions [108]. Therefore, the use of the Bayliss et al. (2003) task allowed the investigation of each function alone and when they are combined to illustrate any differences between participants with CFS/ME and the healthy controls. In this chapter, fMRI was used to investigate the cognitive dysfunction in participants with CFS/ME using the Bayliss et al. (2003) task, which is specifically designed to identify and distinguish subcomponents of working memory [33].

This chapter aimed to illustrate the differences between participants with CFS/ME and the healthy controls in terms of behavioural performance and neural activity during processing and storage tasks separately and combined. By using fMRI and Bayliss et al. (2003) tasks, the residual variance in working memory can be addressed and what it represents beyond processing efficiency and storage capacity. This chapter hypothesised to find differences between the two groups in terms of (1) better performance by the healthy controls group compared to the CFS/ME group, especially in processing speed [455], (2) increased activation in different areas of the brain as evidence of neural compensatory mechanism in participants with CFS/ME once they fatigued [108, 110, 111], and (3) the healthy controls would show overlapping activations within prefrontal, cingulate, and parietal cortices as a result of supporting encoding and maintenance, as well as coordinating the simultaneous loads of storage and processing [49].

7.3 Methods

7.3.1 Participants

Ethics approval for this study was approved by the National Research Ethics Committee (NREC), Wales REC 6 committee (REC reference 17/WA/0401 IRAS project ID 236212) and participants were recruited from the CFS/ME clinic at the Cossham hospital in Bristol. The CDC Criteria was used to recruit participants with CFS/ME [1] into this study. All participants gave informed consent before taking part (Appendix no 3a for CFS/ME and 3b for healthy controls). Participants with neurological disorders from both groups were excluded. Participants with anxiety or depression after assessment with the Hospital Anxiety and Depression Scale (HADS) (appendix no 1) [355] who scored more than 12 in each scale were excluded. All participants fully performed all three tasks.

7.3.2 Questionnaires

The Chalder Fatigue Questionnaire was used as a valid measure for fatigue in adults with CFS/ME [214]. This questionnaire is based on the individual's symptoms during the previous month. The questionnaire provides 11 questions with 5 rating options ranging from 1 (less than usual) to 5 (much more than usual). The result of this questionnaire is then reported as a sum of the 11 items on a 0±3 Likert scale, so it ranges from 0 (less severe fatigue) to 33 (more severe fatigue).

7.3.3 Working Memory fMRI Paradigm

The participants were required to perform three tasks (Figures 10, 11, and 12 above). These three tasks are designed to fractionate working memory components by using processing, storage, and both together in a complex verbal working memory task. MATLAB (2012a) was used to display the stimulus and record responses using MR compatible Lumina response pads. Also, the accuracy and reaction times were recorded. In all tasks, the stimuli were displayed in four blocks of five trials where each block was then separated by 20 seconds to allow the haemodynamic response to return to baseline [456]. The presented stimuli were refreshed after each trial, so to change the location of colours and numbers [456].

7.3.4 Image Acquisition

The MRI scans were performed on 3 Tesla Siemens Magnetom Skyra MRI scanner using a 32 channel radiofrequency head coil. Each participant's scan consisted of 3 fMRI runs, which were acquired using the T2*-weighted gradient echo-planar imaging. Moreover, each run took 8 to 10 minutes, depending on the participant's reaction time. In total, 36 slices covering the whole brain and positioned parallel to the anterior-posterior commissure plane were acquired. The functional scans had the following parameters: time to repetition (TR): 2.5s; time to echo (TE): 30ms; matrix: 64x64; the field of view (FoV): 240; voxel size: 3x3x3mm3; scans were obtained in the axial plane. A T1-weighted inversion recovery gradient echo MPRAGE was also obtained in the sagittal plane, consisting of 192 slices; TR: 1900ms; TE: 2.2ms; 0.9mm isotropic voxel; matrix: 128x128 and used for co-registration.

7.3.5 Behavioural Data Analysis

The correct response in terms of accuracy in percentage and reaction times in seconds were calculated for each task and for the individual participants. Two-sample t-tests were conducted for all recognition phases to compare the accuracy and reaction time of the two groups. The significance level was set at $p<.05$. Also, the correlation between CFQ with accuracy and

reaction time was performed to investigate the effect of fatigue in both the accuracy and reaction time.

7.3.6 Pre-processing and Analysis of fMRI Data

MATLAB (MathWorks Inc., Natick, MA, USA) was used in pre-processing, and the analysis of fMRI data was done using the statistical parametric mapping package SPM12 (Welcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm). Each task: processing, verbal storage and complex verbal tasks, was split into two phases consisting of presentation and recognition so to have processing presentation (PP), processing recognition (PR), verbal storage presentation (VSP), verbal storage recognition (VSR), complex verbal presentation (CVP) and complex verbal recognition (CVR). To correct for head movements, slice timing correction, co-registration, segmentation, image normalisation into standard space based on the MNI template and smoothing using 8mm full-width half-maximum (FWHM) Gaussian kernel, the pre-processing steps were realigned, which also accounts for residual inter-subject differences.

DICOM images were imported to SPM12 so to convert them into NFTI format that SPM can use. Then, images were realigned to account for brain movement effect across and within sessions of each subject. After that, images were co-registered so to link the functional images to the structural images. Then, normalisation was performed to warp each specific subject's images into standard space on the MNI template. Finally, smoothing was performed by applying a smoothing filter to all images to account for individual subject spatial differences, especially at the group analysis stage. Each phase (presentation and recognition) was analysed separately, and the analysis was performed according to the event-related task design.

In the 1st level analysis, each participant's images in the three tasks were separated into two phases, the presentation phase and recognition phase. Unit of the design was set to be seconds, and the interscan interval time, which is the TR used was set on 2.5 seconds. The microtime resolution and onset were left as the default at 16 and 8 respectively. Data and design were replicated six times for each participant at each phase, i.e., presentation and recognition phases for each task. In the presentation phase for each task, the onset time was defined as the time of the first stimulus, while the duration was calculated to be the time between the first and last stimulus of each stimulus group for each participant (before the start of the blank screen). Therefore, it is around 25 seconds for the presentation phase of the processing task, around 7 seconds for the presentation phase of the storage task, and around 13 seconds in the presentation phase of the complex task. This is done to prevent analysing the images while the

blank screen was shown. Movement parameters were added as multiple regressors, and all other parameters, high-pass filter, no model derivation, masking threshold, and serial correlation, were left as the default.

In the recognition part of the 1st level analysis, the unit of the design was set again on seconds with the same interscan interval of 2.5 seconds. All the data was treated the same as the previous part except for the onset and duration of the tasks. In the recognition phase of the processing task, the onset used were all the correctly answered onsets, where the stimulus appeared to the participant. The duration used were the reaction time which represents the time when the participants responded to the stimulus. Movement parameters were added as multiple regressors, and all other fields were left as the default. The same was done to the other two recognition phases, storage recognition and complex recognition. The rest of the fields were left as the defaults used.

In the 2nd level analysis, a comparison between each contrast in the CFS/ME group to the healthy controls group was performed by conducting two-sample t-tests to all contrasts for each group to directly test for differences between participants with CFS/ME and the healthy controls. In addition, one sample-test was performed for each group individually. Also, multiple regression was performed to show the correlation between brain activity change, reaction time and accuracy. The resulting coordinates from all these analyses were converted using GingerALE [356] from MNI space to Talairach space. As Talairach software [357] can be used to label multiple brain regions by knowing their coordinates, these coordinates were converted to brain regions for the comparison between participants with CFS/ME and the healthy controls.

7.4 RESULTS

7.4.1 Participants

The original CFS/ME sample size was 58 participants. However, 16 declined attending the MRI scan due to fatigue and not feeling well. Therefore, forty-two participants with CFS/ME (36 females, six males) met the CDC criteria for CFS/ME [1], and 40 healthy controls (33 females, seven males) were recruited for this study. Participants were well matched on age and sex with the control group. The CFS/ME group had a mean age of 36.57 years, SD=10.80 and the healthy controls group had a mean age of 33.22 years, SD=11.76 (see table 21).

Table 21 shows the mean and standard deviation of participants demographic and the participants with CFS/ME length of illness.

	HC	CFS/ME	t	df	p
Age	33.22 (11.76)	36.57 (10.80)	-1.34	80	.183
Gender	33 females, seven males	36 females, six males			
Length of illness	NA	Mean= 5.69 (5.73) years Median= 3.5 years			

7.4.2 Behavioural results

- Accuracy (ACC)

Figure 22 shows the accuracy performance values, in percentages, for participants with CFS/ME and the healthy controls. Participants with CFS/ME performed significantly worse than the healthy controls in the recognition phase of the complex verbal task with the presence of a very large effect, as shown by the two-sample t-test (see figure 22 and table 22).

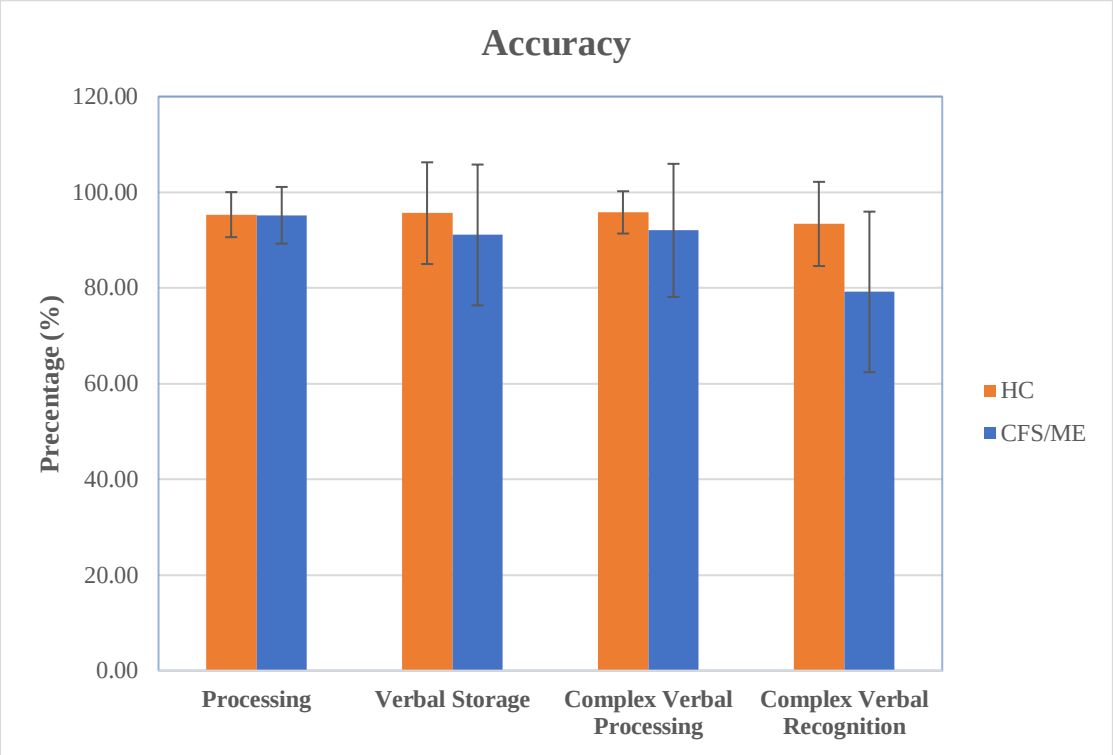


Figure 22 shows participants Accuracy (in percentage) as well as the standard error (in percentage).

Table 22 shows the mean and standard deviation of participants accuracy performance in all tasks.

Accuracy	HC	CFS/ME	t	df	p	Cohen's d
Processing	95.31 (4.70)	95.17 (5.93)	.133	80	.91	.02
Verbal storage	95.62 (10.63)	91.07 (14.71)	1.599	80	.11	.35
Complex Verbal (Presentation)	95.78 (4.41)	92.02 (13.91)	1.634	80	.10	.36
Complex Verbal (Recognition)	93.37 (8.79)	79.16 (16.78)	4.834	62.60	.000	1.06

- Reaction Time (RT)

Figure 23 shows the reaction time values, in seconds, for participants with CFS/ME and healthy controls. Participants with CFS/ME were significantly slower than the healthy controls in the recognition phase of the verbal storage task, the presentation phase of the complex verbal task, and the recognition phase of the complex verbal task (see figure 23 and table 23). However, there was no significant difference between participants with CFS/ME and the healthy controls in the processing task.

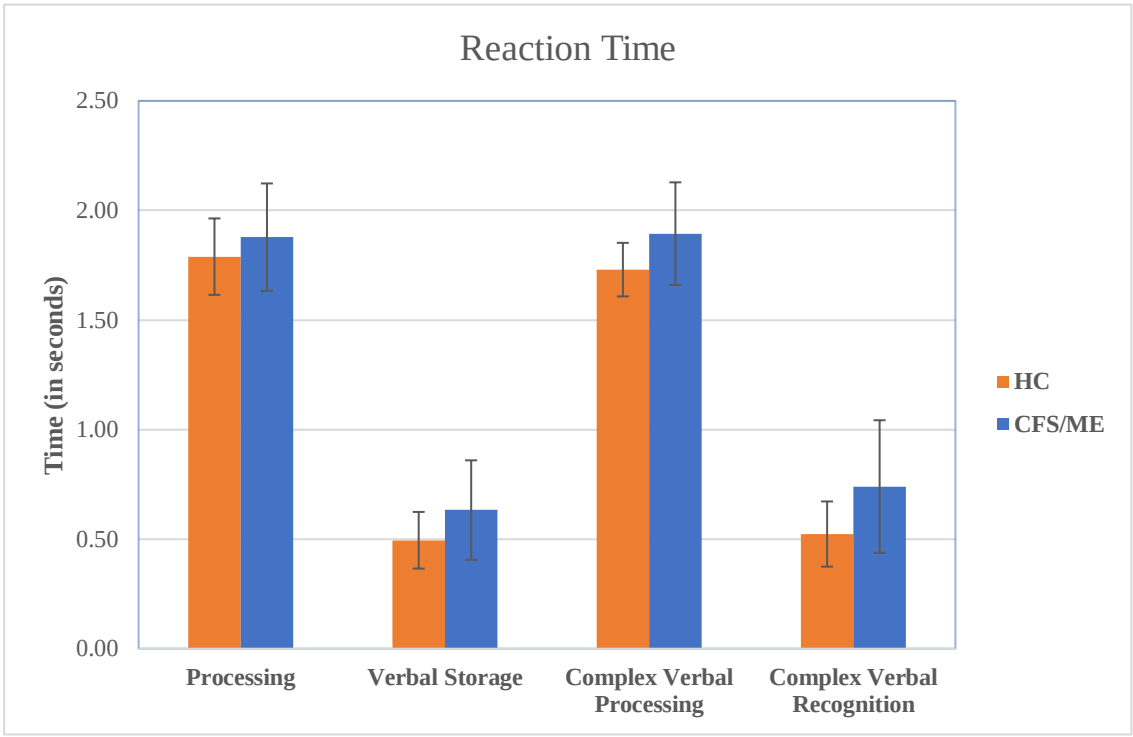


Figure 23 shows participants reaction time (in seconds) as well as their standard error (in seconds).

Table 23 shows the mean and standard deviation of participants reaction times in all tasks.

Reaction time	HC	CFS/ME	t	df	p	Cohen's d
Processing	1.78 (.17)	1.87 (.24)	-1.87	80	.64	.43
Verbal storage	.49 (.12)	.63 (.22)	-3.36	80	.001	.79
Complex Verbal (Presentation)	1.72 (.12)	1.89 (.23)	-4.04	62.38	.000	.92
Complex Verbal (Recognition)	.52 (.14)	.73 (.30)	-4.12	60.37	.000	.88

- Correlation between CFQ and RT

Table 24 below shows the correlation between CFQ and RT in both participants with CFS/ME and the healthy controls. Significant positive correlations were found in all tasks between the fatigue scores and reaction time in both groups. (see table 24).

- Correlation between CFQ and accuracy

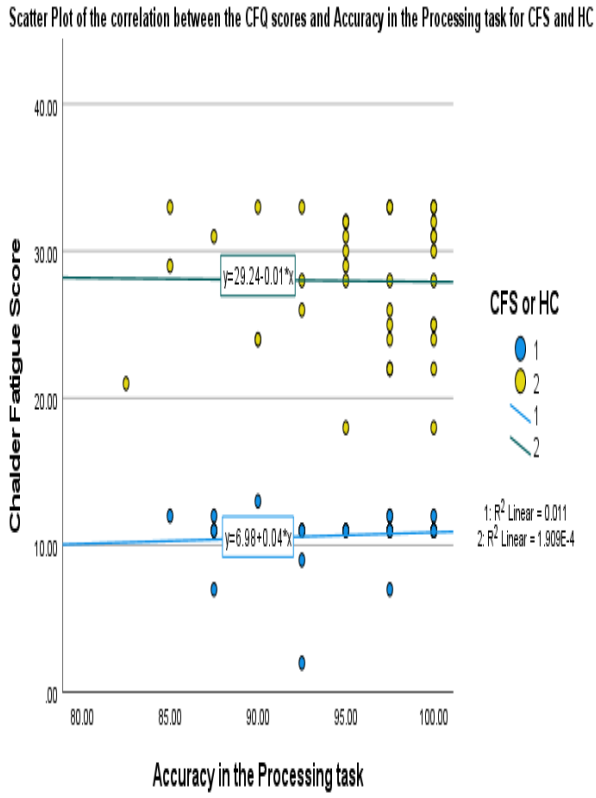
Table 24 shows the correlation between CFQ and reaction time in both participants with CFS/ME and the healthy controls. Significant negative correlations were found between CFQ and accuracy in only the complex task (see table 24).

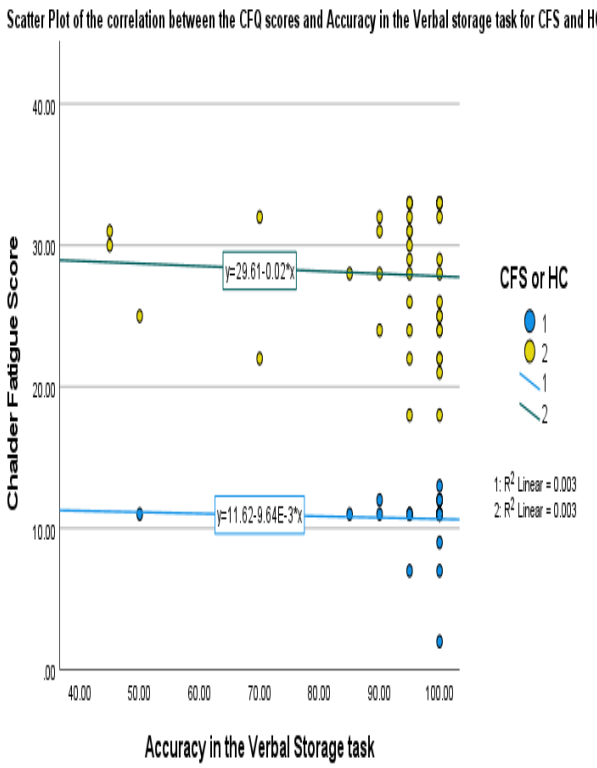
Table 24 shows the correlation between the CFQ and reaction time, as well as the correlation between the CFQ and accuracy in this sample size.

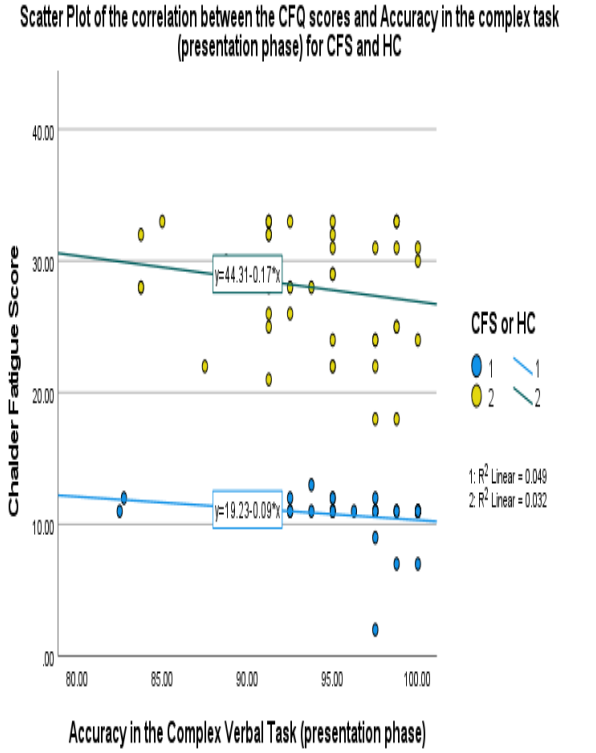
		RT				ACC			
		P	**VS**	**CVP**	**CVR**	**P**	**VS**	**CVP**	**CVR**
CFQ	**Pearson Correlation**	.27	.45	.46	.48	-.02	-.20	-.13	-.52
	Sig. (2-tailed)	.013	.000	.000	.000	.82	.06	.22	.000
	N	82	82	82	82	82	82	82	82

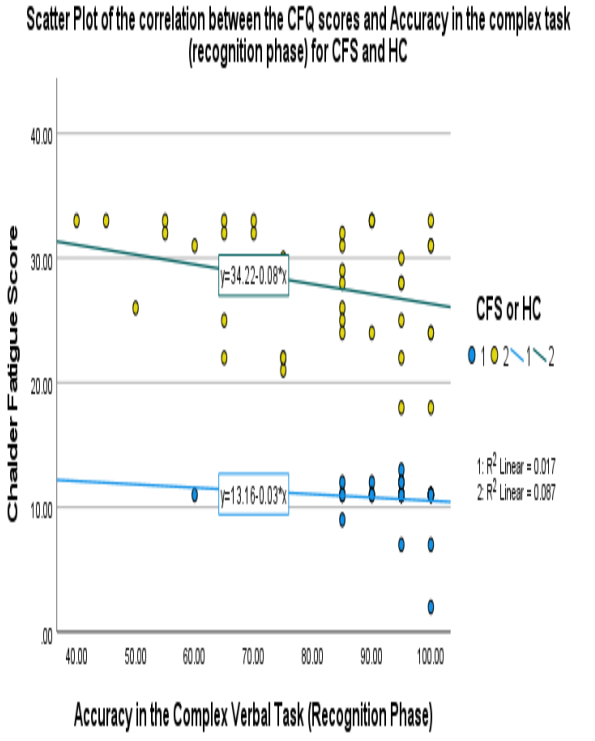
P=Processing. VS=Verbal Storage. CVP=Complex Verbal (presentation phase). CVR=Complex Verbal (recognition phase). ACC=Accuracy. RT=Reaction time. CFQ=Chalder Fatigue Questionnaire score.

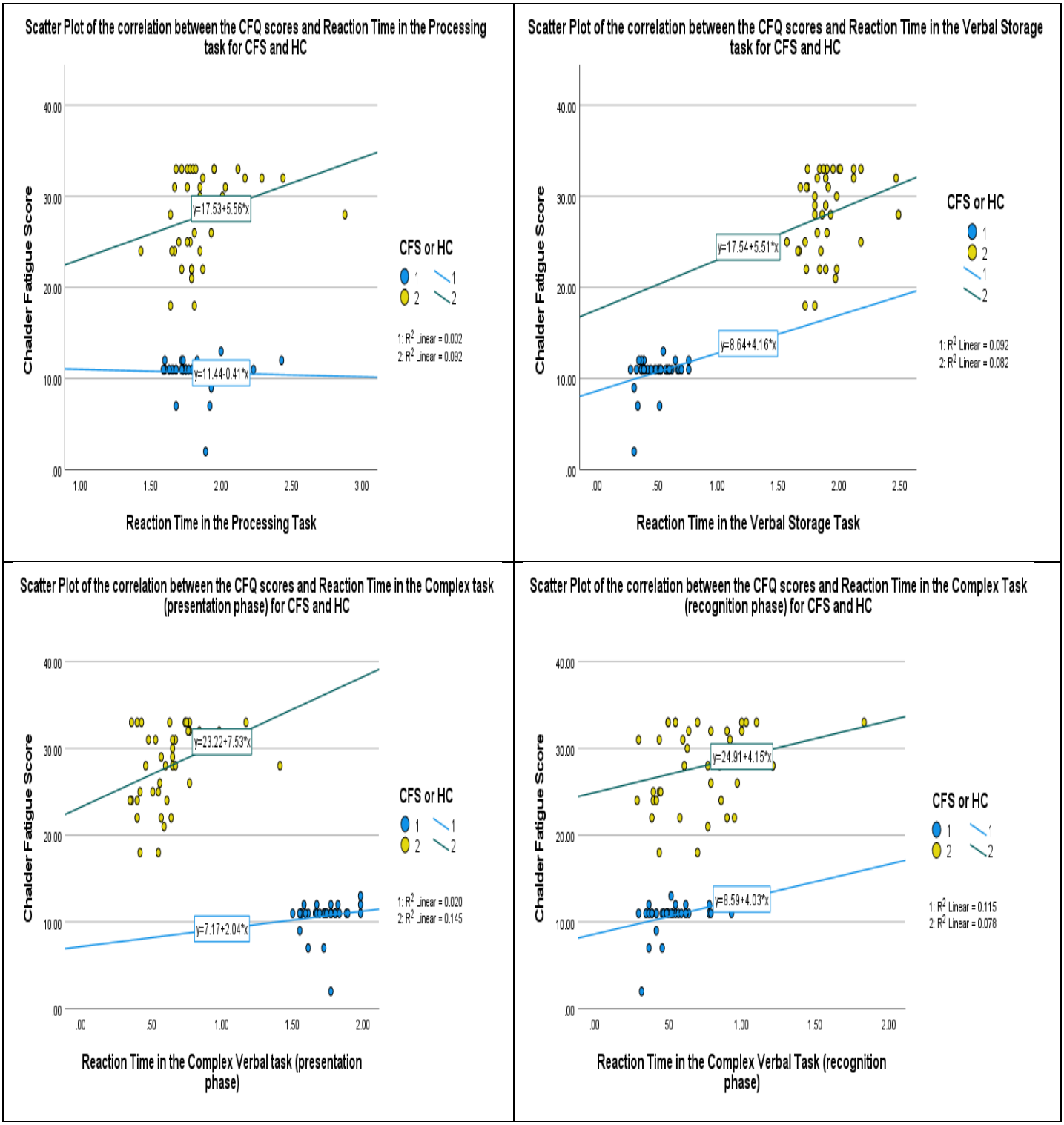
Table 25 shows the correlations between the CFQ with participants' accuracy and reaction time in all tasks for both groups.










Scatter Plot of the correlation between the CFQ scores and Reaction Time in the Processing task for CFS and HC
Chalder Fatigue Score
Reaction Time in the Processing Task
y=17.53+5.56*x
y=11.44-0.41*x
CFS or HC
1: R2 Linear = 0.002
2: R2 Linear = 0.092
Scatter Plot of the correlation between the CFQ scores and Reaction Time in the Verbal Storage task for CFS and HC
Chalder Fatigue Score
Reaction Time in the Verbal Storage Task
y=17.54+5.51*x
y=8.64+4.16*x
CFS or HC
1: R2 Linear = 0.092
2: R2 Linear = 0.082
Scatter Plot of the correlation between the CFQ scores and Reaction Time in the Complex task (presentation phase) for CFS and HC
Chalder Fatigue Score
Reaction Time in the Complex Verbal task (presentation phase)
y=23.22+7.53*x
y=7.17+2.04*x
CFS or HC
1: R2 Linear = 0.020
2: R2 Linear = 0.145
Scatter Plot of the correlation between the CFQ scores and Reaction Time in the Complex Task (recognition phase) for CFS and HC
Chalder Fatigue Score
Reaction Time in the Complex Verbal Task (recognition phase)
y=24.91+4.15*x
y=8.59+4.03*x
CFS or HC
1: R2 Linear = 0.115
2: R2 Linear = 0.078

7.4.3 Neuroimaging Results

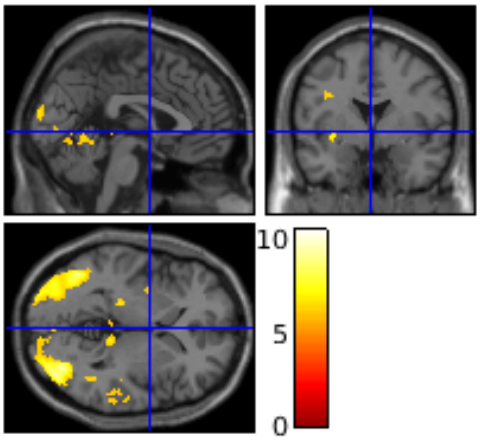
- Comparison Between the Groups

Two sample t-tests were carried out to compare the groups in all the tasks and resulted in no significant differences between the groups in any of the tasks. Therefore, individual groups activated brain regions were reported in images for all tasks.

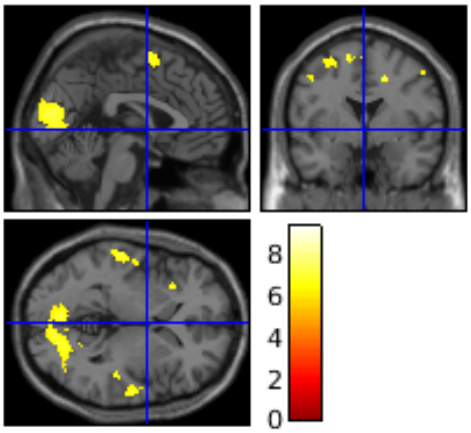
- Processing task

One sample t-test was carried out to map the BOLD activation of the processing task on the brain regions that underlie processing performance in both the presentation and recognition phases, PP and PR, for each group. Figure 24 shows examples of some brain regions activated in the presentation and recognition phases of the processing task mapped on brain anatomy for each group. BOLD signals showed increased activation in bilateral superior temporal gyri in both groups, which is the location of the primary auditory cortex, corresponding well with the study design when the words were presented aurally (see tables 26, 27, 28 and 29). All activations that survived whole-brain family-wise error correction (FWE) at the local maxima $p<0.05$ in t score in the processing task are presented in tables 26 and 27 for participants with CFS/ME and tables 28 and 29 for the healthy controls.

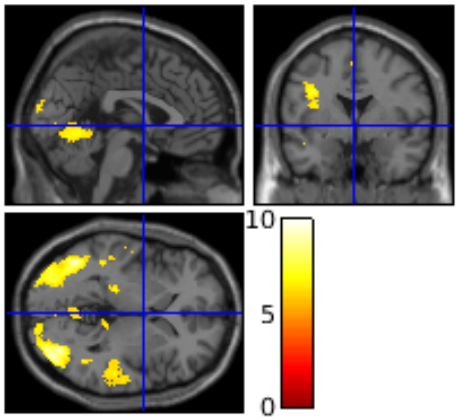
Brain activated regions in the presentation phase of the processing task in participants with CFS/ME



Brain activated regions in the presentation phase of the processing task in HC



Brain activated regions in the recognition phase of the processing task in participants with CFS/ME



Brain activated regions in the recognition phase of the processing task in HC

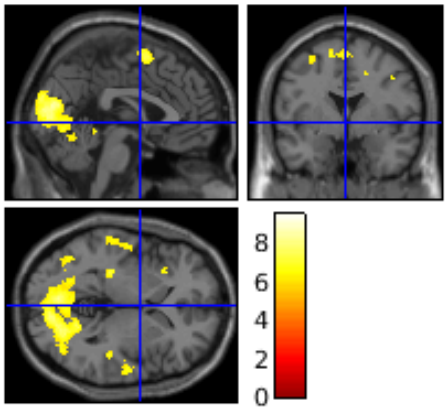


Figure 24 shows brain regions activated during the presentation and recognition phases of the processing task in both participants with CFS/ME and the healthy controls.

Table 26 shows areas of activation in participants with CFS/ME during the presentation phase of the processing task (p threshold= .05 FWE corrected; BA= Brodmann's Area).

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Sub-Gyral	Brodmann area 40	28.5	-69.83	-2.12	10.6
			22.03	-56.16	54.27	7.56
			35.49	-45.5	1.04	6.67
			18.91	-22.16	53.98	6.16
			17.21	-18.7	54.57	6.06
			27.31	-43.54	53.54	5.99
Right	Lingual Gyrus	*	33.71	-72.69	-5.75	9.3
			23.21	-77.59	1.17	9.1
Right	Postcentral Gyrus	Brodmann area 40 Brodmann area 3	52.4	-25.57	16.81	8.39
			22.15	-26.92	59.76	6.11
			48.83	-18.19	24.62	6.05
			23.83	-25.53	61.71	6
Right	Insula	Brodmann area 13	37.03	-25.07	14.74	6.93
Right	Superior Temporal Gyrus	Brodmann area 41	45.75	-29.06	7.74	6.66
Right	Precuneus	Brodmann area 7	18.61	-64.4	50.97	7.76
			20.56	-69.4	40.28	6.91
			25.62	-46.64	51.26	6.28
			20.62	-75.64	35.84	5.98
			24.07	-47.08	44.58	5.97
			23.97	-52.88	46.77	5.95
Right	Declive	*	11.79	-71.61	-19.4	6.35
Right	Declive	*	8.29	-70.48	-16.02	6.01
Right	Extra-Nuclear	*	13	-28.53	14.91	6.31
Right	Thalamus	Pulvinar	13.09	-27.9	11.76	6.1
Right	Fusiform Gyrus	*	37.61	-49.23	-15.97	6.03
Right	Fusiform Gyrus	*	32.19	-57.95	-7.96	5.99
Right	Extra-Nuclear	*	16.43	-28.57	15.02	5.95
Left	Middle Occipital Gyrus	*	-23.51	-82.26	16.73	9.29
Left	Sub-Gyral	*	-41.62	-56.63	-3.67	9.22
			-35.23	-3.29	32.74	6.18
			-41.85	-1.62	24.64	6.15
Left	Fusiform Gyrus	Brodmann area 19	-36.41	-71.22	-11.13	8.69
Left	Extra-Nuclear	*	-31.03	-1.06	3.81	7.5
			-29.69	-20.51	11.69	6.35

			-33.32	-8.61	23.62	5.96
Left	Putamen	*	-29.48	-7.19	7.64	7.06
			-27.91	-13.33	11.45	6.27
Left	Thalamus	*	-19.23	-24.2	3.16	6.89
			-19.33	-31.72	5.03	5.92
Left	Precentral Gyrus	Brodmann area 6	-43.69	-0.84	29.64	6.57
		*	-36.84	-10.93	26.34	6.49
		*	-27.55	-26.64	59.75	6.36
		Brodmann area 4	-30.92	-21.12	59.03	6.35
		*	-30.98	-26.59	59.64	5.99
Left	Culmen	*	-2.09	-52.7	-6.5	6.43
			-1.97	-45.19	-8.37	6.31
			-2.08	-62.7	-10.01	6.11
			-34.36	-50.41	-17	5.96
			-2.21	-61.94	-4.96	5.94
Left	Postcentral Gyrus	Brodmann area	-49.04	-20.65	30.66	6.34
			-36	-29.02	54.1	6.01
			-34.06	-24.01	46.92	5.99
Left	Medial Frontal Gyrus	Brodmann area 6	-10.07	-9.14	52.16	6.07
Left	Insula	Brodmann area 13	-36.71	-8.25	21.94	5.94

Table 27 shows areas of activation in participants with CFS/ME during the recognition phase of the processing task (p threshold= .05 FWE corrected; BA= Brodmann's Area).

Hemisphere	**Brain Region**	**BA**	**MNI Coordinate (x,y,z)**			**T Value**
Right	Sub-Gyral	*	32.09	-72.9	-0.29	9.69
			39.56	-54.2	-0.18	6.71
			26.09	-48.8	41.54	6.01
			20.39	-17	62.47	6.01
Right	Inferior Occipital Gyrus	*	37.68	-76.3	-4.12	9.1
			32.11	-81.9	-4.74	8.89
Right	Transverse Temporal Gyrus	Brodmann area 41	50.6	-25.4	13.53	9.56
			41.35	-23.5	13.56	8.95
Right	Postcentral Gyrus	Brodmann area 40	57.99	-23.8	15.62	8.99
Right	Superior Parietal Lobule	Brodmann area 7	22.12	-61.7	58.26	7.98
			27.68	-50.7	61.2	7.61
			24.06	-64.7	50.8	7.59
Right	Precentral Gyrus	*	25.91	-20.9	64	6.75
			26.03	-18.1	55.25	6.34
			33.34	-18.9	62.51	6.23
Right	Declive	*	13.8	-76.7	-19	6.33
			17.48	-75	-17	6.07
Right	Extra-Nuclear	*	22.81	-29.2	14.51	6.15
Right	Precuneus	Brodmann area 7	24.12	-75.2	42.6	6
Right	Medial Frontal Gyrus	*	16.76	-12.7	57.41	5.98
Right	Culmen	*	4.43	-50.1	-2.19	5.92
Left	Sub-Gyral	Brodmann area 13	-43.7	-61.2	-2.26	10.09
Left			-43.4	-0.15	-10.9	6.16
Left	Middle Occipital Gyrus	*	-27.3	-77.6	10.88	9.69
			-38.2	-75.6	-8.93	9.1
Left	Thalamus	*	-21.5	-24.2	3.42	7.57
			-16.1	-29	13.87	6.36
			-19.7	-31.8	4.53	6.12
Left	Culmen	*	-2.91	-62.4	-10.7	7.17
			-1.09	-55.3	-6.38	7.04
			-4.81	-46.3	-1.99	5.94
			-10.4	-68.1	-9.56	5.93
Left	Culmen of Vermis	*	-1.16	-64.9	-3.7	6.61
Left	Lentiform Nucleus	Putamen	-28.9	-15.4	9.54	7.08
Left	Insula	Brodmann area 13	-29	-21.5	14.36	6.13
			-41.8	-16.8	5.58	6.28

			-41.9	-8.19	13.6	5.99
Left	Extra-Nuclear	*	-30.7	-5.69	6.82	6.51
Left	Precentral Gyrus	*	-27.8	-20.8	64.9	6.05
			-34.9	-11.5	47.64	5.97
Left	Medial Frontal Gyrus	Brodmann area 6	-3.55	-4.95	56	6
Left	Superior Temporal Gyrus	Brodmann area 22	-56.6	-16.4	1.77	5.97
			-52.9	-10.8	2.36	5.94
Left	Lingual Gyrus	Brodmann area 18	-12.4	-78.3	-1.55	5.93

Table 28 shows areas of activation in the healthy controls during the presentation phase of the processing task (p threshold= .05 FWE corrected; BA= Brodmann's Area).

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Inferior Parietal Lobule	*	35.2	-40.72	55.07	9.39
						9.39
Right	Sub-Gyral	*	25.94	-15.11	62.74	7.85
			24.75	24.48	23.23	6.51
			28.44	20.73	22.94	6.14
			15.08	-11.45	44.89	6.03
Right	Precentral Gyrus	Brodmann area 4	33.33	-20.74	62.33	7.43
Right	Transverse Temporal Gyrus	Brodmann area 41	50.62	-28.99	11.4	8.97
			41.37	-25.22	11.6	8.21
Right	Superior Temporal Gyrus	*	54.47	-13.23	3.94	6.8
			39.53	-37.9	6.76	6.03
Right	Lingual Gyrus	*	6.14	-78.54	0.54	8.27
			15.49	-57.75	-0.93	6.21
Right	Cuneus	Brodmann area 17	17.12	-74.06	11.96	7.86
			22.4	-87.01	28.84	6.13
Right	Cingulate Gyrus	Brodmann area 24	15.12	-3.82	43.81	8.1
Right	Inferior Frontal Gyrus	*	52.41	3.49	25.31	6.45
Right	Declive	*	8.21	-64.12	-12.47	6.2
			23.01	-67.92	-12.58	6.01
			24.89	-65.89	-14.16	5.99
Right	Culmen	*	36.12	-52.21	-19.88	6.06
Right	Thalamus	*	11.71	-16.27	17.35	6.06
Right	Extra-Nuclear	*	19.13	-10.72	18	6.02
Right	Postcentral Gyrus	*	46.48	-28.56	45.6	6.02
Left	Sub-Gyral	*	-34.83	-41.82	30.36	8.85
			-39.95	18.06	14.32	8.19
			-40.09	17.01	25.03	7.96
			-29.26	-69.1	20.66	6.19
			-43.67	-60.65	-7.61	6.04
Left	Inferior Parietal Lobule	*	-42.47	-41.66	48.26	8.2
Left	Supramarginal Gyrus	*	-40.45	-38.59	35.97	8.15
Left	Lingual Gyrus	*	-8.73	-77.13	5.83	8.37
Left	Superior Temporal Gyrus	*	-53.1	-39.98	12.2	8.19
			-51.1	-16.94	7.21	8.08
Left	Transverse Temporal Gyrus	Brodmann area 41	-40.09	-24.97	12.04	7.66
Left	Insula	Brodmann area 13	-32.53	16.33	12.48	7.45
Left	Middle Frontal Gyrus	*	-14.71	-7.1	59.21	7.33
			-25.79	-8.73	57.06	7.01
Left	Superior Parietal Lobule	*	-27.8	-61.07	53.87	6.86
Left	Precuneus	*	-18.52	-68.22	49.75	6.63
Left			-22.13	-67.5	42.55	6.22
Left	Parahippocampal Gyrus	*	-23.33	-59.06	-5.32	6.41
Left	Precentral Gyrus	Brodmann area 6	-40.27	2.93	34.5	6.39
			-45.98	-5.54	44.41	6.17
			-22.24	-13.52	67.48	6.06
Left	Inferior Frontal Gyrus	Brodmann area 9	-53.11	3.86	25.37	6.37
Left	Declive	*	-36.14	-56.09	-16.06	6.32

Left	Postcentral Gyrus	Brodmann area 1	-46.14	-23.18	51.75	6.16
			-53.44	-16.86	45.02	6.08
			-38.86	-33.23	58.13	6.06
			-47.97	-21.14	50.11	6
Left	Cuneus	*	-22.03	-85.09	30.08	6.02
Left	Inferior Frontal Gyrus	*	-51.27	9.27	27.71	5.99

Table 29 shows areas of activation in the healthy controls during the recognition phase of the processing task (p threshold= .05 FWE corrected; BA= Brodmann's Area).

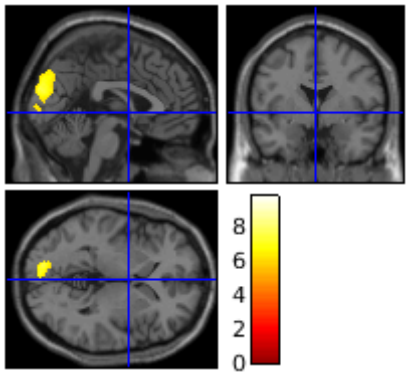
Hemisphere	**Brain Region**	**BA**	**MNI Coordinate (x,y,z)**			**T Value**
Right	Cingulate Gyrus	Brodmann area 24	15.12	-5.69	43.63	6.68
Right	Culmen	*	39.83	-50.4	-19.6	6.06
Right	Extra-Nuclear	*	19.11	-9.03	19.96	6.15
Right	Inferior Frontal Gyrus	*	54.29	5.52	23.74	6.32
			33.33	-40.9	56.82	9.35
Right	Lingual Gyrus	*	6.14	-78.5	0.54	9.24
Right	Postcentral Gyrus	*	44.56	-16.2	53.94	6.21
Right	Precentral Gyrus	Brodmann area 4	37.2	-10.4	52.57	6.69
			39.22	-5.46	40.46	6.47
			35.33	-16	52.01	6.05
Right	Precuneus	Brodmann area 7	26.09	-67.1	36.2	6.88
Right	Sub-Gyral	*	25.94	-15.1	62.74	7.52
			30.16	12.4	31.19	6.73
Right	Superior Parietal Lobule	*	25.88	-59.5	54.93	7.56
Right	Superior Temporal Gyrus	*	54.47	-13.2	3.94	6.79
Right	Thalamus	*	13.56	-18.1	17.2	6.4
Right	Transverse Temporal Gyrus	Brodmann area 41	50.62	-29	11.4	9.24
			41.37	-25.2	11.6	8.19
Left	Cerebellar Lingual	*	0.82	-40.21	-6.72	7.27
Left	Culmen	*	-2.89	-54.92	-9.98	6.07
Left	Cuneus	*	-5.2	-89.37	15.54	8.72
Left	Extra-Nuclear	*	-28.75	18.7	7.36	6.95
			-25.27	-30.46	9.97	6.5
Left	Fusiform Gyrus	*	-32.57	-78.99	-12.77	6.07
Left	Inferior Frontal Gyrus	*	-51.23	4.03	23.61	6.6
Left	Inferior Parietal Lobule	Brodmann area 40	-42.48	-38.11	50.4	9.58
			-40.5	-40.8	39.36	8.43
			-44.01	-37.52	25.2	6.12
Left	Insula	Brodmann area 13	-40.09	-6.69	17.38	6.39
Left	Lingual Gyrus	*	-8.73	-77.13	5.83	9.24
Left	Medial Frontal Gyrus	Brodmann area 6	-9.15	-3.4	59.65	7.23
			-14.69	-10.65	57.07	6.3
			-14.76	-7.45	62.78	6.86
Left	Middle Occipital Gyrus	*	-43.68	-71.65	-10.46	6.31
Left	Postcentral Gyrus	Brodmann area 3	-40.66	-23.73	57.19	6.04
Left	Precentral Gyrus	Brodmann area 6	-31.32	-8.53	55.19	6.91
			-36.93	-12.57	58.31	6.43
			-22.24	-13.52	67.48	6.11
Left	Sub-Gyral	*	-40.07	19.05	23.42	8.29
			-41.82	19.76	16.25	7.48
			-23.94	-16.02	54.6	6.43
			-29.2	-63.16	17.62	6.28
			-30.68	25.46	15.18	6.06
			-30.69	29.02	17.32	6.06
			-30.8	-66.83	-2.57	6.02
			-32.61	-68.33	-6.35	6
			-25.65	-42.39	35.86	6
Left	Superior Temporal Gyrus	*	-53.12	-45.57	11.67	8.29
			-54.95	-38.11	12.35	8.28
			-51.1	-16.94	7.21	8.18

Left	Supramarginal Gyrus	*	-36.77	-47.92	35.15	8.14
Left	Thalamus	*	-17.84	-10.18	13.82	6.23
			-16	-13.91	13.5	6.22
			-17.86	-19.49	12.94	6.13
			-14.18	-17.82	14.96	6.06

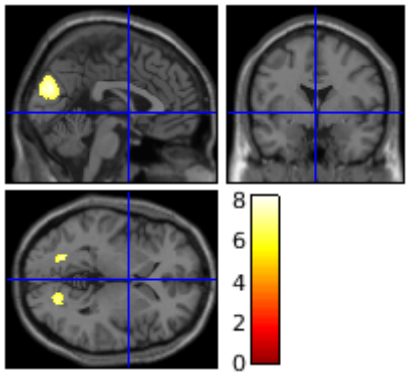
- Verbal Storage

One sample t-test was carried out to map the BOLD activation of the verbal storage task on the brain regions that underlie storage performance in both the presentation and recognition phases, VSP and VSR, for each group. Figure 25 shows examples of some brain regions activated in the presentation and recognition phases of the storage task mapped on brain anatomy for each group. The activations that survived whole-brain family-wise error correction (FWE) at the local maxima $p<0.05$ in t score in the verbal storage task are presented in tables 30 and 31 for participants with CFS/ME and tables 32 and 33 for the healthy controls.

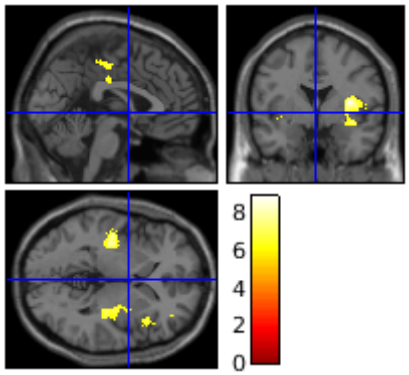
Brain activated regions in the presentation phase of the verbal storage task in participants with CFS/ME



Brain activated regions in the presentation phase of the verbal storage task in HC



Brain activated regions in the recognition phase of the verbal storage task in participants with CFS/ME



Brain activated regions in the recognition phase of the verbal storage task in HC

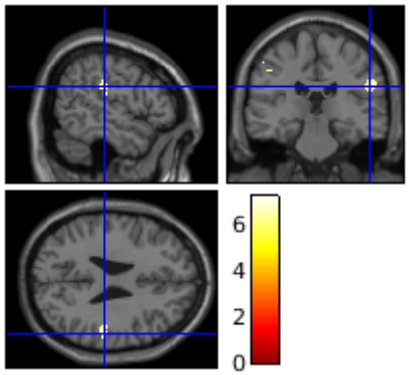


Figure 25 shows brain regions activated during the presentation and recognition phases of the verbal storage task in both participants with CFS/ME and the healthy controls.

Table 30 shows areas of activation in participants with CFS/ME during the presentation phase of the verbal storage task (p threshold= .05 FWE corrected; BA= Brodmann's Area).

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	*	*	-25.53	-3.34	6.06	Right
Right	Cingulate Gyrus	Brodmann area 24	-3.82	43.81	8.1	Right
Right	Culmen	*	-52.21	-19.88	6.06	Right
Right	Cuneus	Brodmann area 17	-74.06	11.96	7.86	Right
			-87.01	28.84	6.13	Right
Right	Declive	*	-64.12	-12.47	6.2	Right
			-67.92	-12.58	6.01	Right
			-65.89	-14.16	5.99	Right
Right	Extra-Nuclear	*	-10.72	18	6.02	Right
Right	Inferior Frontal Gyrus	*	3.49	25.31	6.45	Right
Right	Inferior Parietal Lobule	*	-40.72	55.07	9.39	Right
Right	Lingual Gyrus	*	-78.54	0.54	8.27	Right
			-57.75	-0.93	6.21	Right
Right	Postcentral Gyrus	*	-28.56	45.6	6.02	Right
Right	Precentral Gyrus	Brodmann area 4	-20.74	62.33	7.43	Right
Right	Sub-Gyral	*	-15.11	62.74	7.85	Right
			24.48	23.23	6.51	Right
			20.73	22.94	6.14	Right
			-11.45	44.89	6.03	Right
Right	Superior Temporal Gyrus	*	-13.23	3.94	6.8	Right
Right	Superior Temporal Gyrus	*	-37.9	6.76	6.03	Right
Right	Thalamus	*	-16.27	17.35	6.06	Right
Right	Transverse Temporal Gyrus	Brodmann area 41	-28.99	11.4	8.97	Right
			-25.22	11.6	8.21	Right
Left	Declive	*	-56.09	-16.06	6.32	Left
Left	Sub-Gyral	*	-41.82	30.36	8.85	Left
Left	Inferior Parietal Lobule	*	-41.66	48.26	8.2	Left
Left	Supramarginal Gyrus	*	-38.59	35.97	8.15	Left
Left	Lingual Gyrus	*	-77.13	5.83	8.37	Left
Left	Superior Temporal Gyrus	*	-39.98	12.2	8.19	Left
		*	-16.94	7.21	8.08	Left
Left	Transverse Temporal Gyrus	Brodmann area 41	-24.97	12.04	7.66	Left
Left	Sub-Gyral	*	18.06	14.32	8.19	Left
			17.01	25.03	7.96	Left
Left	Insula	Brodmann area 13	16.33	12.48	7.45	Left
Left	Middle Frontal Gyrus	*	-7.1	59.21	7.33	Left
			-8.73	57.06	7.01	Left
Left	Superior Parietal Lobule	*	-61.07	53.87	6.86	Left
Left	Precuneus	*	-68.22	49.75	6.63	Left

			-67.5	42.55	6.22	Left
Left	Parahippocampal Gyrus	*	-59.06	-5.32	6.41	Left
Left	Precentral Gyrus	*	2.93	34.5	6.39	Left
Left	Inferior Frontal Gyrus	Brodmann area 9	3.86	25.37	6.37	Left
Left	Sub-Gyral	*	-69.1	20.66	6.19	Left
Left	Precentral Gyrus	*	-5.54	44.41	6.17	Left
Left	Postcentral Gyrus	*	-23.18	51.75	6.16	Left
		Brodmann area 1	-16.86	45.02	6.08	Left
		Brodmann area 6	-13.52	67.48	6.06	Left
		*	-33.23	58.13	6.06	Left
Left	Sub-Gyral	*	-60.65	-7.61	6.04	Left
Left	Cuneus	*	-85.09	30.08	6.02	Left
Left	Postcentral Gyrus	*	-21.14	50.11	6	Left
Left	Inferior Frontal Gyrus	*	9.27	27.71	5.99	Left

Table 31 shows areas of activation in participants with CFS/ME during the recognition phase of the verbal storage task (p threshold= .05 FWE corrected; BA= Brodmann's Area).

Hemisphere	**Brain Region**	**BA**	**MNI Coordinate (x,y,z)**			**T Value**
Right	Cingulate Gyrus	Brodmann area 23	11.38	-20.57	42.16	6.71
			4.12	-17.63	31.5	6.43
Right	Extra-Nuclear	*	30.32	-2.8	13.53	8.79
Right	Inferior Parietal Lobule	Brodmann area 40	50.3	-34.98	34.24	8.86
Right	Insula	*	37.89	14.8	6.32	7
			45.32	-3.35	-0.68	5.97
Right	Lentiform Nucleus	Putamen	28.65	-14.27	-3.8	8.79
Right	Middle Frontal Gyrus	Brodmann area 10	36.05	38.68	12.16	7.05
Right	Paracentral Lobule	Brodmann area 31	2.06	-26.46	45.05	7.76
Right	Postcentral Gyrus	*	12.86	-45.2	63.27	6.36
Right	Sub-Gyral	*	16.66	-33.52	59.04	7.62
			36.14	37.52	4.84	6.14
			39.57	11.71	18.67	6.03
Right	Superior Parietal Lobule	*	24	-41.36	62.02	7.66
Right	Superior Temporal Gyrus	*	52.28	-48.68	20.37	6.09
Left	Cingulate Gyrus	Brodmann area 23	-8.95	-18.43	40.22	7.02
			-1.47	-23.36	32.67	6.8
Left	Extra-Nuclear	-34.4	-18.54	3.74	*	8.43
Left	Inferior Parietal Lobule	Brodmann area 40	-36.75	-31.33	38.52	6.89
			-51.65	-39.23	42.93	6.85
			-49.85	-50.59	43.68	6.14
			-51.66	-53.96	39.73	6.13
Left	Insula	Brodmann area 13	-38.2	-19.21	10.82	7.89

			-37.98	0.64	0.09	6.28
Left	Lentiform Nucleus	Putamen	-30.76	-15.35	9.51	7.63
Left	Postcentral Gyrus	*	-25.96	-35.51	61.73	6.96
Left	Sub-Gyral	*	-27.7	-40.22	52.25	7.42
Left	Superior Temporal Gyrus	*	-60.46	-37.73	8.69	6.11
Left	Supramarginal Gyrus	Brodmann area 40	-51.53	-40.22	33.83	6.04
			-55.18	-37.99	30.37	6.16
			-57.05	-54.57	26.97	6.12
			-58.85	-52.35	23.55	5.98
Left	Transverse Temporal Gyrus	*	-34.55	-30.59	11.61	6
Left	Cingulate Gyrus	Brodmann area 23	-8.95	-18.43	40.22	7.02
			-1.47	-23.36	32.67	6.8
Left	Extra-Nuclear	*	-34.4	-18.54	3.74	8.43
Left	Inferior Parietal Lobule	Brodmann area 40	-36.75	-31.33	38.52	6.89
			-51.65	-39.23	42.93	6.85
			-49.85	-50.59	43.68	6.14
			-51.66	-53.96	39.73	6.13
Left	Insula	Brodmann area 13	-38.2	-19.21	10.82	7.89
			-37.98	0.64	0.09	6.28
Left	Lentiform Nucleus	Putamen	-30.76	-15.35	9.51	7.63
Left	Postcentral Gyrus	*	-25.96	-35.51	61.73	6.96
Left	Sub-Gyral	*	-27.7	-40.22	52.25	7.42
Left	Superior Temporal Gyrus	*	-60.46	-37.73	8.69	6.11
Left	Supramarginal Gyrus	Brodmann area 40	-51.53	-40.22	33.83	6.04
			-55.18	-37.99	30.37	6.16
			-57.05	-54.57	26.97	6.12
			-58.85	-52.35	23.55	5.98
Left	Transverse Temporal Gyrus	*	-34.55	-30.59	11.61	6

Table 32 shows areas of activation in the healthy controls during the presentation phase of the verbal storage task (p threshold= .05 FWE corrected; BA= Brodmann's Area).

Hemisphere	**Brain Region**	**BA**	**MNI Coordinate (x,y,z)**			**T Value**
Right	Cuneus	Brodmann area 19	11.42	-84.22	19.91	8.08
			17.06	-78.13	15.18	7.63
			16.79	-81.91	34.64	6.05
Left	Cuneus	*	-3.34	-83.79	16.1	8.24
Left	Superior Parietal Lobule	Brodmann area 7	-20.44	-66.87	55.25	6.53
Left	Precentral Gyrus	Brodmann area 4	-42.43	-10.33	54.83	6.47

Table 33 shows areas of activation in the healthy controls during the recognition phase of the verbal storage task (p threshold= .05 FWE corrected; BA= Brodmann's Area).

Hemisphere	**Brain Region**	**BA**	**MNI Coordinate (x,y,z)**			**T Value**
Right	Inferior Parietal Lobule	*	48.58	-28.51	25.82	7.28

			46.37	-51.26	47.05	6.1
Right	Insula	Brodmann area 13	48.69	-24.09	19.03	6.3
Left	Postcentral Gyrus	Brodmann area 2	-46.02	-27.73	40.51	6.5
Left	Inferior Parietal Lobule	Brodmann area 40	-51.67	-30.26	47.38	6.42
			-56.95	-31.87	25.52	6.2
			-47.65	-31.57	22.1	6.18

- Complex Verbal Storage Task

One sample t-test was carried out to map the BOLD activation of the complex task on the brain regions that underlie complex task performance in both the presentation and recognition phases, CVP and CVR, for each group. Figure 26 shows examples of some brain regions activated in the presentation and recognition phases of the complex task mapped on brain anatomy for each group. The activations that survived whole-brain family-wise error correction (FWE) at the local maxima $p<0.05$ in t score in the complex task are presented in tables 34 and 35 for participants with CFS/ME and tables 36 and 37 for the healthy controls.

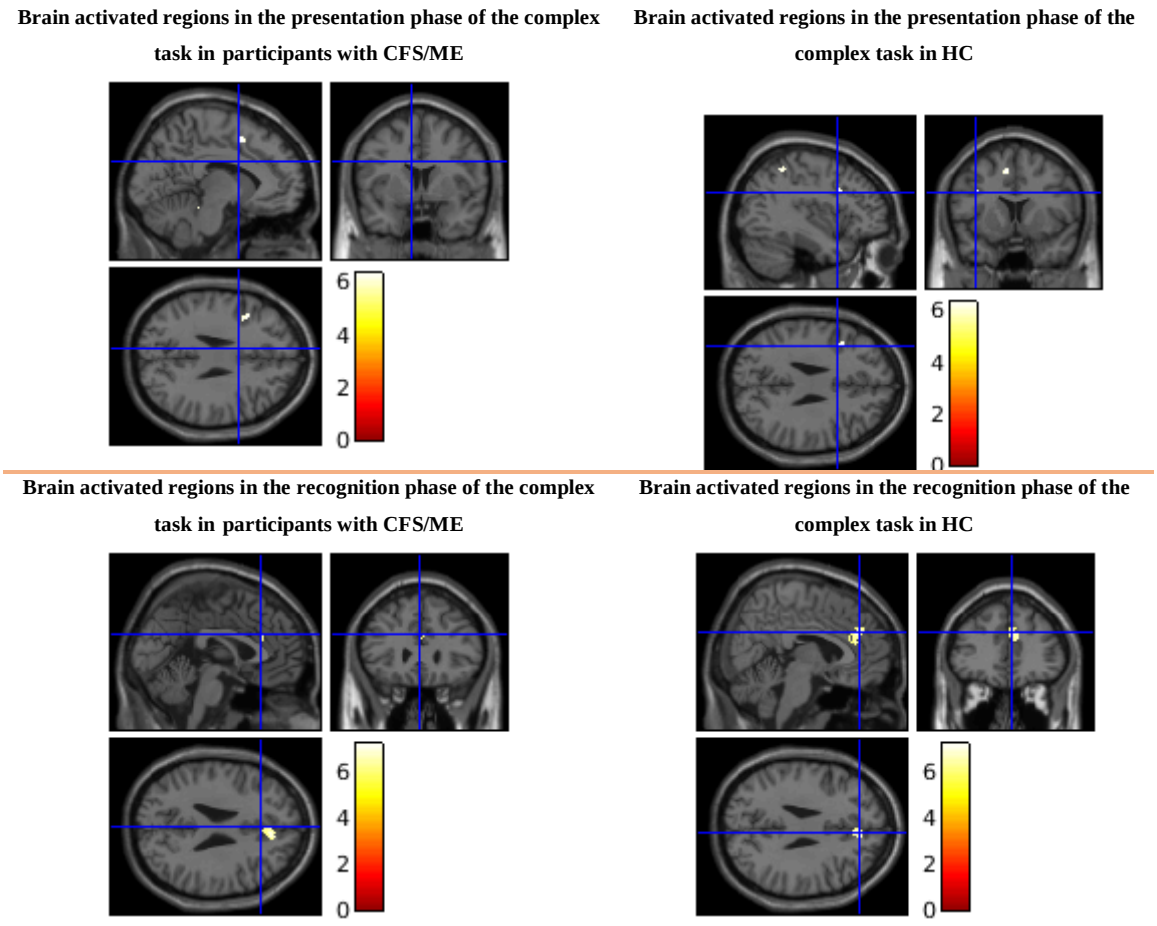


Figure 26 shows brain regions activated during the presentation and recognition phases of the complex task in both participants with CFS/ME and the healthy controls.

Table 34 shows areas of activation in participants with CFS/ME during the presentation phase of the complex verbal storage task (p threshold= .05 FWE corrected; BA= Brodmanns Area).

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Left	Superior Parietal Lobule	*	-22	-71	47	6.4

Table 35 shows areas of activation in participants with CFS/ME during the recognition phase of the complex verbal storage task (p threshold= .05 FWE corrected; BA= Brodmanns Area).

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Medial Frontal Gyrus	Brodmann area 8	11	30	41	5.97

Table 36 shows areas of activation in the healthy controls during the presentation phase of the complex verbal storage task (p threshold= .05 FWE corrected; BA= Brodmanns Area).

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Left	Medial Frontal Gyrus	*	-9	1	49	6.37
Left	Inferior Frontal Gyrus	*	-38	7	31	6.35
Left	Superior Parietal Lobule	Brodmann area 7	-33	-47	50	6.28
Left	Inferior Parietal Lobule	Brodmann area 40	-41	-47	50	5.91

Table 37 shows areas of activation in the healthy controls during the recognition phase of the complex verbal storage task (p threshold= .05 FWE corrected; BA= Brodmanns Area).

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Medial Frontal Gyrus	Brodmann area 9	4	33	33	7.27
Right	Cingulate Gyrus	*	8	30	27	6.85
Right	Sub-Gyral	*	41	-47	2	6.34
Right	Insula	Brodmann area 13	32	-7	19	6.29
			30	-18	19	6.27
Right	Extra-Nuclear	*	27	-3	15	6.21

7.4.4 Correlations Between Activated Brain Regions and the Tasks' Accuracy and Reaction Times

- **Correlation between activated brain regions in the processing task and reaction time and accuracy**

There were no correlations found between activated brain regions and reaction time or accuracy in the processing task.

- **Correlation between activated brain regions in the verbal storage task and reaction time and accuracy**

Positive and negative correlations were found in the CFS/ME group between activated brain regions and accuracy during the verbal storage task (see tables 38 and 39). Only a positive correlation was found between the activated brain regions and accuracy in the healthy controls group (see table 40). No correlation was found between the activated brain regions and reaction time in the presentation phase of the verbal storage task in either group.

Table 38 shows the activated brain regions that are positively correlated with accuracy during the verbal storage task in participants with CFS/ME.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Left	Uvula	*	-12	-95	-25	11.62
Left	Fusiform Gyrus	Brodmann area 20	-47	-33	-25	8.83
			-64	-48	-23	6.1
Left	Inferior Temporal Gyrus	Brodmann area 20	60	-60	-13	7.98
			-60	-27	-28	6.85
			-32	-11	-37	6.26
			-49	-2	-35	6.22

Table 39 shows the activated brain regions that are negatively correlated with accuracy during the verbal storage task in participants with CFS/ME.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Inferior Occipital Gyrus	Brodmann area 18	40	-92	-21	9.46
			40	-88	-14	7.94
Right	Declive	*	51	-67	-22	8.63
Left	Pyramis	*	-34	-86	-33	6.54

Table 40 shows the activated brain regions that are positively correlated with accuracy during the verbal storage task in the healthy controls.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Left	Superior Temporal Gyrus	Brodmann area 42	-66	-31	13	7.85
			-58	5	-3	6.11
			-56	9	-5	6.1

- **Correlation between activated brain regions in the complex verbal storage task and reaction time and accuracy**

Positive and negative correlations were found in the CFS/ME group (in the presentation phase of the complex task) and the healthy controls groups (in the recognition phase of the complex task) between activated brain region and accuracy during the complex task (see tables 41, 42, 43 and 44). No correlation was found between the activated brain regions and reaction time in the complex task.

Table 41 shows the activated brain regions that are positively correlated with accuracy during the presentation phase of the complex task in participants with CFS/ME.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Superior Frontal Gyrus	*	22	52	-12	24.56
Right	Medial Frontal Gyrus	Brodmann area 9&11	4	56	47	14.03
			27	50	-12	6.04
Left	Pyramis	*	-34	-86	-33	23.9
Left	Medial Frontal Gyrus	Brodmann area 9 &10	-2	61	28	25
			-41	51	13	24.6
			-1	61	35	24.6
			-1	60	40	24.6
Left	Inferior Frontal Gyrus	Brodmann area 9 & 46	-60	13	38	24.7
			-54	35	10	24.6
Left	Precentral Gyrus	Brodmann area 1	-64	5	23	24.6
			-66	-19	31	16.3
Left	Superior Temporal Gyrus	Brodmann area 22	-67	-36	14	10.5

Left	Superior Frontal Gyrus	Brodmann area 9	-12	50	23	6.58
Left	Middle Temporal Gyrus	Brodmann area 21	-56	7	-12	6.27

Table 42 shows the activated brain regions that are negatively correlated with accuracy during the presentation phase of the complex task in participants with CFS/ME.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Superior Frontal Gyrus	Brodmann area 10	21	59	35	18.6
Right	Middle Frontal Gyrus	Brodmann area 46	25	53	40	6.7
Left	Middle Frontal Gyrus	Brodmann area 46	-53	34	26	24.72
			-47	48	15	24.56
			-51	40	16	24.56
			-44	49	23	24.56
Left	Precentral Gyrus	Brodmann area 6	-64	-6	33	24.52
			-64	-10	36	24.52

Table 43 shows the activated brain regions that are positively correlated with accuracy during the recognition phase of the complex task in the healthy controls.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Superior Frontal Gyrus	Brodmann area 10	6	59	-9	9.23
Left	Middle Frontal Gyrus	*	-25	30	-17	8.56
Left	Superior Temporal Gyrus	*	-25	12	-26	6.32

Table 44 shows the activated brain regions that are negatively correlated with accuracy during the recognition phase of the complex task in the healthy controls.

Hemisphere	Brain Region	BA	MNI Coordinate (x,y,z)			T Value
Right	Inferior Frontal Gyrus	Brodmann area 47	34	24	-14	9.11
			32	30	-14	8.6
			43	21	-9	7.18
			38	31	-12	6.34
Right	Medial Frontal Gyrus	*	12	54	-10	7.72
			40	46	-6	6.08
Left	Inferior Frontal Gyrus	Brodmann area 47	-26	19	-21	8.63
			-43	20	-14	8.36
Left	Middle Temporal Gyrus	Brodmann area 21	-54	7	-26	7.64
			-56	6	-19	7.01

7.5 Discussion

In order to investigate functional brain differences between participants with CFS/ME and the healthy controls, the Bayliss et al. (2003) task [33] was used with fMRI to measure brain activity differences that would enable us to see differences even if there were no task performance differences. The Bayliss et al. (2003) task was used to fractionate the working memory components and investigate processing and storage, separately and combined. The main finding in this study is the presence of significant behavioural differences between participants with CFS/ME and the healthy controls in their performance in the complex task only. Participants with CFS/ME were slower in the reaction time in the verbal storage recognition phase, the complex verbal presentation phase, and the complex verbal recognition phase. In addition, there were significant positive correlations between CFQ and RT in all tasks and a significant negative correlation between CFQ and accuracy in the complex task.

There were no significant differences in brain activation patterns between the groups when tested directly with two-sample t-test. However, positive and negative correlations were found in the CFS/ME group in the complex task (presentation phase) and the healthy controls groups in the complex task (recognition phase) between activated brain regions and accuracy during the task. Positive and negative correlations were found in the CFS/ME group between activated brain regions and accuracy during the verbal storage task, where the only positive correlation was found in the healthy controls group. The direct correlations between activity and task performance are important, as they provide greater evidence that these regions are involved in task performance. These results show that participants with CFS/ME were slower than the healthy controls, which is supported by previous behavioural studies [444-449]. Although the performance is different between the groups, they still use the same working memory neural network to perform the task as significant brain activation differences were not found between the two groups. This suggests functional networks for tasks performance may not be neurologically compromised in the CFS/ME group. However, there were significant positive correlations between the fatigue scores and reaction time in all tasks, which show evidence that participants with CFS/ME take a longer time due to fatigue.

Previous fMRI studies in CFS/ME showed that when participants with CFS/ME were fatigued, their brains tend to recruit additional brain regions and consume a substantial amount of attentional resources in order to perform at the same level as the healthy controls [109, 159]. Caseras et al. (2006) suggested that patients with CFS/ME find compensatory strategies to overcome their underlying cognitive difficulties [109]. Therefore, it would be reasonable to speculate that with time, participants with CFS/ME would improve their accuracy and reaction time and perform as healthy controls by using the neural compensatory mechanism. This was not found in this study, suggesting the previous finding might be due to other confounders which are known to have an impact on brain function, such as anxiety, depression, illness duration, physical activity and sleep disturbance.

In the previous sMRI chapter (see section 4.4), no anatomical differences were found between participants with CFS/ME and the healthy controls using volumetric MR analyses. Therefore, these structural findings, alongside the functional brain results reported in this chapter showed no differences between the clinical and control groups. These findings might be due to not using phenotyping according to appropriate subgroups, such as Hickie et al. (1995) [176] or

Williams et al. (2017) [188]. According to Hickie et al. (1995), the five main domains for CFS/ME include prolonged fatigue, musculoskeletal pain, impaired neurocognitive function, sleep disturbance, and symptoms suggestive of inflammation [176]. Therefore, the impaired neurocognitive function phenotype would show greater difficulties with these tasks. It might be due to using an easy or short task, as some studies found an association between fatigue and lower performance with increased brain activity while performing a high-effort cognitive task [132, 158, 159]. Therefore, if a more fatiguing task was used, i.e., a longer one (more than 8 minutes) or have more than four digits to remember, it may show some significant differences between the groups. If the current paradigm was to start with the long resting-state scan, it might be enough to induce fatigue as participants were asked to stare at the cross for quite a long time (~15 minutes).

In a previous chapter (see section 6.4.3), there was a significant decrease in intrinsic functional connectivity of the Salience Network (SN) in participants with CFS/ME compared to the healthy controls. The SN plays a key role in many brain functions, including audition, deception, interception, pain, classical conditioning [399, 400] as well as linking cognition and emotion/ interoception [401, 402]. It also influences the performance of other networks, such as the DMN and frontoparietal networks (FPN), by mediating between these networks and the executive control network to guide appropriate responses [403]. Knowing the importance of this network and that it is affected in CFS/ME, it might be informative to focus on using more demanding tasks that are more dependent on the Salience network.

To our knowledge, this is the first study to investigate working memory in participants with CFS/ME and the healthy controls using the Bayliss et al. (2003) task. Several studies have investigated working memory in CFS/ME using different tasks such as finger tapping [110, 170], and auditory monitoring [170], the paced auditory serial addition task [110], and n-back [109]. However, this study used a working memory task that engages the processing and storage aspects of working memory [33]. It has been argued that the complex span tasks engage the processing and storage aspects of working memory, unlike short-term memory tasks such as digit span task, which only engages the storage capacity [457]. While previous studies failed to find behavioural differences between the groups when they use simple tasks, this study did. Performance differences were found between the group in term of accuracy only in the complex task when participants needed to engage both processing efficiency and storage capacity. Also, participants with CFS/ME were slower than the healthy controls in the storage task as well as

the complex span. This highlights the importance of using such a task that combines processing and storage capacity. Studies have demonstrated that such a task may be a better measure of general ability and predictors of performance on a range of complex cognitive activities [458-460]. Also, this task shows that domain-specific storage has a significant contribution to the performance of CFS/ME on the complex span task.

Frontoparietal regions are important in working memory and been highlighted in previous studies in adults and adolescents[461-463]. In all tasks for both groups, the left superior parietal gyrus and the right medial frontal gyrus were activated. The superior parietal gyrus is known to have a close link with the occipital lobe and implicated in many aspects of cognition, such as representing and manipulating objects, parts of visuospatial perception, and attention [464]. The medial frontal gyrus is recognised to be associated with high-level executive functions and decision-related processes [465]. Activation of these brain regions is expected as the tasks used need visuospatial perception and attention. The superior parietal gyrus and right medial frontal gyrus were also activated in CFS/ME studies during processing task in adults [343] and attentional task in adolescents [159]. The left supramarginal gyrus is a region known to be important for phonological store [466]. In the verbal storage and complex tasks, there was no activation in the left supramarginal gyrus. However, activation in the left inferior parietal lobule was found, which supports the Buchsbaum and D'Esposito (2008) theory [467]. They hypothesised that the phonological store is associated with many brain regions that underlie neural processes and do not correspond to a specific functional brain region [467]. Regarding the Baddeley working memory model, the inferior parietal lobule has a contribution in holding retrieved information in a way that makes it accessible for the decision-making processes [468]. It also supports the output buffer hypothesis that assumes the inferior parietal lobule has a role in helping the hold of the qualitative content of retrieved information (such as mental images) [469].

In the healthy controls, results show that there were four brain regions activated during all tasks (the middle frontal gyrus, superior and inferior parietal lobule and insula). The middle frontal gyrus activation has been shown to be involved in the active maintenance of stimuli during memory tasks [100, 470, 471]. Cowan et al. (2011) showed that the activation in the inferior parietal lobule represents domain-general, and it is load-dependent [472]. The parahippocampus activation in the processing task, not seen in the storage, has been linked to the processing component of working memory [473]. It has been linked with successfully

retrieving memories [474], which is supported by finding high behavioural accuracy suggesting that they were able to retrieve the encoded stimuli successfully. Recently, the thalamus has been suggested to mediate between attention and memory systems [475, 476] and its activation in the processing task show evidence that attention network is important within working memory. Therefore, it highlights the importance of attentional networks to accurately encode information that was not required for storing information. In addition, salience network differences were found between the groups (see section 6.4.3), which adds to this finding as the salience network is responsible for detecting and filtering salient stimuli [398]. The limited capacity of the short-term memory has probably influenced the accuracy rate in participants with CFS/ME during the recognition phase of the complex task. The complex task required the participants to memorise a series of numbers while responding to the stimulus that needed processing. As this has not been shown in the processing task and the presentation phase of the complex task, it might mean that the limited capacity of the short-term memory has no effect on the processing task in working memory for participants with CFS/ME. Therefore, these together illustrate that there were two separate neural regions of working memory that underlie processing and storage components.

7.6 Conclusion

Findings in this chapter have provided evidence in the presence of significant behavioural differences between participants with CFS/ME and the healthy controls as they were slower and less accurate. Moreover, it provides evidence that participants with CFS/ME still use the same working memory neural network to perform the task, which suggests that their brain may not be neurologically compromised. Fatigue is potentially responsible for poor performance, given evidence form the correlation between the performance and fatigue scores.

8 Chapter 8: General discussion

8.1 Short Introduction

Persistent and continuous fatigue is the main characteristic that describes the complex illness that is known as CFS/ME. Fatigue, in this illness, lasts for months, depending on the criteria used for diagnosis [1]. Scientists around the world have attempted to investigate CFS/ME to understand its aetiology and pathophysiology (see section 3.5.3. and 3.5.4.). CFS/ME prevalence is 0.76% (95% CI 0.23% to 1.29%); it represents a considerable health burden both financially and societally. It has been reported that >94% of adults' cases suffer from cognitive dysfunction as their most common symptom [144]. Fatigue has an impact on the CFS/ME patients' lifestyle as well as employment status since over 50% of adults are unemployed [145]. CFS/ME has many diagnostic criteria developed to aid in clinics and research. Each criterion has a defined period of the presence of fatigue and other secondary symptoms [1, 5-7] (see section 1.3.1). CFS/ME has many confounders such as length of illness, symptom severity, pain, physical activity, anxiety, depression and sleep disturbance which can have an effect on the brain morphometry and function. The illness duration is an important factor as individuals with CFS/ME often suffer from this illness for a long time before they get diagnosed with CFS/ME. Length of illness has been linked with CFS/ME symptoms and disability. This link implies that early stages might be different from later stages and suggests that individuals with longer illness duration have significantly higher specific cognitive difficulties [361] that are greater in severity [362]. It has been reported that the median length of illness for individuals with CFS/ME in the UK is three and a half years [145] which is almost similar to what this study had (median =3.2 years). This was reported for individuals with CFS/ME who were employed at the time of the study, whereas it is four years among others who ceased working [145]. The average length of illness, according to Nisenbaum et al. (2000), is 7 years [342]; however, the mean illness duration in this thesis was 6.04 years.

In Chapter 3 (see section 3.1), a systematic review was conducted, examining all structural and functional MRI studies, and found that it would be sensible to investigate CFS/ME using MRI and its applications to look for neural biomarkers of CSF/ME. From the systematic review, structural MRI studies gave inconsistent findings, potentially due to different methodologies used [477]. Some studies found differences between CFS/ME [149-153], while others found no differences between the groups [141-143]. Also, it seemed important to use an automated method to analyse the data as these methods were superior due to their ability to link symptoms

to regional structural differences. The importance of voxel-based analysis is that it allows for thorough measurement of differences throughout the entire brain by registering every brain to a template. Then, smoothing brain images so that each voxel is a representation of the average of itself and its neighbours. Lastly, each produced image value is then compared across brains at every voxel [478]. Therefore, this thesis proposed to use Voxel-Based Morphometry (VBM) analysis to analyse the structural data from CSF/ME patients.

Studies identified in the systematic review (see section 3.5.3.) proposed that the non-motor function of the basal ganglia in CFS/ME might have an implication in central fatigue [129, 131]. Moreover, basal ganglia have been linked to fatigue in other studies implicating that it has a significant role in prolonged central fatigue [125, 127, 130]. Therefore, investigation of structural differences as well as functional connectivity in brain networks and basal ganglia was performed. Also, knowing that >94% of adults' with CFS/ME have cognitive dysfunction, which affects their daily functioning, it was essential to develop a reliable and objective measure of cognitive fatigue so it would be possible to relate cognitive function with imaging. Cognitive fatigue can be assessed and measured with reaction time using cognitive tasks such as Bayliss et al. (2003) [33]. From previous studies, the task chosen, as well as its level of difficulty, plays a key role in the findings. A recent meta-analysis on fMRI studies that examined traumatic brain injury patients showed that the main reason behind the discrepancy in activation patterns among studies is attributable to task classification. Also, they reported that hypoactivation could be more prominent in discrete memory tasks, while hyperactivation could be associated with continuous memory tasks [193].

In CFS/ME fMRI studies, a wide variety of tasks had been used to assess differences between participants with CFS/ME and healthy controls. While some studies reported significant differences, others failed to report differences and suggested that the tasks were potentially too difficult for the CFS/ME participant to engage. Generally, rs-fMRI studies showed decreased functional connectivity in various brain regions and networks [129, 131, 154-157, 163, 167, 170]. Tasked-based fMRI studies showed that in more challenging tasks, participants with CFS/ME exhibit widespread increased activation in task-related regions when compared to healthy controls [108-110, 159, 162, 170]. The systematic review found that task difficulty, up to a point, has an impact on brain activation and helped to find differences between the groups. Therefore, the Bayliss et al. (2003) task was chosen to allow the investigation of processing

and storage components of working memory separately and combined [33]. A relatively easy span of Bayliss et al. (2003) was used to prevent further fatigue to participants with CFS/ME.

8.2Thesis Findings

8.2.1 Structural Differences in CFS/ME

The structural morphometry analysis (see section 4.4) showed no statistically significant differences between the patients and controls in global and regional brain volume. Previous studies were inconsistent and found differences in both grey matter volume and white matter volume, ventricular enlargement, white matter hyper-intensities, lesions and cortical thickening [149-153], while others found no differences between the groups [141-143]. Therefore, this illness might be only functional, or another structural imaging technique might be used to evaluate the structural differences.

Diffusion Tensor Imaging (DTI) is a technique used to evaluate the integrity of major white matter fibre tracts and might be useful in this illness in showing differences between the groups. Knowing that this study only investigated the brain volume, future studies might explore structural differences by mapping the diffusion process of water molecules in brain tissue using the DTI technique. Investigating cortical thickness might show differences as seen in the only DTI study by Zeineh et al. (2015) [283]. They found that the fractional anisotropy in the anterior right arcuate fasciculus was higher in participants with CFS/ME compared to healthy controls. Therefore, it might be important to investigate structural differences in this illness using different imaging and analysis techniques. Confounders in this illness, such as illness duration, participants with different symptom severity, pain, physical activity, anxiety, depression and sleep disturbance, might affect the results as these are known to have an impact on brain volume [207, 286, 359, 360]. From the systematic review, only two fMRI studies out of the 16 and seven studies out of the 19 sMRI studies reported their participants' illness duration. Only one study matched their participants' sleep pattern [139], which is known to have a strong association with BOLD signal measured by fMRI, as well as grey matter and white matter volumes [205-208]. This thesis attempted to address some of these confounders such as excluding participants with anxiety and depression using the hospital anxiety and depression scale. Other questionnaires were used to describe other confounders. The level of pain was described using the visual analogue pain rating scale as well as life quality using the SF-36, health outcome using the EQ-5D and sleep pattern using the Epworth sleepiness scale.

However, this thesis did not match for pain, activity, health outcome or sleep pattern as the exclusion criteria for healthy controls would be too long. Therefore, researchers in the future might apply a stratifying by symptoms technique in this illness to enable the comparison with other studies (or healthy controls) and illustrate the impact of these symptoms in this complex illness.

8.2.2 Impact of fatigue on cognition

While a healthy population can overcome the effect of increased fatigue through strategies such as learning and practise [479, 480], CFS/ME population, which already suffer from persistent fatigue, may not be able to compensate to the same extent. Measuring the accuracy and reaction time while performing the working memory task showed that the CFS/ME participants were slower and less accurate with increasing task difficulty. This finding suggests that the acutely induced fatigue due to the task might interrupt cognition in the CFS/ME population. This finding is supported by previous studies that linked lower performance to fatigue [132, 158, 159]. Fatigue scores were positively correlated with the reaction time in all tasks and negatively correlated with accuracy only in the complex task. These results were supported by previous studies as participants with CFS/ME were slower and less accurate [444-449]. Despite the behavioural differences, both groups used the same working memory neural network. This result might provide some evidence that the brain of this cohort may not be neurologically compromised. Using the same network showed no evidence of the compensatory mechanism of additional brain regions or increase activity with increased energy cost as well as no evidence of reorganisation. Fatigue might potentially be responsible for the poor performance giving evidence from the correlations between performance and fatigue score in participants with CFS/ME.

8.2.3 Fatigue and the Basal Ganglia

The investigation of basal ganglia using rs-fMRI revealed no significant differences in global nor local basal ganglia connectivity between participants with CFS/ME and healthy controls. This might show some evidence that the global and local functional connectivity of the basal ganglia is not compromised in CFS/ME. In a recent study, Shan et al. (2018) used the Stroop task and found no differences in functional connectivity between the groups [167]. From the systematic review, there were only five resting-state studies that investigated this illness using rs-fMRI. Two of those studies were in adolescents [131, 377], and one was only on female participants [155]. None of those studies controlled for sleep or physical activity and none of the adult's studies reported their participants' length of illness [155, 167, 169]. These

inconsistencies made it difficult to compare our findings giving the limited information presented in these studies.

8.2.4 Impact of Fatigue on Brain Networks

The current thesis was able to find a significant decrease in intrinsic functional connectivity of the salience network in CFS/ME compared to healthy controls, which was negatively correlated to the Chalder fatigue questionnaire. A decreased FC was found between the BG to the sensorimotor network (SMN) and between the SN to the basal ganglia in CFS/ME compared to healthy controls. Since SMN is known to be corresponding to action and all of our bodily sensations [402], and knowing that patients with CFS/ME suffer from physical impairment as well as cognitive function impairment, it can be argued that the FC patterns either within or between these networks may be impaired in CFS/ME [169, 425]. It might worth noting that with time, the SN alteration might affect the BG function leading to severe fatigue. This thesis found a negative correlation between SN and CFQ. This implies that less fatigue means better network connectivity which then means whenever their fatigue increases, the SN functional connectivity decreases, resulting in a disruption in the cognitively important information. However, future research is necessary to understand the altered relationship of the SN and if this has long term consequences for basal ganglia function. This is supported by previous rs-fMRI studies where they found a decrease in SN when fatigue increases, suggesting that more fatigue means altered connectivity [154, 168]. Also, the alteration in the SN has been reported in previous studies [154, 157, 163], which suggest a strong involvement of SN in the pathophysiology of CFS/ME. The SN has a role in connecting brain regions and networks, switching between default mode network and central executive network [191], and detection and integration of salient sensory information [189, 391]. Any interference in its role may cause a disruption in the cognitively important information [192]. This interference or immature function may add to the brain energy cost [155, 157, 168, 169, 192], which may explain the fatigue as well as some other symptoms such as impaired memory [368, 416]. Furthermore, the SN may influence the working connectivity of other networks, such as the default mode and the frontoparietal networks (DMN and FPN) [403]. The negative correlations found between SN and CFQ implies that less fatigue means better network connectivity which is supported by previous studies [154, 168]. Resting-state fMRI was also used to investigate other brains neural networks in CFS/ME and compare them to healthy controls. A positive correlation was found between CFQ and DMN in healthy controls only. This is supported by the Eichele et al. (2008) work where they suggested that failure in suppressing the DMN during

working memory task increases the error rate [427]. Therefore, the authors suggested that, when fatigued, it results in increased energy expenditure with no gain [427].

Homeostasis is a state of steady internal physical and chemical conditions that are maintained by living systems [436]. Recently, Seeley (2019) suggested that the interaction between cingulate and insula (the regions of the SN) could form an information processing loop that represents and responds to homeostatically relevant stimuli, either the internal or external ones [435]. Also, it could imbue these stimuli with emotional weight [435]. SN regions are known to be enriched with von Economo neurons (VENs). These neurons are proposed to become specialized to support social functioning [481]. Studies have reported that those VENs located in the SN were targeted in behavioural variant frontotemporal dementia [482-484], causing a slow loss of specialized social-emotional capacities as well as deficits in nociceptive and autonomic processing [485]. According to Seeley (2019), SN represents the homeostatic system that engages with the task, such as maintaining the relevant task as long as the stimulus is presented and orchestrating the switching to a new task set [435]. Studies that had been linked to altered connectivity or volume loss in the SN regions are disorders of social-emotional function [486, 487]. These studies include behavioural variant frontotemporal dementia, major depression, schizophrenia, attention deficit and bipolar disorder [486, 487]. SN has an important role in playing a domain-general that can be disrupted and produce different clinical manifestations. These manifestations may depend on the anatomical structure targeted, physiological details and the involvement of other brain regions [435]. Knowing that CFS/ME cohort has SN alteration and given these findings, CFS/ME might be one of these illnesses but has different manifestations. Therefore, future research might use this approach and investigate this network more and might find a link as seen in the previously mentioned illnesses.

8.3Limitations

This illness is complex and heterogeneous and has many confounders which are known to have an impact on brain volume and function. These confounders include using different diagnostic criteria (see section 3.6.3 and 3.6.4), illness duration, participants with different symptom severity, pain, physical activity, anxiety, depression and sleep disturbance which might explain differences in findings across studies. This study had controlled for anxiety and depression. Confounding was defined as “mixing of effects” [488, 489], which represent a mixture of effects between the exposure under investigation with additional factors that eventually distort the true relationship [490]. Confounders need to be considered while designing a study to make

sure that the effect is actually because of exposure or if there are other alternate explanations. The illness duration has been investigated, and results showed that younger patients with less illness duration (less than 10 years) had greater vitality compared to the other group with same-age but longer illness duration [361]. Individuals with CFS/ME with a disease duration of more than two years show more fatigue, more significant concentration problems and more functional disability [362, 363]. It has been shown that with time, patients find different strategies to adapt to their illness [362, 491]. In CFS/ME, brain volume has been found to decrease with the increase in the illness duration by a rate of 1% per year including the normal rate with (p = 0.01) [135, 147, 288]. In normal situations, brain volume reduces by 5% per 10 years after the age of 40 [492]. A longitudinal study of brain volume changes in normal ageing that used magnetic resonance imaging in both cross-sectional and longitudinal studies estimated brain atrophy to be 0.33% every year [493]. Therefore, the decrease in patients with CFS/ME brain volume by 1% every year would be reasonable knowing that this patient groups lack physical activity and have sleep disturbance.

The lack of functional and structural differences between CFS/ME and healthy controls groups in this thesis could potentially be explained by other confounders such as the length of illness, physical activity and sleep patterns which are known to have an impact on the brain. There were no significant structural or functional differences despite participants had a mean illness duration of six years in the structural one and 5.69 years in the functional one. Other studies had reported their participants' length of illness, which varied between two and ten years (see table 45). The table shows that different illness durations had been reported with different findings. Durations such as 6 and 7 years showed no differences [416, 494], where other studies using almost the same duration found decreases in the grey and white matters in different brain regions [287, 288]. A functional MRI study with an illness duration of 6.3 years showed that participants with CFS/ME used the same brain regions as the healthy controls, but there were no brain activation differences [160]. Therefore, this might suggest that CFS/ME is a slow progressing illness and dysfunctional connectivity patterns might not emerge in the first few years or due to the accompanying symptoms. De Lange et al. (2008) found an increase in the lateral prefrontal cortex (grey matter volume) after treatment with cognitive behavioural therapy [286]. Their result shows evidence of intervention/treatment having a direct benefit on the brains of CFS/ME patients. Physical inactivity and sleep disturbance might explain the brain volume decrease over time [207, 286, 359, 360] (see section 4.5). In addition, the sedentary lifestyle of CFS/ME might also explain the brain volume loss as physical activity in

early life can preserve cognition in later life [359, 360] and has been reported to improve the brain volume as well as many cognitive functions such as attention, learning, and memory [359] (see section 4.5). Sleep disturbance has been shown to cause white matter microstructure changes [365] and been linked with brain abnormalities [365-367] (see section 4.5). Therefore, it can be suggested that the earlier the intervention, the better the result as it might reduce symptoms and save brain volume and function.

Table 45 shows MRI studies, CFS/ME illness duration and main findings.

Authors & year	Illness duration (in years)	MRI	Sample size	Country	Main findings
Tanaka et al. (2006)[161]	2.1 (±1.2)	fMRI	6/7	Japan	Attenuated responsiveness of auditory cortices.
De Lange et al. (2004) [160]	6.3 (±4.4)	fMRI	16/16	Netherlands	Participants with CFS/ME were slower and used the same neural networks.
De Lange et al. (2008) [286]	5.8 (±0.79)	sMRI	22/22	Netherlands	Participants with CFS/ME had lower grey matter volume than healthy control.
Okada et al. (2004) [287]	5.81	sMRI	16/49	Japan	Reduced grey-matter volume in the bilateral prefrontal cortex
van der Schaaf et al. (2016) [494]	6.01 (±0.7)	sMRI	89/26	Netherlands	No differences between the groups.
Barnden et al. (2011)[288]	7.4 (±3.5)	sMRI	25/25	Australia	White matter decreased in midbrain with increasing fatigue duration.
Barnden et al. (2016)[416]	7.4 (±3.5)	sMRI	25/25	Australia	No differences between the groups.
Barnden et al. (2015)[147]	7.4 (±3.5)	sMRI	25/25	Australia	Increase T1w signal in the ventrolateral thalamus, internal capsule and prefrontal white matter.
Puri et al. (2012)[136]	10.9 (±1.7)	sMRI			Reduced grey matter volume in the CFS/ME group in the occipital lobes (right and left occipital poles; left lateral occipital cortex, superior division; and left supracalcrine cortex), the right angular gyrus and the posterior division of the left parahippocampal gyrus.

It is important to illustrate that MRI studies investigating CFS/ME did not report a number of important factors. In term of MRI studies, seven structural MRI studies [135, 136, 147, 286,

416, 494, 495] and two fMRI ones [160, 161] had clearly stated their participants' illness duration. However, none of these studies had clearly stated how they calculated their participants' length of illness. A negative correlation was found between the CFS/ME length of illness and the activity of the left putamen [131] and between the length of illness and white and grey matter volumes [135, 147, 285, 286, 288]. Therefore, these results show the importance of the length of illness and how it may affect the results. Also, not all studies had excluded participants with other confounders such as depression, anxiety, nor recorded their sleep pattern or physical activity. Knowing that neuroimaging studies are already expensive and time-consuming, measuring all these factors will make it even more time-consuming and difficult to recruit healthy controls while counting for all these confounders. Therefore, these all together would explain the inconsistencies in the previous studies results and reduce our ability in comparing our results with theirs. Neuroimaging researchers in the future can apply certain strategies such as the use of the views of CFS/ME experts regarding different grouping strategies which can steer CFS/ME research in directions that hold promise and eventually help clinicians in the optimization of their practices. Therefore, there is a need for a consensus on how scientists study this clinical population in the future. This consensus would include well-designed imaging techniques as well as taking these confounders into account and reporting their measures which would help to compare these studies with the future ones.

One of the major concerns in neuroimaging research is the low power in these studies. It has been reported that early neuroimaging studies results may be false due to the low power, and the median power in those studies was only 21% [195, 496]. The sample size calculations for this thesis showed a need for 55 participants for each group in order to reach 80% of the power (see section 2.3.9). Although increasing the sample size is expensive and time-consuming [497], Button et al. (2013) suggested that using small sample sizes would affect the results by reducing the likelihood of detecting true results as well as increasing the likelihood of false positives, which results in inflated positive effect sizes [195]. However, an argument made by Nord et al. (2017) suggests that the power estimates in those neuroimaging studies vary significantly across the different subfields [498]. It has been reported that in 2015, the average sample size per group was 19 participants [499]. In a recent study, Cremers et al. (2017) made a few suggestions to address the low power issue in neurosciences [497]. They suggested using less stringent thresholds, which in turn would lead to a large amount of Type I and Type II errors. Also, they suggested focusing on a particular region of interest which would be very difficult given that the human brain is a complex organisation.

In this thesis, although brain regions used in the power calculation were taken from previous studies [49, 109, 110, 170, 337], it was impractical to perform a region-specific analysis to detect other brain regions that might be involved in CFS/ME due to the heterogeneity nature of the disease. It is because the power calculation performed for the fMRI study using fMRIpower software package (fmripower.org)(see chapter 2.3.8) revealed that 55 participants, per group, were needed to allow for at least 80% power to detect an effect of size .95 s.d. in the anterior cingulate cortex, putamen, pallidum, middle frontal gyrus, thalamus, inferior parietal gyrus, and .60 in the caudate. These regions were chosen based on the findings from a previous study reporting functional differences between the CFS/ME participants and the healthy controls in the systematic review [477]. The present study was only able to recruit 42 CFS/ME participants and 40 healthy controls for the fMRI part over the period of two years and a half (see section 7.4.1). From the fMRI chapter (see section 7.4.3), no brain regions examined survived the multiple comparisons threshold, which suggests that the power in this study might not be adequate to show differences between the groups. Therefore, this study used Cohen's d to emphasise the size of the effect. In addition to presenting the statistical significance of the intervention data [334]. It is known that collecting large sample sizes in a field such as neuroimaging is very expensive and time-consuming. Therefore, it might be time to move towards multicentre or consortia studies that can pool resources. However, this would come with its own limitations as it requires significant collaboration as well as checking the scanner parameters periodically during the study. Therefore, most collaborations focus on anatomical scans as it is hard to ask specific neurocognitive questions.

An alternative solution to find differences between the groups in the present sample size would be increasing the signal-to-noise ratio (SNR). As SNR measures the signal to the background noise, it relies on the hardware, imaging protocols and acquisition sequences [500]. It can be increased using higher magnetic fields such as 7 Tesla. However, it is more expensive and needs extra care when setting up the imaging sequences as well as has its own MRI contraindications such as participants with metallic implants or claustrophobia. SNR also can be improved by acquiring more images; however, this technique has its own limitations. It is even more time consuming and more prone for patient movements which eventually reduces the SNR, so it needs to be utilised with caution [233].

8.4 Future directions

The use of the Bayliss et al. (2003) paradigm has revealed some interesting results. Although the span used in this study was relatively easy, it might worth investigating the effect of fatigue by designing studies with the same task but different task's span. This thesis used four digit-span and revealed that healthy controls found it easy and their accuracy score in the complex task was 93%. On the other hands, participants with CFS/ME had an accuracy of 79%. These results justified our use of such an easy span task and raised questions about whether the increase or decrease of the task span would make major changes in their accuracy. Would increasing the task span to 5 digits would be dramatically difficult for participants with CFS/ME, and they would score around the chance limit (50%). Would the increase in the span task show significant brain activation differences or lead to patients being unable to perform the task?. Another approach that can be used differently from the current one is using the rs-fMRI sequence before the fMRI one. In the rs-fMRI sequence, participants were asked to stare at the fixed cross for about 8 minutes which most of them found difficult and commented on that after the scan. Therefore, it could be suggested to start with the structural then the rs-fMRI sequences then the tasks to induce fatigue in the patient. Those techniques together would enhance the fatigue induced by the paradigm. Also, it might show different functional patterns within brain networks, such as the SN, which liaise between other networks as well as its role in executive control [403]. Other tasks could be suggested to understand further whether the effect of fatigue is specific to working memory or other processes such as attention. Knowing that SN, has a significant role in attention, is affected even in the early stages of this illness, tasks that focus on attention could be incorporated with varying cognitive load. Also, it might be important to increase the task load to see if there is a cut-off point where patients with CFS/ME start to feel fatigued and stop performing the task to a high standard. In previous studies that used working memory tasks, increased widespread activation with difficult tasks were reported [110, 170]. However, another study reported a decrease in brain activation with task difficulty [109]. Therefore, after using different tasks, if the fatiguing effect was only seen in the memory domain, it may be that the working memory domain has a greater deficit in CFS/ME. It would also mean that patients with CFS/ME cannot overcome the effect of fatigue to perform WM tasks as healthy controls. However, if the same effect was seen in all higher cognitive tasks, it would mean that CFS/ME have a deficit in all domains when task load increases.

The inability to reach the targeted sample size as well as the complexity and heterogeneity of this illness, limited our ability to subgroup our patient sample to different phenotypes [176]. Hickie et al. (1995) used symptoms and demographics to define a core group and a smaller polysymptomatic subgroup and found five main domains, including prolonged fatigue, musculoskeletal pain; impaired neurocognitive function; sleep disturbance; and symptoms suggestive of inflammation [176]. These different phenotypes might explain the inconsistencies in previous studies, as some found differences between patients with CFS/ME and healthy controls [149-153], and others did not [141-143]. Therefore, if this study had only included participants with CFS/ME from the impaired neurocognitive function phenotype, it might have shown differences between the groups. Also, this shows the importance of investigating patients with CFS/ME who have different phenotypes. It might show different results with different phenotypes, which will eventually aid in designing different treatment plans for each phenotype.

Addressing confounders as well as subgrouping might aid in understanding this complex illness. Confounders such as physical activity, length of illness, symptom severity, pain, anxiety, depression and sleep disturbance were linked with brain volume loss [207, 286, 359, 360]. For instance, the sample in this thesis was biased to participants with CFS/ME who are more physically mobile and able to travel, prepared to take part in research and complete a 2-hour procedure. When the fatigue is less, it might not show any functional connectivity differences between the groups as there is no need for extra resources. In this thesis, some of the participants with CFS/ME were excluded due to travel difficulties because they suffer from comorbidities and being housebound. Therefore, future studies might consider using larger sample sizes with different phenotypes to allow for comparison between CFS/ME phenotypes and even examining the effect of comorbidities. Given the nature of this illness, it is likely that most of the CFS/ME participants recruited in this thesis had less physical disability associated with CFS/ME, which make the results from this thesis difficult be extrapolated to those who are severely affected. Also, it might worth investigating the other confounders to illustrate their effect. Therefore, it might be reasonable to recruit participants with CFS/ME with and without these confounders to show the impact of these confounders on this patient group.

Importantly, longitudinal studies with these subgroups and confounders may illustrate the effect of length of illness and whether these effects were due to these confounders or from the illness itself. Such studies would help in finding when these structural differences start to

happen to design different treatment plans. De Lange et al. (2008) designed a longitudinal study to investigate the effect of cognitive behaviour therapy (CBT) as a treatment plan [286]. They found a decrease in the grey matter volume at the baseline but an increase in the lateral prefrontal cortex after a year. The increase was correlated with health status, processing speed and physical activity [133]. Also, using longitudinal studies with different subgroups and confounders would help in understanding the inconsistency in the longitudinal studies in this illness. From the systematic review, there were three longitudinal studies with varying periods (6-9 months with CBT, one year and six years) [133, 137, 141]. Shan et al. (2016) found a decrease in the white matter after six years, while Perrin et al. (2010) found no differences nor abnormalities between baseline and 12 months follow up MRI in CFS/ME compared to controls [141].

Diffusion-Weighted Imaging (DWI) is an advanced structural MRI technique that has been developed over the years to provide image contrast of the brain based on differences in the magnitude of diffusion of water molecules. DWI is non-invasive and permits the mapping of the diffusion process of water molecules in brain tissue. DTI, a special case of DWI, measures the restricted diffusion of water in tissue and used as an indirect method to assess the integrity of major white matter fibre tracts. This can be achieved by measuring the orientation, location, and anisotropy of the white matter tract [501]. It measures the directionality of water diffusion, known as fractional anisotropy (FA), which is a reflection of axonal diameter, fibre tract complexity and axonal density [502, 503]. The mobility of water diffusion in white matter is mainly controlled by the cell membrane and myelin sheaths and influenced by the level of myelination and axonal density [503]. So, the reduction in FA can be related to several factors such as degradation of both myelin sheaths and axonal membranes [504, 505], defect of the myelin with sparing of the axonal fibres [506, 507], or a reduction of the density of the axonal fibres [508]. Another term in DTI is the mean diffusivity (MD) which is the water molecules' overall mean-squared displacement restricted by organelles and membranes [509]. It also a reflection of cellular density and extracellular volume [510, 511] and related to the volume fraction of the interstitial space [511]. These terms together, FA and MD, can tell much about the brain's structure, such as if the low FA and high MD was found in the same brain region, it would indicate that higher intracellular or extracellular fluid is associated with less organized myelin and/or axonal structure [512]. A calculation can be done to derive the spatial orientation of fibres from the eigenvalues from the diffusion tensor. In CFS/ME, only one study employing DTI was found. Zeineh et al. (2015) reported an elevated fractional anisotropy (FA) in the right

arcuate fasciculus and in the right inferior longitudinal fasciculus was reported in patients with CFS/ME when compared to healthy controls which was associated with the increase of severity of the CFS/ME [283]. In addition, FA increased in the right anterior arcuate, which was suggested to be a reflection of strengthening of short-range fibres or degeneration of crossing fibres [283]. Moreover, the increase in FA was related to the severity of the disease [283]. Zeineh et al. (2015) suggested that the FA of the right anterior arcuate night be used as CFS/ME biomarker [283]. However, this conclusion may be challenged despite its quality since only one article was used to make this assumption; therefore, it is recommended that further research address this technique in this field. DTI measurements have been used in several studies and correlated with structural measurements in healthy controls [513-515]. According to Banerjee et al. (2016), to assess white matter microstructural damages, DTI might be the most sensitive structural technique as it can show the microstructural changes in brain tissue that appear normal in other sequences [516]. There is a large gap in the DTI literature examining CFS/ME, and further research is needed in order to increase the reliability and validity of the findings and thereby increase our understanding of the disease.

8.5 Conclusion

An important factor that has a detrimental effect on cognition is fatigue. A healthy person can overcome the impact of fatigue to a certain extent; however, those with CFS/ME, who already suffer from persistent fatigue, might not. Therefore, multiple neuroimaging techniques were used to investigate CFS/ME. The combination of techniques used in this thesis provided a better understanding of what may happen with participants with CFS/ME. The result of this thesis and the different methodology used indicates that participants with CFS/ME use the same brain regions to perform the memory task as healthy controls. Also, it shows that the intrinsic functional connectivity of the SN is disrupted in CFS/ME, even in the absence of morphological differences. Knowing the involvement of SN in the pathophysiology of CFS/ME and the function of this network in the detection and integration of salient sensory information [189, 391], any disruption in this network might disrupt cognitively important information [192]. In addition, this disruption or immature function might add to the brain energy cost [155, 157, 168, 169, 192], which may explain the fatigue as well as some other symptoms such as impaired memory [368, 416]. Also, knowing its function in connecting brain regions and networks, switching between default mode network and central executive network [191], any disruption may influence the working connectivity of other networks, such as the DMN and FPN [403]. Currently, it is important to develop accurate and objective measures of

cognitive fatigue, which can explain the neurobiological markers of fatigue in CFS/ME or even in other disorders. The development of such a biomarker would fundamentally improve our understanding of fatigue. It would have implications for research, in which it would allow us to measure the effectiveness of fatigue treatments as well as being able to profile different treatment responses. This profiling could then be used in clinics to refer patients who belong to a certain profile to the appropriate treatment.

References:

1. Fukuda, K., Straus, S. E., Hickie, I., Sharpe, M. C., Dobbins, J. G., and A. Komaroff, *A comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Annals of Internal Medicine.* 1994(121(12)): p. 953-959.
2. Foster, C.a.M., J., *Chronic fatigue syndrome/myalgic encephalopathy.* Dietetic and Nutrition Case Studies, 2016: p. 78-80.
3. Wiborg, J.F., van der Werf, S., Prins, J. B., & Bleijenberg, G., *Being homebound with chronic fatigue syndrome: A multidimensional comparison with outpatients.* Psychiatry research, 2010. **177**(1-2): p. 246-249.
4. Prevention., C.f.D.C.a. *National center for emerging and zoonotic infectious diseases*. 2014 Accessed Jan.].
5. Jason LA, E.M., Porter N and et al., *The Development of a Revised Canadian ME/CFS Case Definition.* Am J Biochem Biotech, 2010(6): p. (2):15.).
6. Baker, R.a.S., E., *Diagnosis and management of chronic fatigue syndrome or myalgic encephalomyelitis (or encephalopathy): summary of NICE guidance.* BMJ, 2007. **335**(7617): p. 446-448.
7. Sharpe, M., Archard, L., Banatvala, J., Borysiewicz, L., Clare, A., David, A., Edwards, R., Hawton, K., Lambert, H. and Lane, R., *A Report-Chronic Fatigue Syndrome: Guidelines for Research.* Journal of the Royal Society of Medicine, 1991. **84**(2): p. 118-121.
8. Shepherd, D.C., & Chaudhuri, A., *ME/CFS/PVFS: An exploration of the key clinical issues*. 2017: ME Association.
9. Carruthers, B.M., M.I. van de Sande, K.L. De Meirleir, N.G. Klimas, G. Broderick, T. Mitchell, D. Staines, A.C. Powles, N. Speight, R. Vallings, L. Bateman, B. Baumgarten-Austrheim, D.S. Bell, N. Carlo-Stella, J. Chia, A. Darragh, D. Jo, D. Lewis, A.R. Light, S. Marshall-Gradisnik, I. Mena, J.A. Mikovits, K. Miwa, M. Murovska, M.L. Pall, and S. Stevens, *Myalgic encephalomyelitis: International Consensus Criteria.* J Intern Med, 2011. **270**(4): p. 327-38.
10. S. Johnston, E.W.B., D. Staines, S. Marshall-Gradisnik, *The prevalence of chronic fatigue syndrome/myalgic encephalomyelitis: a meta-analysis, Clin. Epidemiol.* 2013. **5**: p. 105–110.
11. Nacul, L.C., E.M. Lacerda, D. Pheby, P. Campion, M. Molokhia, S. Fayyaz, J.C. Leite, F. Poland, A. Howe, and M.L. Drachler, *Prevalence of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) in three regions of England: a repeated cross-sectional study in primary care.* BMC Med, 2011. **9**(91): p. 91.
12. Jason, L.A., K.M. Jordan, J.A. Richman, A.W. Rademaker, C.F. Huang, W. McCready, J. Shlaes, C.P. King, D. Landis, S. Torres, T. Haney-Davis, and E.L. Frankenberry, *A Community-based Study of Prolonged Fatigue and Chronic Fatigue.* J Health Psychol, 1999. **4**(1): p. 9-26.
13. Lloyd, A.R., I. Hickie, C.R. Boughton, O. Spencer, and D. Wakefield, *Prevalence of chronic fatigue syndrome in an Australian population.* Med J Aust, 1990. **153**(9): p. 522-8.
14. Rusu, C., M.E. Gee, C. Lagace, and M. Parlor, *Chronic fatigue syndrome and fibromyalgia in Canada: prevalence and associations with six health status indicators.* Health Promot Chronic Dis Prev Can, 2015. **35**(1): p. 3-11.
15. Kawakami, N., N. Iwata, S. Fujihara, and T. Kitamura, *Prevalence of chronic fatigue syndrome in a community population in Japan.* Tohoku J Exp Med, 1998. **186**(1): p. 33-41.
16. Kim, C.H., H.C. Shin, and C.W. Won, *Prevalence of chronic fatigue and chronic fatigue syndrome in Korea: community-based primary care study.* J Korean Med Sci, 2005. **20**(4): p. 529-34.
17. Njoku, M.G., L.A. Jason, and S.R. Torres-Harding, *The prevalence of chronic fatigue syndrome in Nigeria.* J Health Psychol, 2007. **12**(3): p. 461-74.
18. Lim, E.J., Y.C. Ahn, E.S. Jang, S.W. Lee, S.H. Lee, and C.G. Son, *Systematic review and meta-analysis of the prevalence of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME).* J Transl Med, 2020. **18**(1): p. 100.
19. Afari, N. and D. Buchwald, *Chronic fatigue syndrome: a review.* Am J Psychiatry, 2003. **160**(2): p. 221-36.

20. Simon M Collin, R.N., Elise M van de Putte, Sanne L Nijhof, and E. Crawley, *Chronic fatigue syndrome (CFS) or myalgic encephalomyelitis (ME) is different in children compared to in adults : a study of UK and Dutch clinical cohorts.* BMJ Open, 2015. **5**(10): p. p.e008830.
21. Wasson, N., Fu, R., Nelson, H.D. and Medline, P., *Treatment of myalgic encephalomyelitis/chronic fatigue syndrome: a systematic review for a National Institutes of Health Pathways to Prevention Workshop.* Annals of internal medicine, 2015. **162**(12): p. 841.
22. Smith, M.E., E. Haney, M. McDonagh, M. Pappas, M. Daeges, N. Wasson, R. Fu, and H.D. Nelson, *Treatment of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: A Systematic Review for a National Institutes of Health Pathways to Prevention Workshop.* Ann Intern Med, 2015. **162**(12): p. 841-50.
23. Collin, S., Nikolaus, S., Heron, J., Knoop, H., White, P. and Crawley, E., *Chronic fatigue syndrome (CFS) symptom-based phenotypes in two clinical cohorts of adult patients in the UK and The Netherlands.* Journal of Psychosomatic Research, 2016. **81**: p. 14-23.
24. Wilson, A., Hickie, I., Hadzi-Pavlovic, D., Wakefield, D., Parker, G., Straus, S. E., Dale, J., McCluskey, D., Hinds, G., Brickman, A., Goldenberg, D., Demitrack, M., Blakely, T., Wessely, S., Sharpe, M. and Lloyd, A., *What is chronic fatigue syndrome? Heterogeneity within an international multicentre study.* Australian and New Zealand Journal of Psychiatry, 2001. **35**: p. 520-527.
25. Michiels, V. and R. Cluydts, *Neuropsychological functioning in chronic fatigue syndrome: a review.* Acta Psychiatr Scand, 2001. **103**(2): p. 84-93.
26. Grafman, J., *Neuropsychological features of chronic fatigue syndrome.* INFECTIOUS DISEASE AND THERAPY SERIES, 1994. **14**: p. 263-263.
27. Komaroff, A.L. and D. Buchwald, *Symptoms and signs of chronic fatigue syndrome.* Rev Infect Dis, 1991. **13 Suppl 1**: p. S8-11.
28. Euba, R., T. Chalder, A. Deale, and S. Wessely, *A comparison of the characteristics of chronic fatigue syndrome in primary and tertiary care.* Br J Psychiatry, 1996. **168**(1): p. 121-6.
29. Smith, A., Behan, P., Bell, W., Millar, K. and Bakheit, M. , *Behavioural problems associated with the chronic fatigue syndrome.* British Journal of Psychology, 1993. **84**(3): p. 411-423.
30. Cope, H., Pernet, A., Kendall, B. and David, A., *Cognitive functioning and magnetic resonance imaging in chronic fatigue.* British Journal of Psychiatry,, 1995. **167**(01): p. 86-94.
31. Michiels, V., de Gucht, V., Cluydts, R. and Fischler, B., *Attention and Information Processing Efficiency in Patients with Chronic Fatigue Syndrome.* Journal of Clinical and Experimental Neuropsychology, 1999. **21**(5): p. 709-729.
32. Sandman, C., Barron, J., Nackoul, K., Goldstein, J. and Fidler, F., *Memory deficits associated with chronic fatigue immune dysfunction syndrome.* Biological Psychiatry, 1993. **33**(8-9): p. 618-623.
33. D. M. Bayliss, C.J., D. M. Gunn, and A. D. Baddeley, *The complexities of complex span: explaining individual differences in working memory in children and adults.* Journal of Experimental Psychology, 2003. **132**(1): p. 71-92.
34. Marshall, P., Forstot, M, Peterson, PK, & Schenck, CH, *Cognitive slowing and working memory difficulties in chronic fatigue syndrome.* Psychosom Med, 1997. **59**(1): p. 58-66.
35. Deluca, J., C. Christodoulou, B.J. Diamond, E.D. Rosenstein, N. Kramer, and B.H. Natelson, *Working memory deficits in chronic fatigue syndrome: Differentiating between speed and accuracy of information processing.* Journal of the International Neuropsychological Society : JINS, 2004. **10**(1): p. 101–109.
36. Sulheim, C., Fagermoen, E, Sivertsen, Ø, Winger, A, Wyller, VB & ØIE, MG, *Cognitive dysfunction in adolescents with chronic fatigue: a cross-sectional study.* Achieves of Disease in Childhood, 2015. **100**(9): p. 838-844.
37. Cowan, N., *An embedded-processes model of working memory. In Models of Working Memory.* Mechanisms of active maintenance and executive control, 1999. **20**: p. 506.
38. Jones, D.M. and S. Tremblay, *Interference in memory by process or content? A reply to Neath (2000).* Psychon Bull Rev, 2000. **7**(3): p. 550-8.
39. Baddeley, A., *Working Memory: Theories, Models, and Controversies.* Annual Review of Psychology, 2011. **63** (1): p. 1-29.
40. Baddeley, A., *Working memory.* Science, 1992. **255**: p. 556-559.

41. Rypma, B., Berger, J.S., D'Esposito, M., J. Cogn., *The influence of workingmemory demand and subject performance on prefrontal cortical activity.* J. Cogn. Neurosci., 2002. **14**: p. 721-731.
42. Awh, E., Smith, E.E., Jonides, J., *Human rehearsal processes and the frontal lobes: PET evidence.* Ann. N. Y. Acad. Sci. , 1995. **769**(97- 117).
43. Awh, E., Jonides, J., Smith, E.E., Schumacher, E.H., Doeppe, R.A., Katz, S.,, *Dissociation of storage and rehearsal in verbal working memory: evidence from positron emission tomography.* Psychol. Sci, 1996. **7**: p. 25-31.
44. Salmon, E., Van der, L.M., Collette, F., Delfiore, G., Maquet, P., Degueldre, C., Luxen, A., Franck, G.,, *Regional brain activity during working memory tasks.* Brain 1996. **119 (Pt. 5)**: p. 1617-1625.
45. D'Esposito, M., J.A. Detre, D.C. Alsop, R.K. Shin, S. Atlas, and M. Grossman, *The neural basis of the central executive system of working memory.* Nature, 1995. **378**(6554): p. 279-81.
46. Collette, F. and M. Van der Linden, *Brain imaging of the central executive component of working memory.* Neurosci Biobehav Rev, 2002. **26**(2): p. 105-25.
47. Osaka, M., N. Osaka, H. Kondo, M. Morishita, H. Fukuyama, T. Aso, and H. Shibasaki, *The neural basis of individual differences in working memory capacity: an fMRI study.* NeuroImage, 2003. **18**(3): p. 789-797.
48. Owen, A.M., K.M. McMillan, A.R. Laird, and E. Bullmore, *N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies.* Hum Brain Mapp, 2005. **25**(1): p. 46-59.
49. Chein, J.M., A.B. Moore, and A.R. Conway, *Domain-general mechanisms of complex working memory span.* Neuroimage, 2011. **54**(1): p. 550-9.
50. Bates, T.C., M. Luciano, S.E. Medland, G.W. Montgomery, M.J. Wright, and N.G. Martin, *Genetic variance in a component of the language acquisition device: ROBO1 polymorphisms associated with phonological buffer deficits.* Behav Genet, 2011. **41**(1): p. 50-7.
51. Sternberg, R.J., *Cognitive Psychology*. WADSWORTH. 2007.
52. Shallice, T., & Warrington, E. K., *The dissociation between short term retention of meaningful sounds and verbal material.* Neuropsychologia,, 1974. **12**(4): p. 553-555.
53. Farah, M.J., K.M. Hammond, D.N. Levine, and R. Calvanio, *Visual and spatial mental imagery: dissociable systems of representation.* Cogn Psychol, 1988. **20**(4): p. 439-62.
54. Baddeley, A.D., Thomson, N., & Buchanan, M., *Word length and the structure of short-term memory.* ournal of Verbal Learning and Verbal Behavior, 1975. **14**(6): p. 575-589.
55. Sala, J.B., P. Rama, and S.M. Courtney, *Functional topography of a distributed neural system for spatial and nonspatial information maintenance in working memory.* Neuropsychologia, 2003. **41**(3): p. 341-56.
56. Neisser, U., *New vistas in the study of memory*. Remembering reconsidered, ed. I.U.N.E. Winograd. 1988, Cambridge: Cambridge University Press.
57. Baddeley, A.D., Hitch, G. J., & Allen, R. J., *Working memory and binding in sentence recall.* Journal of Memory and Language, 2009. **61**(3): p. 438-456.
58. Baddeley, A., *Working memory: theories, models, and controversies.* Annu Rev Psychol, 2012. **63**(1-29): p. 1-29.
59. Cowan N, D.L., Saults JS, Keller TA, Johnson T, Flores L, *The role of verbal output time and the effects of word-length on immediate memory.* . J. Mem, 1992. **31**: p. 1-17.
60. Lewandowsky, S., & Oberauer, K., *The word length effect provides no evidence for decay in short-term memory.* Psychonomic Bulletin & Review. , 2008. **15**(5): p. 875-888.
61. Jones, D.M., W.J. Macken, and A.C. Murray, *Disruption of visual short-term memory by changing-state auditory stimuli: the role of segmentation.* Mem Cognit, 1993. **21**(3): p. 318-28.
62. Cortis, C., *Comparing the immediate free recall of verbal and visuo-spatial stimuli: List length, capacity and output order effects in single-and dual-modality tasks.* Doctoral dissertation, University of Essex, 2015.
63. Nairne, J.S., *A feature model of immediate memory.* Mem Cognit, 1990. **18**(3): p. 251-69.

64. Furley, P., & Wood, G., *Working memory, attentional control, and expertise in sports: A review of current literature and directions for future research.* Journal of Applied Research in Memory and Cognition, 2016. **5**(4): p. 415-425.
65. Daneman, M., & Carpenter, P. A., *Individual differences in working memory and reading.* Journal of verbal learning and verbal behavior, 1980. **19**(4): p. 450-466.
66. Kane, M.J., & Engle, R. W., *Working-memory capacity and the control of attention: the contributions of goal neglect, response competition, and task set to Stroop interference.* Journal of experimental psychology: General, 2003. **132**(1): p. 47.
67. Turner, M.L., & Engle, R. W., *Is working memory capacity task dependent?* Journal of Memory and Language, 1989. **28**: p. 127-154.
68. Waters, G.S., & Caplan, D., *The measurement of verbal working memory capacity and its relation to reading comprehension.* The Quarterly Journal of Experimental Psychology Section A, 1996. **49**(1): p. 51-79.
69. Kane, M.J., Bleckley, M. K., Conway, A. R., & Engle, R. W., *A controlled-attention view of working-memory capacity.* Journal of experimental psychology: General, 2001. **130**(2): p. 169.
70. Kane, M.J., & Engle, R. W., *Working-memory capacity, proactive interference, and divided attention: Limits on long-term memory retrieval.* Journal of Experimental Psychology: Learning, Memory, and Cognition, 2000. **26**(2): p. 336.
71. Kane, M.J., Hambrick, D. Z., Tuholski, S. W., Wilhelm, O., Payne, T. W., & Engle, R. W., *The generality of working memory capacity: A latent-variable approach to verbal and visuo-spatial memory span and reasoning.* Journal of Experimental Psychology: General, 2004. **133**: p. 189-217.
72. Kane M. J., H.D.Z., Tuholski S. W., Wilhelm O., Payne T. W., Engle R. W., *The domain generality of working-memory capacity: a latent-variable approach to verbal and spatial memory span and reasoning.* J. Exp. Psychol. Gen, 2004. **133**: p. 189–217.
73. Constantinidis, C. and T. Klingberg, *The neuroscience of working memory capacity and training.* Nat Rev Neurosci, 2016. **17**(7): p. 438-49.
74. Kirchner, W.K., *Age differences in short-term retention of rapidly changing information.* J Exp Psychol, 1958. **55**(4): p. 352-8.
75. Gazzaniga, M.S., Ivry, Richard B., and Mangun, George R., *Cognitive Neuroscience: The Biology of the Mind*. 2nd ed ed. 2009.
76. Miller, K.M., Price, C.C., Okun, M.S., Montijo, H., and Bowers, D., *Is the n-back task a valid neuropsychological measure for assessing working memory?* Archives of Clinical Neuropsychology, 2009. **24**: p. 711-717.
77. Bayliss, D.M., and Jarrold, C., *How quickly they forget: The relationship between forgetting and working memory performance.* Journal of Experimental Psychology: Learning, Memory, and Cognition, 2015(41): p. 163-177.
78. PS, G.-R., *The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive, Philosophical Transactions of the Royal Society of London. Series B,.* Biological Sciences, 1996. **351** (1346): p. 1445-53.
79. Fuster, J.M., *Frontal lobe and cognitive development.* Journal of neurocytology, 2002. **31**(3-5): p. 373-385.
80. Fuster JM, B.M., Kroger JK, *Cross-modal and cross-temporal association in neurons of frontal cortex.* Nature Neuroscience, 2000. **405** (6784): p. 347-51.
81. C.F., J., *Studies of cerebral function in primates. The functions of the frontal associations areas in monkeys.* Comp Psychol Monogr, 1936. **13**: p. 3-60.
82. Pribram, K.H.M., M.; Rosvold, H. E.; Kaplan, S. J., *Effects on delayed-response performance of lesions of dorsolateral and ventromedial frontal cortex of baboons.* Journal of comparative and physiological psychology, 1952. **45** (6): p. 565-575.
83. Shimamura, A.P., *The role of the prefrontal cortex in dynamic filtering.* Psychobiology, 2000. **28**: p. 207-218.
84. Kandel, E., Schwartz, J., & Jessell, T., *Principles of Neural Science*. 3rd edition ed. 1991: New York: NY.
85. N., C., *Working Memory Capacity*, ed. Hove. 2005, UK: Psychol Press.

86. Rugg, M.Y., A.P., *Human recognition memory: a cognitive neuroscience perspective.* Trends Cogn. Sci., 2003. **7**(7): p. 313-19.
87. Kolb, B., & Whishaw, I., *Fundamentals of Human Neuropsychology*. 1990, New York: W.H. Freeman and Co.
88. Joseph, T.N., *Developmental differences in the vulnerability to auditory distraction: children vs. adults.* Doctoral dissertation, University of Central Lancashire, 2017.
89. Buckner, R.L., *The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging.* Neuron, 2013. **80**(3): p. 807-815.
90. Wolf, U., M.J. Rapoport, and T.A. Schweizer, *Evaluating the affective component of the cerebellar cognitive affective syndrome.* J Neuropsychiatry Clin Neurosci, 2009. **21**(3): p. 245-53.
91. Zhu, Y., Gao, H., Tong, L., Li, Z., Wang, L., Zhang, C., ... & Yan, B., *Emotion regulation of hippocampus using real-time fMRI neurofeedback in healthy human.* Frontiers in human neuroscience, 2019. **13**: p. 242.
92. Pitkänen, I.A., JP, *Connectivity of the rat amygdaloid complex. The amygdala: A functional analysis.* . England: Oxford University Press, ed. Oxford. 2000.
93. Packard, M.G., & Cahill, L., *Affective modulation of multiple memory systems.* Current opinion in neurobiology, 2001. **11**(6): p. 752-756.
94. Phelps, E.A., *Human emotion and memory: interactions of the amygdala and hippocampal complex.* Current opinion in neurobiology, 2004. **14**(2): p. 198-202.
95. Wixted, J.T. and L.R. Squire, *The role of the human hippocampus in familiarity-based and recollection-based recognition memory.* Behav Brain Res, 2010. **215**(2): p. 197-208.
96. Merkow, M.B., Burke, J. F., & Kahana, M. J., *The human hippocampus contributes to both the recollection and familiarity components of recognition memory.* Proceedings of the National Academy of Sciences, 2015. **112**(46): p. 14378-14383.
97. Corkin, S., Amaral, D. G., González, R. G., Johnson, K. A., & Hyman, B. T., *HM's medial temporal lobe lesion: findings from magnetic resonance imaging.* Journal of Neuroscience, 1997. **17**(10): p. 3964-3979.
98. Clark, R.E., Zola, S. M., & Squire, L. R., *Impaired recognition memory in rats after damage to the hippocampus.* Journal of Neuroscience, 2000. **20**(23): p. 8853-8860.
99. Phelps, E.A., *Human emotion and memory: interactions of the amygdala and hippocampal complex.* Current opinion in neurobiology, 2004. **14**(2): p. 198-202.
100. Cohen, J.D., W.M. Perlstein, T.S. Braver, L.E. Nystrom, D.C. Noll, J. Jonides, and E.E. Smith, *Temporal dynamics of brain activation during a working memory task.* Nature, 1997. **386**(6625): p. 604-8.
101. Mannie, Z.N., Harmer, C. J., Cowen, P. J., & Norbury, R., *A functional magnetic resonance imaging study of verbal working memory in young people at increased familial risk of depression.* Biological psychiatry, 2010. **67**(5): p. 471-477.
102. Goethals, I., K. Audenaert, F. Jacobs, C. Van De Wiele, G. Vermeir, A. Vandierendonck, C. Van Heeringen, and R. Dierckx, *Toward clinical application of neuropsychological activation probes with SPECT: a spatial working memory task.* J Nucl Med, 2002. **43**(11): p. 1426-31.
103. Noël, X., Paternot, J., Van der Linden, M., Sferrazza, R., Verhas, M., Hanak, C., ... & Verbanck, P., *Correlation between inhibition, working memory and delimited frontal area blood flow measured by 99MTC-bicisate spect in alcohol-dependent patients.* Alcohol and Alcoholism, 2001. **36**(6): p. 556-563.
104. Awh, E., Jonides, J., Smith, E. E., Schumacher, E. H., Koeppe, R. A., & Katz, S., *Dissociation of storage and rehearsal in verbal working memory: Evidence from positron emission tomography.* Psychological Science, 1996. **7**(1): p. 25-31.
105. Paulesu, E., C.D. Frith, and R.S. Frackowiak, *The neural correlates of the verbal component of working memory.* Nature, 1993. **362**(6418): p. 342-5.
106. Cohen, J.D., Forman, S. D., Braver, T. S., Casey, B. J., Servan-Schreiber, D., & Noll, D. C., *Activation of the prefrontal cortex in a nonspatial working memory task with functional MRI.* Human brain mapping, 1994. **1**(4): p. 293-304.
107. Baddeley, A.D., Eysenck, M. W., & Anderson, M. C., *Memory.* Psychology Press, ed. Hove. 2009, England.

108. Lange, G., J. Steffener, D.B. Cook, B.M. Bly, C. Christodoulou, W.C. Liu, J. Deluca, and B.H. Natelson, *Objective evidence of cognitive complaints in Chronic Fatigue Syndrome: a BOLD fMRI study of verbal working memory.* Neuroimage, 2005. **26**(2): p. 513-24.
109. Caseras, X., Mataix-Cols, D., Giampietro, V., Rimes, K., Brammer, M., Zelaya, F., Chalder, T. and Godfrey, E. , *Probing the working memory system in chronic fatigue syndrome: A functional magnetic resonance imaging study using the n-back task.* Psychosomatic Medicine, 2006. **68**(6): p. 947-955.
110. Cook, D.B., A.R. Light, K.C. Light, G. Broderick, M.R. Shields, R.J. Dougherty, J.D. Meyer, S. VanRiper, A.J. Stegner, L.D. Ellingson, and S.D. Vernon, *Neural consequences of post-exertion malaise in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome.* Brain Behav Immun, 2017. **62**: p. 87-99.
111. Cook, D.B., O'Connor, P. J., Lange, G., and Steffener, J., *Functional neuroimaging correlates of mental fatigue induced by cognition among chronic fatigue syndrome patients and controls.* Neuroimage, 2007. **36**(1): p. 108-22.
112. Goetz, C.G., *Textbook of clinical neurology* Vol. 355. 2007: Elsevier Health Sciences.
.
113. Fix, J.D., *Basal ganglia and the striatal motor system*. 4th ed. Board Review Series. 2008, Neuroanatomy Baltimore: Wolters Kluwer & Lippincott Williams & Wilkins. 274-281.
114. Ragsdale, C.W., & Graybiel, A. M., *A simple ordering of neocortical areas established by the compartmental organization of their striatal projections.* Proceedings of the National Academy of Sciences, 1990. **87**(16): p. 6196-6199.
115. Schultz, W., *Getting formal with dopamine and reward.* Neuron, 2002. **36**(2): p. 241-263.
116. Williams ZM, E.E., *Selective enhancement of associative learning by microstimulation of the anterior caudate.* Nat Neurosci, 2006. **9**: p. 562–568.
117. Poldrack, R.A., Clark, J., Pare-Blagoev, E. J., Shohamy, D., Moyano, J. C., Myers, C., & Gluck, M. A., *Interactive memory systems in the human brain.* Nature, 2001. **414**(6863): p. 546.
118. Pasupathy, A., & Miller, E. K., *Different time courses of learning-related activity in the prefrontal cortex and striatum.* Nature, 2005. **433**(7028): p. 873.
119. Frank, M.J., Seeberger, L. C., & O'reilly, R. C., *By carrot or by stick: cognitive reinforcement learning in parkinsonism.* Science, 2004. **306**(5703): p. 1940-1943.
120. Fu, S., Zinni, M., Squire, P. N., Kumar, R., Caggiano, D. M., & Parasuraman, R., *When and where perceptual load interacts with voluntary visuospatial attention: An event-related potential and dipole modeling study.* Neuroimage, 2008. **39**(3): p. 1345-1355.
121. Thorn, C.A., Atallah, H., Howe, M., & Graybiel, A. M., *Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning.* Neuron, 2010. **66**(5): p. 781-795.
122. Voytek, B., & Knight, R. T., *Prefrontal cortex and basal ganglia contributions to visual working memory.* Proceedings of the National Academy of Sciences of the United States of America, 2010. **107**(42): p. 18167-18172.
123. Stocco, A.L., Christian; Anderson, John R., *Conditional Routing of Information to the Cortex: A Model of the Basal Ganglia's Role in Cognitive Coordination.* Psychological Review, 2010. **117** (2): p. 541-74.
124. Weyhenmeyer, J.A.G., Eve. A., *Rapid Review of Neuroscience.* Mosby Elsevier. 2007. 102.
125. Chaudhuri, A. and P.O. Behan, *Fatigue and basal ganglia.* J Neurol Sci, 2000. **179**(S 1-2): p. 34-42.
126. Chaudhuri, A.a.B., P.O., *Fatigue in neurological disorders.* The Lancet, 2002. **363**: p. 978-988.
127. Nakagawa, S., H. Takeuchi, Y. Taki, R. Nouchi, Y. Kotozaki, T. Shinada, T. Maruyama, A. Sekiguchi, K. Iizuka, R. Yokoyama, Y. Yamamoto, S. Hanawa, T. Araki, C.M. Miyauchi, D. Magistro, K. Sakaki, H. Jeong, Y. Sasaki, and R. Kawashima, *Basal ganglia correlates of fatigue in young adults.* Sci Rep, 2016. **6**(1): p. 21386.
128. Shanks, L., L.A. Jason, M. Evans, and A. Brown, *Cognitive impairments associated with CFS and POTS.* Front Physiol, 2013. **4**: p. 113.

129. Miller, A.H., J.F. Jones, D.F. Drake, H. Tian, E.R. Unger, and G. Pagnoni, *Decreased basal ganglia activation in subjects with chronic fatigue syndrome: association with symptoms of fatigue.* PLoS One, 2014. **9**(5): p. e98156.
130. Unger, E.R., Miller, A. H., Jones, J. F., Drake, D. F., Tian, H., & Pagnoni, G., *Decreased basal ganglia activation in chronic fatigue syndrome subjects is associated with increased fatigue.* 2012.
131. Mizuno, K., Kawatani, J., Tajima, K., Sasaki, A., Yoneda, T., Komi, M., Hirai, T., Tomoda, A., Joudoi, T. and Watanabe, Y., *Low putamen activity associated with poor reward sensitivity in childhood chronic fatigue syndrome NeuroImage.* NeuroImage Clinical, 2016. **12**: p. 600-606.
132. de Lange, F.P., J.S. Kalkman, G. Bleijenberg, P. Hagoort, J.W. van der Meer, and I. Toni, *Gray matter volume reduction in the chronic fatigue syndrome.* Neuroimage, 2005. **26**(3): p. 777-81.
133. de Lange, F.P., A. Koers, J.S. Kalkman, G. Bleijenberg, P. Hagoort, J.W. van der Meer, and I. Toni, *Increase in prefrontal cortical volume following cognitive behavioural therapy in patients with chronic fatigue syndrome.* Brain, 2008. **131**(Pt 8): p. 2172-80.
134. Okada, T., M. Tanaka, H. Kuratsune, Y. Watanabe, and N. Sadato, *Mechanisms underlying fatigue: a voxel-based morphometric study of chronic fatigue syndrome.* BMC Neurol, 2004. **4**(1): p. 14.
135. Barnden, L.R., B. Crouch, R. Kwiatek, R. Burnet, A. Mernone, S. Chryssidis, G. Scroop, and P. Del Fante, *A brain MRI study of chronic fatigue syndrome: evidence of brainstem dysfunction and altered homeostasis.* NMR Biomed, 2011. **24**(10): p. 1302-12.
136. Puri, B.K., P.M. Jakeman, M. Agour, K.D. Gunatilake, K.A. Fernando, A.I. Gurusinghe, I.H. Treasaden, A.D. Waldman, and P. Gishen, *Regional grey and white matter volumetric changes in myalgic encephalomyelitis (chronic fatigue syndrome): a voxel-based morphometry 3 T MRI study.* Br J Radiol, 2012. **85**(1015): p. e270-3.
137. Shan, Z.Y., R. Kwiatek, R. Burnet, P. Del Fante, D.R. Staines, S.M. Marshall-Gradisnik, and L.R. Barnden, *Progressive brain changes in patients with chronic fatigue syndrome: A longitudinal MRI study.* J Magn Reson Imaging, 2016. **44**(5): p. 1301-1311.
138. Finkelmeyer, A., J. He, L. Maclachlan, S. Watson, P. Gallagher, J.L. Newton, and A.M. Blamire, *Grey and white matter differences in Chronic Fatigue Syndrome - A voxel-based morphometry study.* Neuroimage Clin, 2018. **17**: p. 24-30.
139. Shan, Z.Y., R. Kwiatek, R. Burnet, P. Del Fante, D.R. Staines, S.M. Marshall-Gradisnik, and L.R. Barnden, *Medial prefrontal cortex deficits correlate with unrefreshing sleep in patients with chronic fatigue syndrome.* NMR Biomed, 2017. **30**(10).
140. Zeineh, M.M., J. Kang, S.W. Atlas, M.M. Raman, A.L. Reiss, J.L. Norris, I. Valencia, and J.G. Montoya, *Right arcuate fasciculus abnormality in chronic fatigue syndrome.* Radiology, 2015. **274**(2): p. 517-26.
141. Perrin, R., K. Embleton, V.W. Pentreath, and A. Jackson, *Longitudinal MRI shows no cerebral abnormality in chronic fatigue syndrome.* Br J Radiol, 2010. **83**(989): p. 419-23.
142. Barnden, L.R., R. Kwiatek, B. Crouch, R. Burnet, and P. Del Fante, *Autonomic correlations with MRI are abnormal in the brainstem vasomotor centre in Chronic Fatigue Syndrome.* Neuroimage Clin, 2016. **11**: p. 530-537.
143. van der Schaaf, M.E., F.P. De Lange, I.C. Schmits, D.E.M. Geurts, K. Roelofs, J.W.M. van der Meer, I. Toni, and H. Knoop, *Prefrontal Structure Varies as a Function of Pain Symptoms in Chronic Fatigue Syndrome.* Biol Psychiatry, 2017. **81**(4): p. 358-365.
144. Collin, S.M., S. Nikolaus, J. Heron, H. Knoop, P.D. White, and E. Crawley, *Chronic fatigue syndrome (CFS) symptom-based phenotypes in two clinical cohorts of adult patients in the UK and The Netherlands.* J Psychosom Res, 2016. **81**: p. 14-23.
145. Collin, S.M., E. Crawley, M.T. May, J.A. Sterne, W. Hollingworth, and U.C.M.N.O. Database, *The impact of CFS/ME on employment and productivity in the UK: a cross-sectional study based on the CFS/ME national outcomes database.* BMC Health Serv Res, 2011. **11**(1): p. 217.
146. Nichols, T., Das, S., Eickhoff, S., Evans, A., Glatard, T., Hanke, M., Kriegeskorte, N., Milham, M., Poldrack, R., Poline, J., Proal, E., Thirion, B., Van Essen, D., White, T. and B. Yeo, . *Best practices in data analysis and sharing in neuroimaging using MRI. Nature Neuroscience.* 2017. **20**(3): p. 299–303.

147. Barnden, L., Benjamin C., Richard K., Richard B., Anacleto M., Steve C., Scroop. G., Del Fante P., *Evidence in chronic fatigue syndrome for severity-dependent upregulation of prefrontal myelination that is independent of anxiety and depression.* NMR in Biomedicine, 2015. **28**(3): p. 404-413.
148. Barnden, L.R., Z.Y. Shan, D.R. Staines, S. Marshall-Gradisnik, K. Finegan, T. Ireland, and S. Bhuta, *Hyperintense sensorimotor T1 spin echo MRI is associated with brainstem abnormality in chronic fatigue syndrome.* Neuroimage Clin, 2018. **20**: p. 102-109.
149. Natelson, B.H., J.M. Cohen, I. Brassloff, and H.J. Lee, *A controlled study of brain magnetic resonance imaging in patients with the chronic fatigue syndrome.* J Neurol Sci, 1993. **120**(2): p. 213-7.
150. Greco, A., C. Tannock, J. Brostoff, and D.C. Costa, *Brain MR in chronic fatigue syndrome.* AJNR Am J Neuroradiol, 1997. **18**(7): p. 1265-9.
151. Schwartz, R., Garada, BM., Komaroff, AL, Tice, HM, Gleit, M., Jolesz, F., and Holman, BL., *Detection of Intracracial Abnormalities in Patients with Chronic Fatigue Syndrome: Comparison of MR Imaging and SPECT.* American Journal of Roentgenology, 1993. **162**(4): p. 935-941.
152. Lange, G., J. DeLuca, J.A. Maldjian, H. Lee, L.A. Tiersky, and B.H. Natelson, *Brain MRI abnormalities exist in a subset of patients with chronic fatigue syndrome.* J Neurol Sci, 1999. **171**(1): p. 3-7.
153. Lange, G., A.I. Holodny, J. DeLuca, H.J. Lee, X.H. Yan, J. Steffener, and B.H. Natelson, *Quantitative assessment of cerebral ventricular volumes in chronic fatigue syndrome.* Appl Neuropsychol, 2001. **8**(1): p. 23-30.
154. Gay, C.W., M.E. Robinson, S. Lai, A. O'Shea, J.G. Craggs, D.D. Price, and R. Staud, *Abnormal Resting-State Functional Connectivity in Patients with Chronic Fatigue Syndrome: Results of Seed and Data-Driven Analyses.* Brain Connect, 2016. **6**(1): p. 48-56.
155. Kim, B., Namkoong, K., Kim, J., Lee, S., Yoon, K., Choi, M. and Jung, Y., *Altered resting-state functional connectivity in women with chronic fatigue syndrome. Psychiatry Research.* Neuroimaging, 2015. **234**(3): p. 292-297.
156. Wortinger, L., Endestad, T., Melinder, A., Øie, M., Sulheim, D., Fagermoen, E. and Wyller, V., *Emotional conflict processing in adolescent chronic fatigue syndrome: A pilot study using functional magnetic resonance imaging.* Journal of clinical and experimental neuropsychology, 2016. **39**(4): p. 355-368.
157. Wortinger, L.A., M. Glenne Oie, T. Endestad, and V. Bruun Wyller, *Altered right anterior insular connectivity and loss of associated functions in adolescent chronic fatigue syndrome.* PLoS One, 2017. **12**(9): p. e0184325.
158. Cook, D.B., O'Connor, P. J., Lange, G., and Steffener, J., *Functional neuroimaging correlates of mental fatigue induced by cognition among chronic fatigue syndrome patients and controls.* Neuroimage, 2007. **36**(1): p. 108-122.
159. Mizuno, K., M. Tanaka, H.C. Tanabe, T. Joudoi, J. Kawatani, Y. Shigihara, A. Tomoda, T. Miike, K. Imai-Matsumura, N. Sadato, and Y. Watanabe, *Less efficient and costly processes of frontal cortex in childhood chronic fatigue syndrome.* Neuroimage Clin, 2015. **9**: p. 355-68.
160. de Lange, F.P., J.S. Kalkman, G. Bleijenberg, P. Hagoort, S.P. van der Werf, J.W. van der Meer, and I. Toni, *Neural correlates of the chronic fatigue syndrome--an fMRI study.* Brain, 2004. **127**(Pt 9): p. 1948-57.
161. Tanaka, M., N. Sadato, T. Okada, K. Mizuno, T. Sasabe, H.C. Tanabe, D.N. Saito, H. Onoe, H. Kuratsune, and Y. Watanabe, *Reduced responsiveness is an essential feature of chronic fatigue syndrome: a fMRI study.* BMC Neurol, 2006. **6**: p. 9.
162. Caseras, X., D. Mataix-Cols, K.A. Rimes, V. Giampietro, M. Brammer, F. Zelaya, T. Chalder, and E. Godfrey, *The neural correlates of fatigue: an exploratory imaginal fatigue provocation study in chronic fatigue syndrome.* Psychol Med, 2008. **38**(7): p. 941-51.
163. Wortinger, L.A., T. Endestad, A.M. Melinder, M.G. Oie, A. Sevenius, and V. Bruun Wyller, *Aberrant Resting-State Functional Connectivity in the Salience Network of Adolescent Chronic Fatigue Syndrome.* PLoS One, 2016. **11**(7): p. e0159351.

164. Scheltens, P., F. Barkhof, D. Leys, J.P. Pruvo, J.J. Nauta, P. Vermersch, M. Steinling, and J. Valk, *A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging.* J Neurol Sci, 1993. **114**(1): p. 7-12.
165. Geha, P.Y., M.N. Baliki, R.N. Harden, W.R. Bauer, T.B. Parrish, and A.V. Apkarian, *The brain in chronic CRPS pain: abnormal gray-white matter interactions in emotional and autonomic regions.* Neuron, 2008. **60**(4): p. 570-81.
166. Kuchinad, A., P. Schweinhardt, D.A. Seminowicz, P.B. Wood, B.A. Chizh, and M.C. Bushnell, *Accelerated brain gray matter loss in fibromyalgia patients: premature aging of the brain?* J Neurosci, 2007. **27**(15): p. 4004-7.
167. Shan, Z.Y., K. Finegan, S. Bhuta, T. Ireland, D.R. Staines, S.M. Marshall-Gradisnik, and L.R. Barnden, *Decreased Connectivity and Increased Blood Oxygenation Level Dependent Complexity in the Default Mode Network in Individuals with Chronic Fatigue Syndrome.* Brain Connect, 2018. **8**(1): p. 33-39.
168. Wortinger, L., Endestad, T., Melinder, A., Øie, M., Sevenius, A. and Bruun Wyller, V., *Aberrant Resting-State Functional Connectivity in the Salience Network of Adolescent Chronic Fatigue Syndrome.* PLOS ONE, 2016. **11**(7): p. e0159351.
169. Gay, C., Robinson, M., Lai, S., O'Shea, A., Craggs, J., Price, D. and Staud, R., *Abnormal Resting-State Functional Connectivity in Patients with Chronic Fatigue Syndrome.* Brain Connectivity, 2016. **6**(1): p. 48-56.
170. Cook, D.B., P.J. O'Connor, G. Lange, and J. Steffener, *Functional neuroimaging correlates of mental fatigue induced by cognition among chronic fatigue syndrome patients and controls.* Neuroimage, 2007. **36**(1): p. 108-22.
171. Menon, V., *Salience network. In A. W. Toga (Ed.), Brain mapping: An encyclopedic reference.* London: Academic Press, 2015. **2**: p. 597-611.
172. Schwartz, R., Garada, BM., Komaroff, AL, Tice, HM, Gleit, M., Jolesz, F., Holman, BL., *Detection of Intracracial Abnormalities in Patients with Chronic Fatigue Syndrome: Comparison of MR Imaging and SPECT*. 1993.
173. Filley, C.M. and R.D. Fields, *White matter and cognition: making the connection.* J Neurophysiol, 2016. **116**(5): p. 2093-2104.
174. Martino, J., C. Brogna, S.G. Robles, F. Vergani, and H. Duffau, *Anatomic dissection of the inferior fronto-occipital fasciculus revisited in the lights of brain stimulation data.* Cortex, 2010. **46**(5): p. 691-9.
175. Burgmans S., v.B.M.P., van den Berg K. E., Gronenschild E.H., Jacobs H. I., and Jolles J., *The posterior parahippocampal gyrus is preferentially affected in agerelated memory decline.* Neurobiol Aging, 2009. **32**: p. 1572-8.
176. Hickie, I., Lloyd, A., Hadzi-Pavlovic, D., Parker, G., Bird, K., & Wakefield, D., *Can the chronic fatigue syndrome be defined by distinct clinical features?* Psychological medicine, 1995. **25**(5): p. 925-935.
177. Woolf, C.J. and M.W. Salter, *Neuronal plasticity: increasing the gain in pain.* Science, 2000. **288**(5472): p. 1765-9.
178. Apkarian, A.V., Sosa, Y., Sonty, S., Levy, R. M., Harden, R. N., Parrish, T. B., & Gitelman, D. R., *Chronic back pain is associated with decreased prefrontal and thalamic gray matter density.* Journal of Neuroscience, 2004. **24**(46): p. 10410–10415.
179. Buckalew, N., Haut, M. W., Morrow, L., & Weiner, D., *Chronic pain is associated with brain volume loss in older adults: preliminary evidence.* Pain Medicine, 2008. **9**(2): p. 240-248.
180. Schmidt-Wilcke, T., E. Leinisch, S. Ganssbauer, B. Draganski, U. Bogdahn, J. Altmeppen, and A. May, *Affective components and intensity of pain correlate with structural differences in gray matter in chronic back pain patients.* Pain, 2006. **125**(1-2): p. 89-97.
181. Schmidt-Wilcke, T., E. Leinisch, A. Straube, N. Kampfe, B. Draganski, H.C. Diener, U. Bogdahn, and A. May, *Gray matter decrease in patients with chronic tension type headache.* Neurology, 2005. **65**(9): p. 1483-6.
182. Hsu, M.C., Harris, R. E., Sundgren, P. C., Welsh, R. C., Fernandes, C. R., Clauw, D. J., & Williams, D. A., *No consistent difference in gray matter volume between individuals with fibromyalgia and age-matched healthy subjects when controlling for affective disorder.* PAIN, 2009. **143**(3): p. 262-267.

183. Burgmer, M., M. Gaubitz, C. Konrad, M. Wrenger, S. Hilgart, G. Heuft, and B. Pfleiderer, *Decreased gray matter volumes in the cingulo-frontal cortex and the amygdala in patients with fibromyalgia.* Psychosom Med, 2009. **71**(5): p. 566-73.
184. M. Valet, H.G.u., T. Sprenger et al., *Patients with pain disorder show gray-matter loss in pain-processing structures: a voxel-basedmorphometric study.* Psychosomatic Medicine, 2009. **71**(1): p. 49–56.
185. Hickie, I., T. Davenport, S.D. Vernon, R. Nisenbaum, W.C. Reeves, D. Hadzi-Pavlovic, A. Lloyd, and G. International Chronic Fatigue Syndrome Study, *Are chronic fatigue and chronic fatigue syndrome valid clinical entities across countries and health-care settings?* Aust N Z J Psychiatry, 2009. **43**(1): p. 25-35.
186. Komaroff, A.L., *Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: A Real Illness.* Ann Intern Med, 2015. **162**(12): p. 871-2.
187. Wakefield, J.C., *The DSM-5 debate over the bereavement exclusion: psychiatric diagnosis and the future of empirically supported treatment.* Clin Psychol Rev, 2013. **33**(7): p. 825-45.
188. Williams, T.E., Chalder, T., Sharpe, M., & White, P. D., *Heterogeneity in chronic fatigue syndrome–empirically defined subgroups from the PACE trial.* Psychological medicine, 2017. **47**(8): p. 1454-1465.
189. Seeley, W.W., V. Menon, A.F. Schatzberg, J. Keller, G.H. Glover, H. Kenna, A.L. Reiss, and M.D. Greicius, *Dissociable intrinsic connectivity networks for salience processing and executive control.* J Neurosci, 2007. **27**(9): p. 2349-56.
190. Chen, T., L. Michels, K. Supekar, J. Kochalka, S. Ryali, and V. Menon, *Role of the anterior insular cortex in integrative causal signaling during multisensory auditory-visual attention.* Eur J Neurosci, 2015. **41**(2): p. 264-74.
191. Sridharan D, L.D., Menon V. . Proc Natl Acad Sci U S A., *A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks.* 2008. **105**(34): p. 12569-74.
192. Toga, A.W., *Brain mapping: An encyclopedic reference*, ed. A. Press. 2015.
193. Bryer, E.J., Medaglia, J. D., Rostami, S., & Hillary, F. G., *Neural recruitment after mild traumatic brain injury is task dependent: a meta-analysis.* Journal of the International Neuropsychological Society, 2013. **19**(7): p. 751-762.
194. Desmond, J.E. and G.H. Glover, *Estimating sample size in functional MRI (fMRI) neuroimaging studies: statistical power analyses.* J Neurosci Methods, 2002. **118**(2): p. 115-28.
195. Button, K.S., J.P. Ioannidis, C. Mokrysz, B.A. Nosek, J. Flint, E.S. Robinson, and M.R. Munafo, *Power failure: why small sample size undermines the reliability of neuroscience.* Nat Rev Neurosci, 2013. **14**(5): p. 365-76.
196. Turner, B.O., E.J. Paul, M.B. Miller, and A.K. Barbey, *Small sample sizes reduce the replicability of task-based fMRI studies.* Commun Biol, 2018. **1**(1): p. 62.
197. Welvaert, M. and Y. Rosseel, *On the definition of signal-to-noise ratio and contrast-to-noise ratio for FMRI data.* PLoS One, 2013. **8**(11): p. e77089.
198. Leuthardt, E.C., Guzman, G., Bandt, S. K., Hacker, C., Vellimana, A. K., Limbrick, D., ... & Benzinger, T. L., *Integration of resting state functional MRI into clinical practice-A large single institution experience.* PLoS One, 2018. **13**(6): p. e0198349.
199. Davatzikos, C., *Why voxel-based morphometric analysis should be used with greatcaution when characterizing group differences.* NeuroImage 2004. **23** p. 17–20.
200. Miller, E.K., Cohen, J. D., Logothetis, N. K., Bobbert, M., Gómez Álvarez, C., van Weeren, R., & Weishaupt, M. , *What we can do and what we cannot do with fMRI.* Nature Neuroscience, 2008 **24**(7197): p. 869-78.
201. Radua, J. and D. Mataix-Cols, *Meta-analytic methods for neuroimaging data explained.* Biol Mood Anxiety Disord, 2012. **2**(1): p. 6.
202. Muller, V.I., E.C. Cieslik, A.R. Laird, P.T. Fox, J. Radua, D. Mataix-Cols, C.R. Tench, T. Yarkoni, T.E. Nichols, P.E. Turkeltaub, T.D. Wager, and S.B. Eickhoff, *Ten simple rules for neuroimaging meta-analysis.* Neurosci Biobehav Rev, 2018. **84**: p. 151-161.

203. Turkeltaub, P.E., G.F. Eden, K.M. Jones, and T.A. Zeffiro, *Meta-analysis of the functional neuroanatomy of single-word reading: method and validation.* Neuroimage, 2002. **16**(3 Pt 1): p. 765-80.
204. Eickhoff, S.B., T.E. Nichols, A.R. Laird, F. Hoffstaedter, K. Amunts, P.T. Fox, D. Bzdok, and C.R. Eickhoff, *Behavior, sensitivity, and power of activation likelihood estimation characterized by massive empirical simulation.* Neuroimage, 2016. **137**: p. 70-85.
205. Sexton, C.E., J.F. Betts, N. Demnitz, H. Dawes, K.P. Ebmeier, and H. Johansen-Berg, *A systematic review of MRI studies examining the relationship between physical fitness and activity and the white matter of the ageing brain.* Neuroimage, 2016. **131**: p. 81-90.
206. Burzynska, A., Wong, C., Voss, M., Cooke, G., Gothe, N., Fanning, J., McAuley, E. and Kramer, A., *Physical Activity Is Linked to Greater Moment-To-Moment Variability in Spontaneous Brain Activity in Older Adults.* PLOS ONE, 2015. **10**(8): p. e0134819.
207. Erickson, K.I., R.L. Leckie, and A.M. Weinstein, *Physical activity, fitness, and gray matter volume.* Neurobiol Aging, 2014. **35 Suppl 2**: p. S20-8.
208. Krause, A.J., E.B. Simon, B.A. Mander, S.M. Greer, J.M. Saletin, A.N. Goldstein-Piekarski, and M.P. Walker, *The sleep-deprived human brain.* Nat Rev Neurosci, 2017. **18**(7): p. 404-418.
209. Giedd, J.N., J. Blumenthal, N.O. Jeffries, F.X. Castellanos, H. Liu, A. Zijdenbos, T. Paus, A.C. Evans, and J.L. Rapoport, *Brain development during childhood and adolescence: a longitudinal MRI study.* Nat Neurosci, 1999. **2**(10): p. 861-3.
210. Collin, S., Norris, T., Nuevo, R., Tilling, K., Joinson, C., Sterne, J. and Crawley, E., *Chronic Fatigue Syndrome at Age 16 Years.* Pediatrics, 2016. **137**(2): p. e20153434-e20153434.
211. Jack, C.R., Jr., M.A. Bernstein, N.C. Fox, P. Thompson, G. Alexander, D. Harvey, B. Borowski, P.J. Britson, L.W. J, C. Ward, A.M. Dale, J.P. Felmlee, J.L. Gunter, D.L. Hill, R. Killiany, N. Schuff, S. Fox-Bosetti, C. Lin, C. Studholme, C.S. DeCarli, G. Krueger, H.A. Ward, G.J. Metzger, K.T. Scott, R. Mallozzi, D. Blezek, J. Levy, J.P. Debbins, A.S. Fleisher, M. Albert, R. Green, G. Bartzokis, G. Glover, J. Mugler, and M.W. Weiner, *The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods.* J Magn Reson Imaging, 2008. **27**(4): p. 685-91.
212. Guidelines, M.S.C.f.C.P., *Fatigue and Multiple Sclerosis: Evidence-based Management Strategies for Fatigue in Multiple Sclerosis.* Clinical Practice Guidelines, 1998. **Washington D.C.**
213. Jackson, C., *The Chalder fatigue scale (CFQ 11).* Occupational medicine, 2015. **65**(1): p. 86.
214. Chalder, T., G. Berelowitz, T. Pawlikowska, L. Watts, S. Wessely, D. Wright, and E.P. Wallace, *Development of a fatigue scale.* J Psychosom Res, 1993. **37**(2): p. 147-53.
215. Chilcot, J., Norton, S., Kelly, M. E., & Moss-Morris, R., *The Chalder Fatigue Questionnaire is a valid and reliable measure of perceived fatigue severity in multiple sclerosis.* Multiple Sclerosis Journal, 2016. **22**(5): p. 677-684.
216. De Vries, J., H.J. Michielsen, and G.L. Van Heck, *Assessment of fatigue among working people: a comparison of six questionnaires.* Occup Environ Med, 2003. **60 Suppl 1**: p. i10-5.
217. Jason, L.A., M. Evans, M. Brown, N. Porter, A. Brown, J. Hunnell, V. Anderson, and A. Lerch, *Fatigue Scales and Chronic Fatigue Syndrome: Issues of Sensitivity and Specificity.* Disabil Stud Q, 2011. **31**(1): p. 1.
218. Devaux M, S.F., *Social disparities in hazardous alcohol use: Self-report bias may lead to incorrect estimates.* Eur J Public Health, 2016. **26**(1): p. 129-134.
219. Stouten, B., *Identification of ambiguities in the 1994 chronic fatigue syndrome research case definition and recommendations for resolution.* BMC health services research, 2005. **5**(37).
220. Chalder, T., Berelowitz, G., Pawlikowska, T., Watts, L., Wessely, S., Wright, D., & Wallace, E. P., *Development of a fatigue scale. Journal of psychosomatic research.* 1993. **37**(2): p. 147-153.
221. Fox, M.D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E., *The human brain is intrinsically organized into dynamic, anticorrelated functional networks.* Proceedings of the National Academy of Sciences, 2005. **102**(27): p. 9673-9678.
222. Dyson M, B.D., Stewart M,, *Exploring the Brain.* The Open University, 2006: p. 24-27.
223. Bloch, F., *Nuclear induction.* Physical review, 1946. **70**(7-8): p. 460.

224. Purcell, E.M., Torrey, H. C., & Pound, R. V., *Resonance absorption by nuclear magnetic moments in a solid.* Physical review, 1946. **69**(1-2): p. 37.
225. Plewes, D.B. and W. Kucharczyk, *Physics of MRI: a primer.* J Magn Reson Imaging, 2012. **35**(5): p. 1038-54.
226. Sprawls, P., *Magnetic resonance imaging: principles, methods, and techniques*. Medical Physics Publishing. 2000.
227. Hahn, E.L., *Spin echoes.* Phys. Rev., 1950. **80**(4): p. 580-594,.
228. Tang, M.Y., T.W. Chen, X.M. Zhang, and X.H. Huang, *GRE T2 *-weighted MRI: principles and clinical applications.* Biomed Res Int, 2014. **2014**: p. 312142.
229. Biswal, B., Zerrin Yetkin, F., Haughton, V. M., & Hyde, J. S., *Functional connectivity in the motor cortex of resting human brain using echo-planar MRI.* Magnetic resonance in medicine, 1995. **34**(4): p. 537-541.
230. Chavhan, G.B., P.S. Babyn, B. Thomas, M.M. Shroff, and E.M. Haacke, *Principles, techniques, and applications of T2*-based MR imaging and its special applications.* Radiographics, 2009. **29**(5): p. 1433-49.
231. Jones, J., and F. Gaillard., *MRI sequences (overview).* 2017.
232. Heeger, D.J., & Ress, D., *What does fMRI tell us about neuronal activity?* Nature Reviews Neuroscience, 2002. **3**(2): p. 142.
233. Amaro, E., Jr. and G.J. Barker, *Study design in fMRI: basic principles.* Brain Cogn, 2006. **60**(3): p. 220-32.
234. Huettel, S.A.S., A. W.; McCarthy, G., *Functional Magnetic Resonance Imaging*. 2 ed ed, ed. M. Sinauer. 2009.
235. Niendorf, T., Pohlmann, A., Arakelyan, K., Flemming, B., Cantow, K., Hentschel, J., Grosenick, D., Ladwig, M., Reimann, H., Klix, S. and Waiczies, S.,, *How bold is blood oxygenation level-dependent (BOLD) magnetic resonance imaging of the kidney? Opportunities, challenges and future directions.* Acta Physiologica, 2015. **213**(1): p. 19-38.
236. F. Varela, J.P.L., E. Rodriguez, and J. Martinerie., *The brainweb: phase synchronization and largescale integration.* Nature Rev. Neuro, 2001.
237. C, A.-M., *Electrical activity of the brain: slow waves and neuronal activity.* Isr J Med Sci, 1965. **1**: p. 104-117.
238. Buchwald JS, H.E., Schramm S, *A comparison of multi-unit activity and EEG activity recorded from the same brain site in chronic cats during behavioral conditioning.* Nature 1965. **205**: p. 1012-1014.
239. Fromm GH, B.H., *The relationship between neuron activity and cortical steady potentials.* Electroencephalogr Clin Neurophysiol, 1967. **22**: p. 159-166.
240. Logothetis, N.K., & Panzeri, S., *Local field potential, relationship to BOLD signal.* Encyclopedia of Computational Neuroscience, 2015: p. 1560-1568.
241. Carlson, D.E., Borg, J. S., Dzirasa, K., & Carin, L., *On the relationship between LFP & spiking data.* Advances in Neural Information Processing Systems, 2014.
242. B. Pesaran, J.S.P., M. Sahani, P. P. Mitra, and R. A. Andersen., *Temporal structure in neuronal activity during working memory in macaque parietal cortex.* Nature neuroscience, 2002. **5**: p. 805-811.
243. C. Mehring, J.R., E. Vaadia, S. Cardosa de Oliveira, A. Aertsen, and S. Rotter., *Inference of hand movements from local field potentials in monkey motor cortex.* Nature neuroscience, 2003. **6**(12): p. 1253-1254.
244. Logothetis, N.K., J. Pauls, M. Augath, T. Trinath, and A. Oeltermann, *Neurophysiological investigation of the basis of the fMRI signal.* Nature, 2001. **412**(6843): p. 150-7.
245. Rauch A, R.G., Logothetis NK, *The effect of a serotonin-induced dissociation between spiking and perisynaptic activity on BOLD functional MRI.* Proceedings of the National Academy of Sciences, 2008 **105**(18): p. 6759-64.
246. Viswanathan, A., & Freeman, R. D., *Neurometabolic coupling in cerebral cortex reflects synaptic more than spiking activity.* Nature neuroscience, 2007. **10**(10): p. 1308-1312.
247. Ekstrom, A., *How and when the fMRI BOLD signal relates to underlying neural activity: the danger in dissociation.* Brain research reviews, 2010. **62**(2): p. 233-244.

248. Goense, J.B. and N.K. Logothetis, *Neurophysiology of the BOLD fMRI signal in awake monkeys.* Curr Biol, 2008. **18**(9): p. 631-40.
249. Shmuel, A., M. Augath, A. Oeltermann, and N.K. Logothetis, *Negative functional MRI response correlates with decreases in neuronal activity in monkey visual area V1.* Nat Neurosci, 2006. **9**(4): p. 569-77.
250. Devor A, T.P., Nishimura N, Teng IC, Hillman EM, Narayanan SN, Ulbert I, Boas DA, Kleinfeld D, Dale AM, *Suppressed neuronal activity and concurrent arteriolar vasoconstriction may explain negative blood oxygenation level-dependent signal.* J Neurosci, 2007 **27**(16): p. 4452-9.
251. Mathiesen, C., K. Caesar, N. Akgoren, and M. Lauritzen, *Modification of activity-dependent increases of cerebral blood flow by excitatory synaptic activity and spikes in rat cerebellar cortex.* J Physiol, 1998. **512 (Pt 2)**: p. 555-66.
252. Mathiesen C, C.K., Lauritzen M, *Temporal coupling between neuronal activity and blood flow in rat cerebellar cortex as indicated by field potential analysis.* J Physiol Pharmacol, 2000 **1**: p. 235-46.
253. Magri C, S.U., Murayama Y, Panzeri S, Logothetis NK, *The amplitude and timing of the BOLD signal reflects the relationship between local field potential power at different frequencies.* J Neurosci, 2012. **32**: p. 1395-1407.
254. Kesavadas, C., *Resting state functional magnetic resonance imaging: an emerging clinical tool.* Neurol India, 2013. **61**(2): p. 103-4.
255. Fox, M.D. and M. Greicius, *Clinical applications of resting state functional connectivity.* Front Syst Neurosci, 2010. **4**: p. 19.
256. Henson, R.N.A., *Event-related fMRI: Introduction, stastistical modelling, design optimisation and examples.* Japanese Journal of Cognitive Neuroscience, 2000.
257. Chee, M.W., V. Venkatraman, C. Westphal, and S.C. Siong, *Comparison of block and event-related fMRI designs in evaluating the word-frequency effect.* Hum Brain Mapp, 2003. **18**(3): p. 186-93.
258. Pfleiderer, B., J. Ostermann, N. Michael, and W. Heindel, *Visualization of auditory habituation by fMRI.* Neuroimage, 2002. **17**(4): p. 1705-10.
259. Michael N, O.J., Sörös P, Schwindt W, Pfleiderer B., *A subgroup of depressed patients presented altered habituation in the auditory cortex as measured by fMRI.* Neuropsychobiology 2004. **49**: p. 5–9.
260. Logothetis, N.K. and J. Pfeuffer, *On the nature of the BOLD fMRI contrast mechanism.* Magn Reson Imaging, 2004. **22**(10): p. 1517-31.
261. Friston, K.J., *Statistical parametric mapping.* In Neuroscience databases, 2003: p. 237-250.
262. K.J. Friston, A.P.H., K.J. Worsley, J.P. Poline, C.D. Frith, R.S.J. Frackowiak,, *Statistical parametric maps in functional imaging: a general linear approach.* Human Brain Mapping, 1995. **2**: p. 189-210,.
263. K.J. Friston, C.D.F., P.F. Liddle and R.S.J. Frackowiak, *Comparing functional (PET) images: the assessment of significant change.* Journal of Cerebral Blood Flow and Metabolism, 1991. **11**: p. 690-699.
264. Flandin, G., & Friston, K. J., *Analysis of family-wise error rates in statistical parametric mapping using random field theory.* Human brain mapping, 2019. **40**(7): p. 2052-2054.
265. Flandin, G., & Friston, K. J., *Statistical parametric mapping (SPM).* Scholarpedia, 2008. **3**(4).
266. Adler, N.J., *Re-entry: Managing cross-cultural transitions.* Group & Organization Studies, 1981. **6**(3): p. 341-356.
267. Worsley, K.J., A.C. Evans, S. Marrett, and P. Neelin, *A three-dimensional statistical analysis for CBF activation studies in human brain.* J Cereb Blood Flow Metab, 1992. **12**(6): p. 900-18.
268. Worsley, K.J., S. Marrett, P. Neelin, A.C. Vandal, K.J. Friston, and A.C. Evans, *A unified statistical approach for determining significant signals in images of cerebral activation.* Hum Brain Mapp, 1996. **4**(1): p. 58-73.
269. Roels, S.P., H. Bossier, T. Loeys, and B. Moerkerke, *Data-analytical stability of cluster-wise and peak-wise inference in fMRI data analysis.* J Neurosci Methods, 2015. **240**: p. 37-47.

270. Li, H., Nickerson, L. D., Zhao, X., Nichols, T. E., & Gao, J. H., *A voxelationcorrected non-stationary 3D cluster-size test based on random field theory.* NeuroImage, 2015. **118**: p. 676–682. .
271. Li, H., L.D. Nickerson, J. Xiong, Q. Zou, Y. Fan, Y. Ma, T. Shi, J. Ge, and J.H. Gao, *A high performance 3D cluster-based test of unsmoothed fMRI data.* Neuroimage, 2014. **98**: p. 537-46.
272. Flandin, G., & Friston, K. J., *Analysis of family-wise error rates in statistical parametric mapping using random field theory.* Arxiv, 2016: p. 1–4.
273. Tierney, T.M., Clark, C. A., & Carmichael, D. W., *Is Bonferroni correction more sensitive than random field theory for most fMRI studies?* arXiv preprint arXiv, 2016.
274. Shimizu, S., D. Hirose, H. Hatanaka, N. Takenoshita, Y. Kaneko, Y. Ogawa, H. Sakurai, and H. Hanyu, *Role of Neuroimaging as a Biomarker for Neurodegenerative Diseases.* Frontiers in Neurology, 2018. **9**: p. 265.
275. Turner, R. and T. Jones, *Techniques for imaging neuroscience.* Br Med Bull, 2003. **65**(1): p. 3-20.
276. Jack, C.R., Jr., M.S. Albert, D.S. Knopman, G.M. McKhann, R.A. Sperling, M.C. Carrillo, B. Thies, and C.H. Phelps, *Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimers Dement, 2011. **7**(3): p. 257-62.
277. McKhann, G.M., D.S. Knopman, H. Chertkow, B.T. Hyman, C.R. Jack, Jr., C.H. Kawas, W.E. Klunk, W.J. Koroshetz, J.J. Manly, R. Mayeux, R.C. Mohs, J.C. Morris, M.N. Rossor, P. Scheltens, M.C. Carrillo, B. Thies, S. Weintraub, and C.H. Phelps, *The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimers Dement, 2011. **7**(3): p. 263-9.
278. McKeith, I.G., B.F. Boeve, D.W. Dickson, G. Halliday, J.P. Taylor, D. Weintraub, D. Aarsland, J. Galvin, J. Attems, C.G. Ballard, A. Bayston, T.G. Beach, F. Blanc, N. Bohnen, L. Bonanni, J. Bras, P. Brundin, D. Burn, A. Chen-Plotkin, J.E. Duda, O. El-Agnaf, H. Feldman, T.J. Ferman, D. Ffytche, H. Fujishiro, D. Galasko, J.G. Goldman, S.N. Gomperts, N.R. Graff-Radford, L.S. Honig, A. Iranzo, K. Kantarci, D. Kaufer, W. Kukull, V.M.Y. Lee, J.B. Leverenz, S. Lewis, C. Lippa, A. Lunde, M. Masellis, E. Masliah, P. McLean, B. Mollenhauer, T.J. Montine, E. Moreno, E. Mori, M. Murray, J.T. O'Brien, S. Orimo, R.B. Postuma, S. Ramaswamy, O.A. Ross, D.P. Salmon, A. Singleton, A. Taylor, A. Thomas, P. Tiraboschi, J.B. Toledo, J.Q. Trojanowski, D. Tsuang, Z. Walker, M. Yamada, and K. Kosaka, *Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium.* Neurology, 2017. **89**(1): p. 88-100.
279. Sima, D.M., Loeckx, D., Smeets, D., Jain, S., Parizel, P. M., & Van Hecke, W., *Use Case I: Imaging Biomarkers in Neurological Disease. Focus on Multiple Sclerosis. In Imaging Biomarkers*. Cham, ed. Springer. 2017.
280. Ashburner, J., & Friston, K. J., *Voxel-based morphometry-the methods.* Neuroimage, 2000. **11**(6): p. 805-821.
281. Maguire, E.A., D.G. Gadian, I.S. Johnsrude, C.D. Good, J. Ashburner, R.S. Frackowiak, and C.D. Frith, *Navigation-related structural change in the hippocampi of taxi drivers.* Proc Natl Acad Sci U S A, 2000. **97**(8): p. 4398-403.
282. Shan, Z., Kwiatek, R., Burnet, R., Del Fante, P., Staines, D., Marshall-Gradisnik, S. and Barnden, L., *Progressive Brain Changes in Patients With Chronic Fatigue Syndrome: A Longitudinal MRI Study.* Journal of Magnetic Resonance Imaging, 2016. **44**(5): p. 1301-1311.
283. Zeineh, M., Kang, J., Atlas, S., Raman, M., Reiss, A., Norris, J., Valencia, I. and Montoya, J., *Right Arcuate Fasciculus Abnormality in Chronic Fatigue Syndrome.* Radiology, 2015. **274**(2): p. 517-526.
284. Barnden, L.R., Shan, Z. Y., Staines, D. R., Marshall-Gradisnik, S., Finegan, K., Ireland, T., & Bhuta, S., *Hyperintense sensorimotor T1 spin echo MRI is associated with brainstem abnormality in chronic fatigue syndrome.* NeuroImage: Clinical, 2018. **20**: p. 102-109.

285. de Lange, F., Kalkman, J., Bleijenberg, G., Hagoort, P., van der Meer, J. and Toni, I., *Gray matter volume reduction in the chronic fatigue syndrome.* NeuroImage, 2005. **26**(3): p. 777–781.
286. de Lange, F., Koers, A., Kalkman, J., Bleijenberg, G., Hagoort, P., van der Meer, J. and Toni, I., *Increase in prefrontal cortical volume following cognitive behavioural therapy in patients with chronic fatigue syndrome.* 2008. **131**: p. 2172-2180.
287. Okada, T., Tanaka, M., Kuratsune, H., Watanabe, Y., Sadato, N., *Mechanisms underlying fatigue: A voxel-based morphometric study of chronic fatigue syndrome.* BMC Neurology, 2004. **4**(1): p. 14.
288. Barnden, L., Benjamin C., Richard K., Richard B., Anacleto M., Steve C., Scroop. G., Del Fante P., *A brain MRI study of chronic fatigue syndrome Evidence of brainstem dysfunction and altered homeostasis.* NMR in biomedicine, 2011. **24**: p. 1302–1312.
289. Kana, R.K., L.Q. Uddin, T. Kenet, D. Chugani, and R.A. Muller, *Brain connectivity in autism.* Front Hum Neurosci, 2014. **8**: p. 349.
290. Cole, D.M., S.M. Smith, and C.F. Beckmann, *Advances and pitfalls in the analysis and interpretation of resting-state FMRI data.* Front Syst Neurosci, 2010. **4**: p. 8.
291. Rubinov, M. and O. Sporns, *Complex network measures of brain connectivity: uses and interpretations.* Neuroimage, 2010. **52**(3): p. 1059-69.
292. Friston, K.J., L. Harrison, and W. Penny, *Dynamic causal modelling.* Neuroimage, 2003. **19**(4): p. 1273-302.
293. Stephan, K.E., W.D. Penny, J. Daunizeau, R.J. Moran, and K.J. Friston, *Bayesian model selection for group studies.* Neuroimage, 2009. **46**(4): p. 1004-17.
294. Butts, C.T., *Revisiting the foundations of network analysis.* Science, 2009. **325**(5939): p. 414-6.
295. Park, B., Ko, J. H., Lee, J. D., & Park, H. J., *Correction: Evaluation of Node-Inhomogeneity Effects on the Functional Brain Network Properties Using an Anatomy-Constrained Hierarchical Brain Parcellation.* Plos one, 2013. **8**(10).
296. Smith, S.M., C.F. Beckmann, J. Andersson, E.J. Auerbach, J. Bijsterbosch, G. Douaud, E. Duff, D.A. Feinberg, L. Griffanti, M.P. Harms, M. Kelly, T. Laumann, K.L. Miller, S. Moeller, S. Petersen, J. Power, G. Salimi-Khorshidi, A.Z. Snyder, A.T. Vu, M.W. Woolrich, J. Xu, E. Yacoub, K. Ugurbil, D.C. Van Essen, M.F. Glasser, and W.U.-M.H. Consortium, *Resting-state fMRI in the Human Connectome Project.* Neuroimage, 2013. **80**: p. 144-68.
297. Kaiser, M. and S. Varier, *Evolution and development of brain networks: from Caenorhabditis elegans to Homo sapiens.* Network, 2011. **22**(1-4): p. 143-7.
298. Garrison, K.A., D. Scheinost, E.S. Finn, X. Shen, and R.T. Constable, *The (in)stability of functional brain network measures across thresholds.* Neuroimage, 2015. **118**: p. 651-61.
299. Jalili, M., *Graph theoretical analysis of Alzheimer's disease: Discrimination of AD patients from healthy subjects.* Information Sciences, 2017. **384**: p. 145-156.
300. Zalesky, A., A. Fornito, and E.T. Bullmore, *On the use of correlation as a measure of network connectivity.* Neuroimage, 2012. **60**(4): p. 2096-2106.
301. De Vico Fallani, F., J. Richiardi, M. Chavez, and S. Achard, *Graph analysis of functional brain networks: practical issues in translational neuroscience.* Philos Trans R Soc Lond B Biol Sci, 2014. **369**(1653): p. 20130521.
302. Rubinov, M. and O. Sporns, *Weight-conserving characterization of complex functional brain networks.* Neuroimage, 2011. **56**(4): p. 2068-79.
303. Bullmore, E. and O. Sporns, *Complex brain networks: graph theoretical analysis of structural and functional systems.* Nat Rev Neurosci, 2009. **10**(3): p. 186-98.
304. Watts, D.J. and S.H. Strogatz, *Collective dynamics of 'small-world' networks.* Nature, 1998. **393**(6684): p. 440-442.
305. Stanley, M.L., M.N. Moussa, B.M. Paolini, R.G. Lyday, J.H. Burdette, and P.J. Laurienti, *Defining nodes in complex brain networks.* Front Comput Neurosci, 2013. **7**: p. 169.
306. Barnes, K.A., Nelson, S. M., Cohen, A. L., Power, J. D., Coalson, R. S., Miezin, F. M., & Schlaggar, B. L., *Parcellation in left lateral parietal cortex is similar in adults and children.* Cerebral Cortex, 2012. **22**: p. 1148-1158.

307. Nelson, S.M., A.L. Cohen, J.D. Power, G.S. Wig, F.M. Miezin, M.E. Wheeler, K. Velanova, D.I. Donaldson, J.S. Phillips, B.L. Schlaggar, and S.E. Petersen, *A parcellation scheme for human left lateral parietal cortex.* Neuron, 2010. **67**(1): p. 156-70.
308. Craddock, R.C., G.A. James, P.E. Holtzheimer, 3rd, X.P. Hu, and H.S. Mayberg, *A whole brain fMRI atlas generated via spatially constrained spectral clustering.* Hum Brain Mapp, 2012. **33**(8): p. 1914-28.
309. Mumford, J.A., S. Horvath, M.C. Oldham, P. Langfelder, D.H. Geschwind, and R.A. Poldrack, *Detecting network modules in fMRI time series: a weighted network analysis approach.* Neuroimage, 2010. **52**(4): p. 1465-76.
310. Thirion, B., E. Duchesnay, E. Hubbard, J. Dubois, J.B. Poline, D. Lebihan, and S. Dehaene, *Inverse retinotopy: inferring the visual content of images from brain activation patterns.* Neuroimage, 2006. **33**(4): p. 1104-16.
311. Blumensath, T., S. Jbabdi, M.F. Glasser, D.C. Van Essen, K. Ugurbil, T.E. Behrens, and S.M. Smith, *Spatially constrained hierarchical parcellation of the brain with resting-state fMRI.* Neuroimage, 2013. **76**: p. 313-24.
312. Tzourio-Mazoyer, N., B. Landeau, D. Papathanassiou, F. Crivello, O. Etard, N. Delcroix, B. Mazoyer, and M. Joliot, *Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain.* Neuroimage, 2002. **15**(1): p. 273-289.
313. Ciftci, K., *Minimum spanning tree reflects the alterations of the default mode network during Alzheimer's disease.* Ann Biomed Eng, 2011. **39**(5): p. 1493-504.
314. Cole, M.W., J.R. Reynolds, J.D. Power, G. Repovs, A. Anticevic, and T.S. Braver, *Multi-task connectivity reveals flexible hubs for adaptive task control.* Nat Neurosci, 2013. **16**(9): p. 1348-55.
315. Power, J.D., A.L. Cohen, S.M. Nelson, G.S. Wig, K.A. Barnes, J.A. Church, A.C. Vogel, T.O. Laumann, F.M. Miezin, B.L. Schlaggar, and S.E. Petersen, *Functional network organization of the human brain.* Neuron, 2011. **72**(4): p. 665-78.
316. Stevens, A.A., S.C. Tappon, A. Garg, and D.A. Fair, *Functional brain network modularity captures inter- and intra-individual variation in working memory capacity.* PLoS One, 2012. **7**(1): p. e30468.
317. He, Y. and A. Evans, *Graph theoretical modeling of brain connectivity.* Curr Opin Neurol, 2010. **23**(4): p. 341-50.
318. van den Heuvel, M.P. and H.E. Hulshoff Pol, *Exploring the brain network: a review on resting-state fMRI functional connectivity.* Eur Neuropsychopharmacol, 2010. **20**(8): p. 519-34.
319. Woodward, N.D. and C.J. Cascio, *Resting-State Functional Connectivity in Psychiatric Disorders.* JAMA Psychiatry, 2015. **72**(8): p. 743-4.
320. Andrews-Hanna, J.R., A.Z. Snyder, J.L. Vincent, C. Lustig, D. Head, M.E. Raichle, and R.L. Buckner, *Disruption of large-scale brain systems in advanced aging.* Neuron, 2007. **56**(5): p. 924-35.
321. Damoiseaux, J.S., C.F. Beckmann, E.J. Arigita, F. Barkhof, P. Scheltens, C.J. Stam, S.M. Smith, and S.A. Rombouts, *Reduced resting-state brain activity in the "default network" in normal aging.* Cereb Cortex, 2008. **18**(8): p. 1856-64.
322. Newman, M.E. and M. Girvan, *Finding and evaluating community structure in networks.* Phys Rev E Stat Nonlin Soft Matter Phys, 2004. **69**(2 Pt 2): p. 026113.
323. Wang, J., X. Zuo, and Y. He, *Graph-based network analysis of resting-state functional MRI.* Front Syst Neurosci, 2010. **4**: p. 16.
324. Latora, V. and M. Marchiori, *Efficient behavior of small-world networks.* Phys Rev Lett, 2001. **87**(19): p. 198701.
325. Bassett, D.S., and Bullmore, E. T., *Small-world brain networks.* Neuroscientist, 2006. **12**: p. 512–523.
326. Stam, C.J. and J.C. Reijneveld, *Graph theoretical analysis of complex networks in the brain.* Nonlinear Biomed Phys, 2007. **1**(1): p. 3.
327. J. Friedman, T.H., and R. Tibshirani,, *Sparse inverse covariance estimation with the graphical LASSO.* Biostatistics, 2008. **9**(3): p. 432–441.

328. J. Mei and J. M. F Moura, *Signal processing on graphs: Estimating the structure of a graph.* Speech and Signal Processing (ICASSP). 2015 p. 5495–5499.
329. F. Lin, M.F., and M. R Jovanovic,, *Identification of sparse communication graphs in consensus networks.* in Communication, Control, and Computing (Allerton), 2012 p. 85–89.
330. G. Gnecco, R.M., and A. Bemporad,, *Sparse solutions to the average consensus problem via l1-norm regularization of the fastest mixing markov-chain problem.* Decision and Control (CDC), 2014 p. 2228–2233.
331. Barbey, A.K., *Network Neuroscience Theory of Human Intelligence.* Trends Cogn Sci, 2018. **22**(1): p. 8-20.
332. Nichols, T. and S. Hayasaka, *Controlling the familywise error rate in functional neuroimaging: a comparative review.* Stat Methods Med Res, 2003. **12**(5): p. 419-46.
333. Brett, M., Penny, W., & Kiebel, S., *Introduction to random field theory.* Human brain function, 2003. **2**.
334. Cohen, J., *Statistical Power Analysis for the Behavioral Sciences*. Lawrence Erlbaum Associates., ed. N. Hillsdale. Vol. 2nd ed.
. 1998.
335. Coe, R., *It's the effect size, stupid: What effect size is and why it is important.* In Paper presented at the British Educational Research Association annual conference, 2002. **12**: p. 14.
336. Mumford, J.A. and T.E. Nichols, *Power calculation for group fMRI studies accounting for arbitrary design and temporal autocorrelation.* Neuroimage, 2008. **39**(1): p. 261-8.
337. Moore, A.B., Z. Li, C.E. Tyner, X. Hu, and B. Crosson, *Bilateral basal ganglia activity in verbal working memory.* Brain Lang, 2013. **125**(3): p. 316-23.
338. Pourhoseingholi, M.A., Baghestani, A. R., & Vahedi, M., *How to control confounding effects by statistical analysis.* Gastroenterology and hepatology from bed to bench, 2012. **5**(2): p. 79.
339. Deale, A., T. Chalder, I. Marks, and S. Wessely, *Cognitive behavior therapy for chronic fatigue syndrome: a randomized controlled trial.* Am J Psychiatry, 1997. **154**(3): p. 408-14.
340. Powell, P., R.P. Bentall, F.J. Nye, and R.H. Edwards, *Randomised controlled trial of patient education to encourage graded exercise in chronic fatigue syndrome.* BMJ, 2001. **322**(7283): p. 387-90.
341. Wearden, A.J.D., Christopher; Chew-Graham, Carolyn; Bentall, Richard P.; Morriss, Richard K.; Peters, Sarah; Riste, Lisa; Richardson, Gerry; Lovell, Karina, *Nurse led, home based self help treatment for patients in primary care with chronic fatigue syndrome: randomised controlled trial.* BMJ 2010. **340**: p. 1756-1833.
342. Nisenbaum, R., A. Jones, J. Jones, and W. Reeves, *Longitudinal analysis of symptoms reported by patients with chronic fatigue syndrome.* Ann Epidemiol, 2000. **10**(7): p. 458.
343. Shan, Z.Y., K. Finegan, S. Bhuta, T. Ireland, D.R. Staines, S.M. Marshall-Gradisnik, and L.R. Barnden, *Brain function characteristics of chronic fatigue syndrome: A task fMRI study.* Neuroimage Clin, 2018. **19**: p. 279-286.
344. Kim, B.J., H.G. Kang, H.J. Kim, S.H. Ahn, N.Y. Kim, S. Warach, and D.W. Kang, *Magnetic resonance imaging in acute ischemic stroke treatment.* J Stroke, 2014. **16**(3): p. 131-45.
345. Traboulsee, A.L. and D.K. Li, *The role of MRI in the diagnosis of multiple sclerosis.* Adv Neurol, 2006. **98**: p. 125-46.
346. Chalela, J.A., C.S. Kidwell, L.M. Nentwich, M. Luby, J.A. Butman, A.M. Demchuk, M.D. Hill, N. Patronas, L. Latour, and S. Warach, *Magnetic resonance imaging and computed tomography in emergency assessment of patients with suspected acute stroke: a prospective comparison.* Lancet, 2007. **369**(9558): p. 293-8.
347. Good, C.D., I.S. Johnsrude, J. Ashburner, R.N. Henson, K.J. Friston, and R.S. Frackowiak, *A voxel-based morphometric study of ageing in 465 normal adult human brains.* Neuroimage, 2001. **14**(1 Pt 1): p. 21-36.
348. Scarpazza, C., & De Simone, M. S., *Voxel-based morphometry: current perspectives.* Neuroscience and Neuroeconomics, 2016. **5**: p. 19-35.
349. Wang, W.Y., J.T. Yu, Y. Liu, R.H. Yin, H.F. Wang, J. Wang, L. Tan, J. Radua, and L. Tan, *Voxel-based meta-analysis of grey matter changes in Alzheimer's disease.* Transl Neurodegener, 2015. **4**(1): p. 6.

350. Focke N, T.S., Paulus W, Falkai P, Gruber O., *Do manual and voxel-based morphometry measure the same? A proof of concept study.* Front Psychiatry, 2014. **5**: p. 39.
351. Matsuda H, M.S., Nemoto K, et al., *Automatic voxel-based morphometry of structural MRI by SPM8 plus diffeomorphic anatomic registration through exponentiated lie algebra improves the diagnosis of probable Alzheimer disease.* AJNR Am J Neuroradiol, 2012. **33**(6): p. 1109–1114.
352. Guo Y, Z.Z., Zhou B, et al., *Grey-matter volume as a potential feature for the classification of Alzheimer's disease and mild cognitive impairment: an exploratory study.* Neurosci Bull, 2014. **30**(3): p. 477–489.
353. Roelcke, U., L. Kappos, J. Lechner-Scott, H. Brunnschweiler, S. Huber, W. Ammann, A. Plohmann, S. Dellas, R.P. Maguire, J. Missimer, E.W. Radu, A. Steck, and K.L. Leenders, *Reduced glucose metabolism in the frontal cortex and basal ganglia of multiple sclerosis patients with fatigue: a 18F-fluorodeoxyglucose positron emission tomography study.* Neurology, 1997. **48**(6): p. 1566-71.
354. Roberts, D., *Chronic fatigue syndrome and quality of life.* Patient Relat Outcome Meas, 2018. **9**: p. 253-262.
355. Zigmond, A.S. and R.P. Snaith, *The hospital anxiety and depression scale.* Acta Psychiatr Scand, 1983. **67**(6): p. 361-70.
356. Eickhoff, S.B., A.R. Laird, C. Grefkes, L.E. Wang, K. Zilles, and P.T. Fox, *Coordinate-based activation likelihood estimation meta-analysis of neuroimaging data: a random-effects approach based on empirical estimates of spatial uncertainty.* Hum Brain Mapp, 2009. **30**(9): p. 2907-26.
357. Lancaster, J.L., M.G. Woldorff, L.M. Parsons, M. Liotti, C.S. Freitas, L. Rainey, P.V. Kochunov, D. Nickerson, S.A. Mikiten, and P.T. Fox, *Automated Talairach atlas labels for functional brain mapping.* Hum Brain Mapp, 2000. **10**(3): p. 120-31.
358. Evans, A.C., Collins, D.L., Milner, B., *An MRI-based stereotaxic atlas from 250 young normal subjects.* Proc 22nd Annual Symposium, Society for Neuroscience, 1992. **18**: p. 408.
359. Ratey, J.J. and J.E. Loehr, *The positive impact of physical activity on cognition during adulthood: a review of underlying mechanisms, evidence and recommendations.* Rev Neurosci, 2011. **22**(2): p. 171-85.
360. Å berg, M.A., Pedersen, N.L., Tor é n, K., Svartengren, M., B ä ckstrand, B., Johnsson, T., et al., *Cardiovascular fi tness is associated with cognition in young adulthood.* Proc. Natl. Acad. Sci. USA, 2009. **106**: p. 20906 – 20911.
361. Kidd, E., Brown, A., McManimen, S., Jason, L. A., Newton, J. L., & Strand, E. B., *The relationship between age and illness duration in chronic fatigue syndrome.* Diagnostics, 2016. **6**(2): p. 16.
362. Friedberg, F., L. Dechene, M.J. McKenzie, 2nd, and R. Fontanetta, *Symptom patterns in long-duration chronic fatigue syndrome.* J Psychosom Res, 2000. **48**(1): p. 59-68.
363. van der Werf, S.P., B. de Vree, M. Alberts, J.W. van der Meer, G. Bleijenberg, and N. Netherlands Fatigue Research Group, *Natural course and predicting self-reported improvement in patients with chronic fatigue syndrome with a relatively short illness duration.* J Psychosom Res, 2002. **53**(3): p. 749-53.
364. Spartano, N.L., K.L. Davis-Plourde, J.J. Himali, C. Andersson, M.P. Pase, P. Maillard, C. DeCarli, J.M. Murabito, A.S. Beiser, R.S. Vasan, and S. Seshadri, *Association of Accelerometer-Measured Light-Intensity Physical Activity With Brain Volume: The Framingham Heart Study.* JAMA Netw Open, 2019. **2**(4): p. e192745.
365. Elvsashagen, T., L.B. Norbom, P.O. Pedersen, S.H. Quraishi, A. Bjornerud, U.F. Malt, I.R. Groote, and L.T. Westlye, *Widespread changes in white matter microstructure after a day of waking and sleep deprivation.* PLoS One, 2015. **10**(5): p. e0127351.
366. McKinnon AC, L.J., Terpening Z, et al., *Sleep disturbance in mild cognitive impairment is associated with alterations in the brain's default mode network.* Behav Neurosci, 2016. **130**(3): p. 305-315.
367. Mander, B.A., V. Rao, B. Lu, J.M. Saletin, J.R. Lindquist, S. Ancoli-Israel, W. Jagust, and M.P. Walker, *Prefrontal atrophy, disrupted NREM slow waves and impaired hippocampal-dependent memory in aging.* Nat Neurosci, 2013. **16**(3): p. 357-64.

368. Boissoneault, J., J. Letzen, S. Lai, A. O'Shea, J. Craggs, M.E. Robinson, and R. Staud, *Abnormal resting state functional connectivity in patients with chronic fatigue syndrome: an arterial spin-labeling fMRI study.* Magn Reson Imaging, 2016. **34**(4): p. 603-8.
369. DeLuca, J., Genova, H. M., Hillary, F. G., & Wylie, G., *Neural correlates of cognitive fatigue in multiple sclerosis using functional MRI.* Journal of the neurological sciences, 2008. **270**(1-2): p. 28-39.
370. Finke, C., J. Schlichting, S. Papazoglou, M. Scheel, A. Freing, C. Soemmer, L.M. Pech, A. Pajkert, C. Pfuller, J.T. Wuerfel, C.J. Ploner, F. Paul, and A.U. Brandt, *Altered basal ganglia functional connectivity in multiple sclerosis patients with fatigue.* Mult Scler, 2015. **21**(7): p. 925-34.
371. WJH., N., *The relationship of basal ganglia to the limbic system*. Handbook of clinical neurology, ed. B.G. Vinken PJ, eds. Vol. 49. 1986, Amsterdam: Elsevier Science.
372. Ghika, J., F. Ghika-Schmid, H. Fankhauser, G. Assal, F. Vingerhoets, A. Albanese, J. Bogousslavsky, and J. Favre, *Bilateral contemporaneous posteroventral pallidotomy for the treatment of Parkinson's disease: neuropsychological and neurological side effects. Report of four cases and review of the literature.* J Neurosurg, 1999. **91**(2): p. 313-21.
373. Petersen, S.E., H. van Mier, J.A. Fiez, and M.E. Raichle, *The effects of practice on the functional anatomy of task performance.* Proc Natl Acad Sci U S A, 1998. **95**(3): p. 853-60.
374. Koch, K., G. Wagner, K. von Consbruch, I. Nenadic, C. Schultz, C. Ehle, J. Reichenbach, H. Sauer, and R. Schlosser, *Temporal changes in neural activation during practice of information retrieval from short-term memory: an fMRI study.* Brain Res, 2006. **1107**(1): p. 140-50.
375. Vuilleumier, P., R.N. Henson, J. Driver, and R.J. Dolan, *Multiple levels of visual object constancy revealed by event-related fMRI of repetition priming.* Nat Neurosci, 2002. **5**(5): p. 491-9.
376. Bandettini, P.A., K.K. Kwong, T.L. Davis, R.B. Tootell, E.C. Wong, P.T. Fox, J.W. Belliveau, R.M. Weisskoff, and B.R. Rosen, *Characterization of cerebral blood oxygenation and flow changes during prolonged brain activation.* Hum Brain Mapp, 1997. **5**(2): p. 93-109.
377. Mizuno, K., Tanaka, M., Tanabe, H. C., Joudoi, T., Kawatani, J., Shigihara, Y., Tomoda, A., Miike, T., Imai-Matsumura, K., Sadato, N.,and Watanabe, Y., *Less efficient and costly processes of frontal cortex in childhood chronic fatigue syndrome.* Neuroimage Clin, 2015. **9**: p. 355-68.
378. Whitfield-Gabrieli, S. and A. Nieto-Castanon, *Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks.* Brain Connect, 2012. **2**(3): p. 125-41.
379. Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B. and Joliot, M., *Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain.* NeuroImage, 2002. **15**: p. 273-289.
380. Muldoon, S.F., E.W. Bridgeford, and D.S. Bassett, *Small-World Propensity and Weighted Brain Networks.* Sci Rep, 2016. **6**: p. 22057.
381. Liao, X., A.V. Vasilakos, and Y. He, *Small-world human brain networks: Perspectives and challenges.* Neurosci Biobehav Rev, 2017. **77**: p. 286-300.
382. Sporns, O. and C.J. Honey, *Small worlds inside big brains.* Proc Natl Acad Sci U S A, 2006. **103**(51): p. 19219-20.
383. Achard, S. and E. Bullmore, *Efficiency and cost of economical brain functional networks.* PLoS Comput Biol, 2007. **3**(2): p. e17.
384. D.J. Watts, S.H.S., *Collective dynamics of 'small-world' networks.* Nature Neuroscience, 1998. **393** p. 440-442.
385. Z.-J. Dai, Y.H., *Disrupted structural and functional brain connectomes in mild cognitive impairment and Alzheimer's disease.* Neurosci. Bull., 2014. **30** p. 217-232.
386. M. Filippi, M.P.v.d.H., A. Fornito, Y. He, H.E.H. Pol, F. Agosta, G. Comi, M.A. Rocca, *Assessment of system dysfunction in the brain through MRI-based connectomics.* Lancet Neurol, 2013. **12** p. 1189-1199.
387. K.J. Friston, A.P.H., K.J. Worsley, J.P. Poline, C.D. Frith, R.S. Frackowiak, *Statistical parametric maps in functional imaging: a general linear approach.* Hum. Brain Mapp, 1994: p. 189-210.

388. O., K.H.H., *Distinct Basal Ganglia circuits controlling behaviors guided by flexible and stable values.* Neuron, 2013. **79**: p. 1001-10.
389. Kim, H.F. and O. Hikosaka, *Parallel basal ganglia circuits for voluntary and automatic behaviour to reach rewards.* Brain, 2015. **138**(Pt 7): p. 1776-800.
390. S., H.O.K.H.Y.M.Y., *Basal Ganglia circuits for reward value-guided behavior.* Annu Rev Neurosci, 2014. **37**: p. 289–306.
391. Chen T, M.L., Supekar K, Kochalka J, Ryali S, Menon V., *Role of the anterior insular cortex in integrative causal signaling during multisensory auditory-visual attention.* Eur J Neurosci, 2015. **41**(2): p. 264-74.
392. Cook, D.B.O.C., Patrick J. Lange, Gudrun and Steffener, Jason, *Functional neuroimaging correlates of mental fatigue induced by cognition among chronic fatigue syndrome patients and controls.* NeuroImage, 2007. **36**(1): p. 108–122.
393. Caseras, X., Mataix-Cols, D., Rimes, KA., Giampietro, V., Brammer, M., Zelaya, F., Chalder, T. and Godfrey, E., *The neural correlates of fatigue: an exploratory imaginal fatigue provocation study in chronic fatigue syndrome.* Psychological Medicine, 2008. **38**(7).
394. Raichle, M.E., A.M. MacLeod, A.Z. Snyder, W.J. Powers, D.A. Gusnard, and G.L. Shulman, *A default mode of brain function.* Proc Natl Acad Sci U S A, 2001. **98**(2): p. 676-82.
395. Shulman, G.L., J.A. Fiez, M. Corbetta, R.L. Buckner, F.M. Miezin, M.E. Raichle, and S.E. Petersen, *Common Blood Flow Changes across Visual Tasks: II. Decreases in Cerebral Cortex.* J Cogn Neurosci, 1997. **9**(5): p. 648-63.
396. Gusnard, D.A., M.E. Raichle, and M.E. Raichle, *Searching for a baseline: functional imaging and the resting human brain.* Nat Rev Neurosci, 2001. **2**(10): p. 685-94.
397. Raichle, M.E., *Two views of brain function.* Trends Cogn Sci, 2010. **14**(4): p. 180-90.
398. Menon, V. and L.Q. Uddin, *Saliency, switching, attention and control: a network model of insula function.* Brain Struct Funct, 2010. **214**(5-6): p. 655-67.
399. Borsook, D., R. Edwards, I. Elman, L. Becerra, and J. Levine, *Pain and analgesia: the value of salience circuits.* Prog Neurobiol, 2013. **104**: p. 93-105.
400. Kucyi, A. and K.D. Davis, *The dynamic pain connectome.* Trends Neurosci, 2015. **38**(2): p. 86-95.
401. Smith, S.M., P.T. Fox, K.L. Miller, D.C. Glahn, P.M. Fox, C.E. Mackay, N. Filippini, K.E. Watkins, R. Toro, A.R. Laird, and C.F. Beckmann, *Correspondence of the brain's functional architecture during activation and rest.* Proc Natl Acad Sci U S A, 2009. **106**(31): p. 13040-5.
402. Lois G, L.J., Wessa M., *Altered functional connectivity between emotional and cognitive resting state networks in euthymic bipolar I disorder patients.* PLoS One, 2014. **9**: p. e107829.
403. Uddin, L.Q., *Salience processing and insular cortical function and dysfunction.* Nat Rev Neurosci, 2015. **16**(1): p. 55-61.
404. Yuan, K., W. Qin, D. Yu, Y. Bi, L. Xing, C. Jin, and J. Tian, *Core brain networks interactions and cognitive control in internet gaming disorder individuals in late adolescence/early adulthood.* Brain Struct Funct, 2016. **221**(3): p. 1427-42.
405. Demertzi, A., Gomez, F., Crone, J. S., Vanhaudenhuyse, A., Tshibanda, L., Noirhomme, Q., ... & Soddu, A., *Multiple fMRI system-level baseline connectivity is disrupted in patients with consciousness alterations.* Cortex, 2014. **52**: p. 35-46.
406. Marek, S. and N.U.F. Dosenbach, *The frontoparietal network: function, electrophysiology, and importance of individual precision mapping.* Dialogues Clin Neurosci, 2018. **20**(2): p. 133-140.
407. V., M., *Large-scale brain networks and psychopathology: a unifying triple network model.* Trends in cognitive sciences., 2011. **15**(10): p. 483–506.
408. Holgate, S.T., A.L. Komaroff, D. Mangan, and S. Wessely, *Chronic fatigue syndrome: understanding a complex illness.* Nat Rev Neurosci, 2011. **12**(9): p. 539-44.
409. Raichle, M.E. and A.Z. Snyder, *A default mode of brain function: a brief history of an evolving idea.* Neuroimage, 2007. **37**(4): p. 1083-90; discussion 1097-9.
410. Buckner, R.L., J.R. Andrews-Hanna, and D.L. Schacter, *The brain's default network: anatomy, function, and relevance to disease.* Ann N Y Acad Sci, 2008. **1124**: p. 1-38.
411. Mak, L.E., L. Minuzzi, G. MacQueen, G. Hall, S.H. Kennedy, and R. Milev, *The Default Mode Network in Healthy Individuals: A Systematic Review and Meta-Analysis.* Brain Connect, 2017. **7**(1): p. 25-33.

412. Persson, J., S. Pudas, L.G. Nilsson, and L. Nyberg, *Longitudinal assessment of default-mode brain function in aging.* Neurobiol Aging, 2014. **35**(9): p. 2107-17.
413. Iraji, A., Calhoun, V. D., Wiseman, N. M., Davoodi-Bojd, E., Avanaki, M. R., Haacke, E. M., & Kou, Z., *The connectivity domain: Analyzing resting state fMRI data using feature-based data-driven and model-based methods.* Neuroimage, 2016. **134**: p. 494-507.
414. D.V. Calhoun, T.A., K.L. Hansen, J. Larsen, J.J. Pekar, *ICA of Functional MRI Data: An Overview*. 2003.
415. Newman, M.E., *Analysis of weighted networks.* Phys Rev E Stat Nonlin Soft Matter Phys, 2004. **70**(5 Pt 2): p. 056131.
416. Barnden, L., Kwiatek, R., Crouch, B., Burnet, R. and Del Fante, P., *Autonomic correlations with MRI are abnormal in the brainstem vasomotor centre in Chronic Fatigue Syndrome.* NeuroImage. Clinical, 2016. **11**: p. 530–537.
417. Uddin, L.Q., *Salience network of the human brain*. 2016: Academic press.
418. Petersen, S.E. and M.I. Posner, *The attention system of the human brain: 20 years after*. Annu Rev Neurosci, 2012. **35**: p. 73-89.
419. V., M., *Salience network: An Encyclopedia Reference.* Brain Mapping, 2015. **2**.
420. Voss, M.W., L. Chaddock, J.S. Kim, M. Vanpatter, M.B. Pontifex, L.B. Raine, N.J. Cohen, C.H. Hillman, and A.F. Kramer, *Aerobic fitness is associated with greater efficiency of the network underlying cognitive control in preadolescent children.* Neuroscience, 2011. **199**: p. 166-76.
421. Voss, M.W., L.S. Nagamatsu, T. Liu-Ambrose, and A.F. Kramer, *Exercise, brain, and cognition across the life span.* J Appl Physiol (1985), 2011. **111**(5): p. 1505-13.
422. Supekar, K. and V. Menon, *Developmental maturation of dynamic causal control signals in higher-order cognition: a neurocognitive network model.* PLoS Comput Biol, 2012. **8**(2): p. e1002374.
423. Craig, A.D., *How do you feel--now? The anterior insula and human awareness.* Nat Rev Neurosci, 2009. **10**(1): p. 59-70.
424. Craig, A.D., *Significance of the insula for the evolution of human awareness of feelings from the body.* Ann N Y Acad Sci, 2011. **1225**: p. 72-82.
425. Laird, A.R., P.M. Fox, S.B. Eickhoff, J.A. Turner, K.L. Ray, D.R. McKay, D.C. Glahn, C.F. Beckmann, S.M. Smith, and P.T. Fox, *Behavioral interpretations of intrinsic connectivity networks.* J Cogn Neurosci, 2011. **23**(12): p. 4022-37.
426. Piccoli, T., Valente, G., Linden, D. E., Re, M., Esposito, F., Sack, A. T., & Di Salle, F., *The default mode network and the working memory network are not anti-correlated during all phases of a working memory task.* PloS one, 2015. **10**(4): p. e0123354.
427. Eichele, T., S. Debener, V.D. Calhoun, K. Specht, A.K. Engel, K. Hugdahl, D.Y. von Cramon, and M. Ullsperger, *Prediction of human errors by maladaptive changes in event-related brain networks.* Proc Natl Acad Sci U S A, 2008. **105**(16): p. 6173-8.
428. Lv, H., Wang, Z., Tong, E., Williams, L. M., Zaharchuk, G., Zeineh, M., ... & Wintermark, M., *Resting-state functional MRI: everything that nonexperts have always wanted to know.* American Journal of Neuroradiology, 2018. **39**(8): p. 1390-1399.
429. Wyller, V.B., H.R. Eriksen, and K. Malterud, *Can sustained arousal explain the Chronic Fatigue Syndrome?* Behav Brain Funct, 2009. **5**(10): p. 10.
430. Pani, L., A. Porcella, and G.L. Gessa, *The role of stress in the pathophysiology of the dopaminergic system.* Mol Psychiatry, 2000. **5**(1): p. 14-21.
431. Burkey, A.R., E. Carstens, and L. Jasmin, *Dopamine reuptake inhibition in the rostral agranular insular cortex produces antinociception.* J Neurosci, 1999. **19**(10): p. 4169-79.
432. Tanaka, M., A. Ishii, and Y. Watanabe, *Neural mechanisms underlying chronic fatigue.* Rev Neurosci, 2013. **24**(6): p. 617-28.
433. Wyller, V.B. and I.B. Helland, *Relationship between autonomic cardiovascular control, case definition, clinical symptoms, and functional disability in adolescent chronic fatigue syndrome: an exploratory study.* Biopsychosoc Med, 2013. **7**(1): p. 5.
434. Beissner, F., K. Meissner, K.J. Bar, and V. Napadow, *The autonomic brain: an activation likelihood estimation meta-analysis for central processing of autonomic function.* J Neurosci, 2013. **33**(25): p. 10503-11.

435. Seeley, W.W., *The Salience Network: A Neural System for Perceiving and Responding to Homeostatic Demands.* J Neurosci, 2019. **39**(50): p. 9878-9882.
436. Gordon., B., J. DeSaix, Peter., Johnson, Eddie., Johnson, Jody E., Korol, Oksana., Kruse, Dean H., Poe, Brandon., *Anatomy and physiology.* 2017: Houston, Texas : OpenStax College, Rice University.
437. Capuron, L., L. Welberg, C. Heim, D. Wagner, L. Solomon, D.A. Papanicolaou, R.C. Craddock, A.H. Miller, and W.C. Reeves, *Cognitive dysfunction relates to subjective report of mental fatigue in patients with chronic fatigue syndrome.* Neuropsychopharmacology, 2006. **31**(8): p. 1777-84.
438. Costigan, A., C. Elliott, C. McDonald, and J.L. Newton, *Orthostatic symptoms predict functional capacity in chronic fatigue syndrome: implications for management.* QJM, 2010. **103**(8): p. 589-95.
439. DeBattista, C., J. Belanoff, S. Glass, A. Khan, R.L. Horne, C. Blasey, L.L. Carpenter, and G. Alva, *Mifepristone versus placebo in the treatment of psychosis in patients with psychotic major depression.* Biol Psychiatry, 2006. **60**(12): p. 1343-9.
440. Santamarina-Perez, P., F.J. Eiroa-Orosa, A. Rodriguez-Urrutia, A. Qureshi, and J. Alegre, *Neuropsychological impairment in female patients with chronic fatigue syndrome: a preliminary study.* Appl Neuropsychol Adult, 2014. **21**(2): p. 120-7.
441. Cockshell, S.J. and J.L. Mathias, *Cognitive functioning in chronic fatigue syndrome: a meta-analysis.* Psychol Med, 2010. **40**(8): p. 1253-67.
442. Cvejic, E., R.C. Birch, and U. Vollmer-Conna, *Cognitive Dysfunction in Chronic Fatigue Syndrome: a Review of Recent Evidence.* Curr Rheumatol Rep, 2016. **18**(5): p. 24.
443. Reeves, W.C., A. Lloyd, S.D. Vernon, N. Klimas, L.A. Jason, G. Bleijenberg, B. Evengard, P.D. White, R. Nisenbaum, E.R. Unger, and G. International Chronic Fatigue Syndrome Study, *Identification of ambiguities in the 1994 chronic fatigue syndrome research case definition and recommendations for resolution.* BMC Health Serv Res, 2003. **3**(1): p. 25.
444. Cockshell, S.J. and J.L. Mathias, *Cognitive deficits in chronic fatigue syndrome and their relationship to psychological status, symptomatology, and everyday functioning.* Neuropsychology, 2013. **27**(2): p. 230-42.
445. Ickmans, K., M. Meeus, D. Kos, P. Clarys, G. Meersdom, L. Lambrecht, N. Pattyn, and J. Nijs, *Cognitive performance is of clinical importance, but is unrelated to pain severity in women with chronic fatigue syndrome.* Clin Rheumatol, 2013. **32**(10): p. 1475-85.
446. Meeus, M., K. Ickmans, F. Struyf, D. Kos, L. Lambrecht, B. Willekens, P. Cras, and J. Nijs, *What is in a name? Comparing diagnostic criteria for chronic fatigue syndrome with or without fibromyalgia.* Clin Rheumatol, 2016. **35**(1): p. 191-203.
447. Ickmans, K., P. Clarys, J. Nijs, M. Meeus, D. Aerenhouts, E. Zinzen, S. Aelbrecht, G. Meersdom, L. Lambrecht, and N. Pattyn, *Association between cognitive performance, physical fitness, and physical activity level in women with chronic fatigue syndrome.* J Rehabil Res Dev, 2013. **50**(6): p. 795-810.
448. Ickmans, K., M. Meeus, M. De Kooning, L. Lambrecht, N. Pattyn, and J. Nijs, *Can recovery of peripheral muscle function predict cognitive task performance in chronic fatigue syndrome with and without fibromyalgia?* Phys Ther, 2014. **94**(4): p. 511-22.
449. Ickmans K, M.M., De Kooning M, Lambrecht LJ, Pattyn N, Nijs J., *Associations between cognitive performance and pain in chronic fatigue syndrome: comorbidity with fibromyalgia does matter.* Pain Physician, 2015. **18**(5): p. E841–E52. .
450. Neu, D., O. Mairesse, X. Montana, M. Gilson, F. Corazza, N. Lefevre, P. Linkowski, O. Le Bon, and P. Verbanck, *Dimensions of pure chronic fatigue: psychophysical, cognitive and biological correlates in the chronic fatigue syndrome.* Eur J Appl Physiol, 2014. **114**(9): p. 1841-51.
451. Cockshell, S.J. and J.L. Mathias, *Test effort in persons with Chronic Fatigue Syndrome when assessed using the Validity Indicator Profile.* J Clin Exp Neuropsychol, 2012. **34**(7): p. 679-87.
452. Lange, G.S., J. Cook, D. Bly, B. Christodoulou, C. Liu, W. DeLuca, J. and Natelson, B., *Objective evidence of cognitive complaints in Chronic Fatigue Syndrome: A BOLD fMRI study of verbal working memory.* NeuroImage, 2005. **26**(2): p. 513–524.

453. Tombaugh, T.N., *A comprehensive review of the Paced Auditory Serial Addition Test (PASAT).* Arch Clin Neuropsychol, 2006. **21**(1): p. 53-76.
454. Baddeley, A., *Exploring the central executive.* The Quarterly Journal of Experimental Psychology Section A,, 1996. **49**(1): p. 5-28.
455. Tiersky, L.A., S.K. Johnson, G. Lange, B.H. Natelson, and J. DeLuca, *Neuropsychology of chronic fatigue syndrome: a critical review.* J Clin Exp Neuropsychol, 1997. **19**(4): p. 560-86.
456. Hernandez, L., Badre, D., Noll, D., and Jonides, J., *Temporal sensitivity of event-related fMRI.* NeuroImage, 2002. **17**: p. 1018-1026.
457. Daneman, M., & Carpenter, P. A., *Individual differences in working memory and reading.* Journal of Memory and Language, 1980. **19**(4): p. 450.
458. Engle, R.W., Kane, M. J., & Tuholski, S. W., *Individual differences in working memory capacity and what they tell us about controlled attention, general fluid intelligence, and functions of the prefrontal cortex.* 1999.
459. Baddeley, A.E., Aggleton, J. P., & Conway, M. A., *Episodic memory: New directions in research*, ed. O.U. Press. 2002.
460. Kyllonen, P.C., & Christal, R. E., *Reasoning ability is (little more than) working-memory capacity?!* Intelligence, 1990. **14**(4): p. 389-433.
461. Klingberg T, D.F., *The role of fronto-parietal and fronto-striatal networks in the development of working memory: a longitudinal study.* Cereb Cortex, 2015. **25**: p. 1587–1595.
462. Rosenberg, M.D., S.A. Martinez, K.M. Rapuano, M.I. Conley, A.O. Cohen, M.D. Cornejo, D.J. Hagler, Jr., W.J. Meredith, K.M. Anderson, T.D. Wager, E. Feczko, E. Earl, D.A. Fair, D.M. Barch, R. Watts, and B.J. Casey, *Behavioral and Neural Signatures of Working Memory in Childhood.* J Neurosci, 2020. **40**(26): p. 5090-5104.
463. Klingberg, T., H. Forssberg, and H. Westerberg, *Increased brain activity in frontal and parietal cortex underlies the development of visuospatial working memory capacity during childhood.* J Cogn Neurosci, 2002. **14**(1): p. 1-10.
464. Olson, C.R., & Colby, C. L., *Spatial cognition*. In Fundamental Neuroscience. 2013: Academic Press.
465. Talati, A., & Hirsch, J., *Functional specialization within the medial frontal gyrus for perceptual go/no-go decisions based on "what," "when," and "where" related information: an fMRI study.* Journal of cognitive neuroscience, 2005. **17**(7): p. 981-993.
466. Deschamps, I., S.R. Baum, and V.L. Gracco, *On the role of the supramarginal gyrus in phonological processing and verbal working memory: evidence from rTMS studies.* Neuropsychologia, 2014. **53**: p. 39-46.
467. Buchsbaum, B.R. and M. D'Esposito, *The search for the phonological store: from loop to convolution.* J Cogn Neurosci, 2008. **20**(5): p. 762-78.
468. Cabeza, R., E. Ciaramelli, I.R. Olson, and M. Moscovitch, *The parietal cortex and episodic memory: an attentional account.* Nat Rev Neurosci, 2008. **9**(8): p. 613-25.
469. Wagner, A.D., B.J. Shannon, I. Kahn, and R.L. Buckner, *Parietal lobe contributions to episodic memory retrieval.* Trends Cogn Sci, 2005. **9**(9): p. 445-53.
470. Leung, H.C., J.C. Gore, and P.S. Goldman-Rakic, *Sustained mnemonic response in the human middle frontal gyrus during on-line storage of spatial memoranda.* J Cogn Neurosci, 2002. **14**(4): p. 659-71.
471. Rypma, B., V. Prabhakaran, J.E. Desmond, G.H. Glover, and J.D. Gabrieli, *Load-dependent roles of frontal brain regions in the maintenance of working memory.* Neuroimage, 1999. **9**(2): p. 216-26.
472. Cowan, N., D. Li, A. Moffitt, T.M. Becker, E.A. Martin, J.S. Saults, and S.E. Christ, *A neural region of abstract working memory.* J Cogn Neurosci, 2011. **23**(10): p. 2852-63.
473. Duzel, E., Habib, R., Rottel, M., Guderian, S., Tulving, E., and Heinze, H.J., *Human hippocampal and parahippocampal activity during visual associative recognition memory for spatial and non-spatial stimulus configurations.* The Journal of Neuroscience, 2003. **23**: p. 9439-9444.
474. Wagner, A.D., Schacter, D.L., Rotte, M., Koutstaal, W., Maril, A., Dale, A.M., Rosen, B.R., and Buckner, R.L., *Building memories: remembering and forgetting of verbal experiences as predicted by brain activity.* Science, 1998. **281**: p. 1188-1191.

475. de Bourbon-Teles, J., P. Bentley, S. Koshino, K. Shah, A. Dutta, P. Malhotra, T. Egner, M. Husain, and D. Soto, *Thalamic control of human attention driven by memory and learning.* Curr Biol, 2014. **24**(9): p. 993-9.
476. Leszcznski, M., and Staudigl, T., *Memory-guided attention in the anterior thalamus.* Neuroscience and Biobehavioural Reviews,, 2016. **66**: p. 163-165.
477. Almutairi, B., Langley, C., Crawley, E., & Thai, N. J., *Using structural and functional MRI as a neuroimaging technique to investigate chronic fatigue syndrome/myalgic encephalopathy: a systematic review.* BMJ open, 2020. **10**(8): p. e031672.
478. Ashburner, J., *Computational anatomy with the SPM software.* Magn Reson Imaging, 2009. **27**(8): p. 1163-74.
479. Agarwal, P.K., Karpicke, J.D., Kang, S.H., Roediger III, H.L., and McDermott, K.B., *Examining the testing effect with open- and closed-book tests.* Applied Cognitive Psychology, 2007. **22**: p. 861-876.
480. Roediger, H.L., 3rd and A.C. Butler, *The critical role of retrieval practice in long-term retention.* Trends Cogn Sci, 2011. **15**(1): p. 20-7.
481. Allman JM, H.A., Erwin JM, Nimchinsky E, Hof P, *The anterior cingulate cortex: the evolution of an interface between emotion and cognition.* Ann N Y Acad Sci 2001. **935**: p. 107–117.
482. Seeley, W.W., D.A. Carlin, J.M. Allman, M.N. Macedo, C. Bush, B.L. Miller, and S.J. Dearmond, *Early frontotemporal dementia targets neurons unique to apes and humans.* Ann Neurol, 2006. **60**(6): p. 660-7.
483. Kim, E.J., M. Sidhu, S.E. Gaus, E.J. Huang, P.R. Hof, B.L. Miller, S.J. DeArmond, and W.W. Seeley, *Selective frontoinsular von Economo neuron and fork cell loss in early behavioral variant frontotemporal dementia.* Cereb Cortex, 2012. **22**(2): p. 251-9.
484. Nana, A.L., M. Sidhu, S.E. Gaus, J.L. Hwang, L. Li, Y. Park, E.J. Kim, L. Pasquini, I.E. Allen, K.P. Rankin, G. Toller, J.H. Kramer, D.H. Geschwind, G. Coppola, E.J. Huang, L.T. Grinberg, B.L. Miller, and W.W. Seeley, *Neurons selectively targeted in frontotemporal dementia reveal early stage TDP-43 pathobiology.* Acta Neuropathol, 2019. **137**(1): p. 27-46.
485. Snowden, J.S., D. Bathgate, A. Varma, A. Blackshaw, Z.C. Gibbons, and D. Neary, *Distinct behavioural profiles in frontotemporal dementia and semantic dementia.* J Neurol Neurosurg Psychiatry, 2001. **70**(3): p. 323-32.
486. Goodkind, M., S.B. Eickhoff, D.J. Oathes, Y. Jiang, A. Chang, L.B. Jones-Hagata, B.N. Ortega, Y.V. Zaiko, E.L. Roach, M.S. Korgaonkar, S.M. Grieve, I. Galatzer-Levy, P.T. Fox, and A. Etkin, *Identification of a common neurobiological substrate for mental illness.* JAMA Psychiatry, 2015. **72**(4): p. 305-15.
487. Sha Z, W.T., Mechelli A, He Y, *Common dysfunction of largescale neurocognitive networks across psychiatric disorders.* Biol Psychiatry, 2019. **85**: p. 379 –388.
488. KJ, R., *A potential bias in safety evaluation during open-label extensions of randomized clinical trials.* Pharmacoepidemiol Drug Saf;, 2004. **13**(5): p. 295–298.
489. NS, W., *Clinical Epidemiology: The Study of the Outcome of Illness.* 2nd ed. 2006, New York: Oxford University Press: Oxford University.
490. Skelly, A.C., J.R. Dettori, and E.D. Brodt, *Assessing bias: the importance of considering confounding.* Evid Based Spine Care J, 2012. **3**(1): p. 9-12.
491. Jason, L., Jessen, T., Porter, N., Boulton, A., & Gloria-Njoku, M., *Examining types of fatigue among individuals with ME/CFS.* Disability Studies Quarterly, 2009. **29**(3).
492. Svennerholm, L., K. Bostrom, and B. Jungbjer, *Changes in weight and compositions of major membrane components of human brain during the span of adult human life of Swedes.* Acta Neuropathol, 1997. **94**(4): p. 345-52.
493. Scahill, R.I., Frost, C., Jenkins, R., Whitwell, J. L., Rossor, M. N., & Fox, N. C., *A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging.* Archives of neurology, 2003. **60**(7): p. 989-994.
494. van, d., L. De, I.C. Schmits, D.E.M. Geurts, K. Roelofs, d. van, I. Toni, and H. Knoop, *Prefrontal Structure Varies as a Function of Pain Symptoms in Chronic Fatigue Syndrome.* Biological Psychiatry, 2017. **81**(4): p. 358–365.
495. Okada, T., Tanaka, M., Kuratsune, H., Watanabe, Y., and N. Sadato, *Mechanisms underlying fatigue A voxel-based morphometric study of chronic fatigue syndrome*. 2004.

496. Ioannidis, J.P., *Why most published research findings are false.* PLoS Medicine, 2005. **2**.
497. Cremers, H.R., Tor, D., and Yarkoni, T., *The relation between statistical power and inference in fMRI.* Plos One, 2017. **12**.
498. Nord, C.L., Valton, V., Wood, J. and Roiser, J.P., *Power-up: a reanalysis of 'power failure' in neuroscience using mixture modelling.* Journal of Neuroscience, 2017: p. pp.3592-3616.
499. Poldrack, R.A., C.I. Baker, J. Durnez, K.J. Gorgolewski, P.M. Matthews, M.R. Munafo, T.E. Nichols, J.B. Poline, E. Vul, and T. Yarkoni, *Scanning the horizon: towards transparent and reproducible neuroimaging research.* Nat Rev Neurosci, 2017. **18**(2): p. 115-126.
500. Redpath, T.W., *Signal-to-noise ratio in MRI.* Br J Radiol, 1998. **71**(847): p. 704-7.
501. Manenti, G., M. Carlani, S. Mancino, V. Colangelo, M. Di Roma, E. Squillaci, and G. Simonetti, *Diffusion tensor magnetic resonance imaging of prostate cancer.* Invest Radiol, 2007. **42**(6): p. 412-9.
502. Pierpaoli, C. and P.J. Basser, *Toward a quantitative assessment of diffusion anisotropy.* Magn Reson Med, 1996. **36**(6): p. 893-906.
503. C., B., *The basis of anisotropic water diffusion in the nervous system - a technical review.* NMR in Biomedicine, 2002. **15**: p. 435-455.
504. Werring DJ, T.A., Clark CA, Parker GJ, Barker GJ, Miller DH, Thompson AJ. . . ;, *Diffusion tensor imaging can detect and quantify corticospinal tract degeneration after stroke.* Journal of Neurology, Neurosurgery, and Psychiatry, 2000. **69**: p. 269-272.
505. Pierpaoli C, B.A., Pajevic S, Chen R, Penix LR, Virta A, Basser P., *Water diffusion changes in Wallerian degeneration and their dependence on white matter architecture.* Neuroimage, 2001. **13**: p. 1174-1185.
506. Gulani, V., A.G. Webb, I.D. Duncan, and P.C. Lauterbur, *Apparent diffusion tensor measurements in myelin-deficient rat spinal cords.* Magn Reson Med, 2001. **45**(2): p. 191-5.
507. Song SK, S.S., Ramsbottom MJ, Chang C, Russell J, Cross AH., *Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water.* Neuroimage, 2002. **17**: p. 1429-1436.
508. Takahashi, M., D.B. Hackney, G. Zhang, S.L. Wehrli, A.C. Wright, W.T. O'Brien, H. Uematsu, F.W. Wehrli, and M.E. Selzer, *Magnetic resonance microimaging of intraaxonal water diffusion in live excised lamprey spinal cord.* Proc Natl Acad Sci U S A, 2002. **99**(25): p. 16192-6.
509. Neil JJ, S.S., McKinstry RC, Schefft GL, Snyder AZ, Almli CR, Akbudak E, Aronovitz JA, Miller JP, Lee BC, Conturo TE., *Normal brain in human newborns: apparent diffusion coefficient and diffusion anisotropy measured by using diffusion tensor MR imaging.* Radiology, 1998. **209**: p. 57-66.
510. Gass A, N.T., Hirsch JG., *Acute and chronic changes of the apparent diffusion coefficient in neurological disorders--biophysical mechanisms and possible underlying histopathology.* Journal of the Neurological Sciences, 2001. **186(Suppl 1):S**: p. 15-23.
511. CH., S., *Nuclear magnetic resonance (NMR) measurement of the apparent diffusion coefficient (ADC) of tissue water and its relationship to cell volume changes in pathological states.* Neurochemistry International, 2004. **45**: p. 569-582.
512. Pfefferbaum, A. and E.V. Sullivan, *Disruption of brain white matter microstructure by excessive intracellular and extracellular fluid in alcoholism: evidence from diffusion tensor imaging.* Neuropsychopharmacology, 2005. **30**(2): p. 423-32.
513. Takao, H., O. Abe, H. Yamasue, S. Aoki, H. Sasaki, K. Kasai, N. Yoshioka, and K. Ohtomo, *Gray and white matter asymmetries in healthy individuals aged 21-29 years: a voxel-based morphometry and diffusion tensor imaging study.* Hum Brain Mapp, 2011. **32**(10): p. 1762-73.
514. Rovaris, M., G. Iannucci, M. Cercignani, M.P. Sormani, N. De Stefano, S. Gerevini, G. Comi, and M. Filippi, *Age-related changes in conventional, magnetization transfer, and diffusion-tensor MR imaging findings: study with whole-brain tissue histogram analysis.* Radiology, 2003. **227**(3): p. 731-8.
515. Bennett, I.J., D.J. Madden, C.J. Vaidya, D.V. Howard, and J.H. Howard, Jr., *Age-related differences in multiple measures of white matter integrity: A diffusion tensor imaging study of healthy aging.* Hum Brain Mapp, 2010. **31**(3): p. 378-90.

516. Banerjee, G., D. Wilson, H.R. Jager, and D.J. Werring, *Novel imaging techniques in cerebral small vessel diseases and vascular cognitive impairment.* Biochim Biophys Acta, 2016. **1862**(5): p. 926-38.

Appendix no 1

Study ID: Flag

HADS

This questionnaire is designed to help describe how you feel. Please read each item and then place a cross in the box next to the reply that comes closest to how you have been feeling in the past week. Try to give your first reaction. This will probably be more accurate than spending a long time thinking about an answer.

Please cross only one box for each question

1.1	I feel tense / wound up:	A	
	Most of the time	3	☐
	A lot of the time	2	☐
	Occasionally	1	☐
	Not at all	0	☐
1.2	I still enjoy things I used to:	D	
	Definitely as much	0	☐
	Not quite as much	1	☐
	Only a little	2	☐
	Hardly at all	3	☐
1.3	I get a sort of frightened feeling as if something awful is about to happen:	A	
	Very definitely and quite badly	3	☐
	Not too badly	2	☐
	A little, but it doesn't worry me	1	☐
	Not at all	0	☐
1.4	I can laugh and see the funny side of things:	D	
	As much as I ever could	0	☐
	Not quite as much now	1	☐
	Definitely not so much	2	☐
	Not at all	3	☐
1.5	Worrying thoughts go through my mind:	A	
	A great deal of the time	3	☐
	A lot of the time	2	☐
	From time to time	1	☐
	Only occasionally	0	☐
1.6	I feel cheerful	D	
	Not at all	3	☐
	Not often	2	☐
	Sometimes	1	☐
	Most of the time	0	☐
1.7	I can sit at ease and feel relaxed:	A	
	Definitely	0	☐
	Usually	1	☐
	Not often	2	☐
	Not at all	3	☐
1.8	I feel as if I am slowed down:	D	
	Nearly all of the time	3	☐
	Very often	2	☐
	Sometimes	1	☐
	Not at all	0	☐
1.9	I get a frightened feeling like 'butterflies' in my stomach:	A	
	Not at all	0	☐
	Occasionally	1	☐
	Quite often	2	☐
	Very often	3	☐
1.10	I have lost interest in my appearance:	D	
	Definitely	3	☐
	I don't take as much care as I should	2	☐
	I may not take quite as much care	1	☐
	I take just as much care as ever	0	☐
1.11	I feel restless as if I have to be on the move:	A	
	Very much indeed	3	☐
	Quite a lot	2	☐
	Not very much	1	☐
	Not at all	0	☐
1.12	I look forward with enjoyment to things:	D	
	As much as I ever did	0	☐
	Rather less than I used to	1	☐
	Definitely less than I used to	2	☐
	Hardly at all	3	☐
1.13	I get sudden feelings of panic:	A	
	Very often indeed	3	☐
	Quite often	2	☐
	Not very often	1	☐
	Not at all	0	☐
1.14	I can enjoy a good book, radio or TV programme:	D	
	Often	0	☐
	Sometimes	1	☐
	Not often	2	☐
	Very seldom	3	☐

Appendix no 2

CRiCBristol
Clinical Research & Imaging Centre

MAGNETIC RESONANCE IMAGING INITIAL SCREENING FORM

NAME OF PARTICIPANT..... Sex: M / F

Date of birth..... Weight in kg..... or Stones/lbs Height in cm or m.....

Please read the questions on this screening form CAREFULLY. Your safety in the magnetic environment is our primary concern. THIS IS VERY IMPORTANT. For a very small number of individuals, being scanned can be uncomfortable, or endanger health or even life. The purpose of these questions is to make sure that you are not such a person. The information you provide will be treated as strictly confidential and will be held in secure conditions. If you are unsure of the answer to any of the questions, please ASK the person who gave you this form or the person who will be performing the scan. Definitions of some of the more technical terms are given overleaf. I wish to be screened by the same gender YES/NO*

Please answer all questions	*Circle answer*
1. Have you been fitted with a pacemaker, artificial heart valve, cochlear implant or any other implanted device?	YES/NO
2. Have you any surgical clips, aneurysm clips, shunts or stents in your body?	YES/NO
3. Have you ever had any metal fragments in your eyes?	YES/NO
4. Have you been exposed in your life to metal debris as a result of welding, grinding, filing, sawing or drilling of metal either occupationally or recreationally?	YES/NO
5. Do you wear a hearing aid?	YES/NO
6. Have you ever had any metal fragments, e.g. shrapnel in any other part of your body?	YES/NO
7. Have you any surgically implanted metal in any part of your body (e.g. joint replacement or bone reconstruction)?	YES/NO
8. Have you ever had any surgery that might have involved metal implants of which you are not aware?	YES/NO
9. Is there any possibility that you might be pregnant?	YES/NO
10. Do you have a contraceptive coil (IUD) ?	YES/NO
11. Have you been sterilised using clips?	YES/NO
12. Do you have **any** dental work (including dentures, crowns, bridgework, braces) in your mouth, other than simple fillings?	YES/NO
13. Have you ever suffered from any of: epilepsy, diabetes or thermoregulatory problems?	YES/NO
14. Have you ever suffered from any heart disease?	YES/NO
15. Do you have any tattoos? Do you have any permanent eye makeup?	YES/NO
16. Are you wearing any skin patches? (eg. Nicotine)	YES/NO

I have read and understood the questions above and have answered them correctly.

SIGNED..... DATE.....

In the presence of ..... (Name) ..... (Signature)

Appendix no 3a

University of BRISTOL

(Appendix 3a)

CRiCBristol
Clinical Research & Imaging Centre

Study Number:

Participant Identification Number:

CONSENT FORM

Title of Project: Understanding the neural mechanisms of Fatigue and memory impairments in patients with Chronic Fatigue Syndrome and Multiple Sclerosis: An MRI study

Name of Researcher: **Basim S Almutairi**

Please initial all boxes

1. I confirm that I have read and understand the CFS/ME participant information sheet version 2 (05/12/17) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to my questionnaire data to be used for the purposes of this study ☐
4. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from Research Team and from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
5. I agree to perform a memory task on a computer for the purposes of this study. ☐
6. I agree that, in the unlikely event that some abnormal/unusual appearance is discovered on the scan, CRICBristol staff can contact my GP in writing. ☐
7. I understand that information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐
8. I agree to undergo an MRI scan of my brain for the purposes of this study ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature.

Appendix no 3b

University of BRISTOL

(Appendix 3c)

CRiCBristol
Clinical Research & Imaging Centre

Study Number:

Participant Identification:

CONSENT FORM

Title of Project: Understanding the neural mechanisms of fatigue and memory impairments in patients with Chronic Fatigue Syndrome and Multiple Sclerosis: An MRI study

Name of Researcher: **Basim S Almutairi**

Please initial all boxes

1. I confirm that I have read and understand the healthy participant information sheet version 2 (05/12/17) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to completing questionnaire pack to be used for the purposes of this study ☐
4. I agree to perform a memory task on a computer for the purposes of this study. ☐
5. I agree that, in the unlikely event that some abnormal/unusual appearance is discovered on the scan, CRICBristol staff can contact my GP in writing. ☐
6. I understand that information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐
7. I agree to undergo a MRI scan of my brain for the purposes of this study ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature.

Appendix no 4

(Appendix 4)

Study ID

Visual Analogue Pain Rating Scale

Please mark the line to describe the severity of your pain

NO PAIN ——————————— **PAIN AS BAD AS POSSIBLE**

Study ID:

SF-36

The following questions are about ACTIVITIES you might do during a typical day.
Does your health now limit you in these activities? If so, how much?

Please cross only one box in each line ☒

		Yes, limited a lot	Yes, limited a little	No, not limited at all
1.1	Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	1 ☐	2 ☐	3 ☐
1.2	Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1 ☐	2 ☐	3 ☐
1.3	Lifting or carrying groceries	1 ☐	2 ☐	3 ☐
1.4	Climbing several flights of stairs	1 ☐	2 ☐	3 ☐
1.5	Climbing one flight of stairs	1 ☐	2 ☐	3 ☐
1.6	Bending, kneeling, or stooping	1 ☐	2 ☐	3 ☐
1.7	Walking more than a mile	1 ☐	2 ☐	3 ☐
1.8	Walking half a mile	1 ☐	2 ☐	3 ☐
1.9	Walking one hundred yards	1 ☐	2 ☐	3 ☐
1.10	Bathing or dressing yourself	1 ☐	2 ☐	3 ☐

Study ID: _____

1 - Pre	5 - 24 mth
2 - 6 wk	6 - Misc
3 - 6 mth	7 - D/C
4 - 12 mth	

EQ-5D™

By placing a tick in one box in each group below, please indicate which statements describe your own health state today

1.1 Mobility	
I have no problems in walking about	☐ 1
I have some problems in walking about	☐ 2
I am confined to bed	☐ 3
1.2 Self-Care	
I have no problems with self-care	☐ 1
I have some problems with washing or dressing myself	☐ 2
I am unable to wash or dress myself	☐ 3
1.3 Usual Activities *(e.g. work, studies, housework, families or leisure)*	
I have no problems with performing my usual activities	☐ 1
I have some problems with performing my usual activities	☐ 2
I am unable to perform my usual activities	☐ 3
1.4 Pain/Discomfort	
I have no pain or discomfort	☐ 1
I have moderate pain or discomfort	☐ 2
I have extreme pain or discomfort	☐ 3
1.5 Anxiety/Depression	
I am not anxious or depressed	☐ 1
I am moderately anxious or depressed	☐ 2
I am extremely anxious or depressed	☐ 3

(Appendix 4)

Study ID: ____

Epworth Sleepiness Scale

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Please cross only one box in each line ☒

It is important that you answer each question as best you can

		would never doze	slight chance of dozing	moderate chance of dozing	high chance of dozing
1.1	Sitting and reading	0 ☐	1 ☐	2 ☐	3 ☐
1.2	Watching TV	0 ☐	1 ☐	2 ☐	3 ☐
1.3	Sitting, inactive in a public place like a theatre or meeting	0 ☐	1 ☐	2 ☐	3 ☐
1.4	As a passenger in a car for an hour without a break	0 ☐	1 ☐	2 ☐	3 ☐
1.5	Lying down to rest in the afternoon when circumstances permit	0 ☐	1 ☐	2 ☐	3 ☐
1.6	Sitting and talking to someone	0 ☐	1 ☐	2 ☐	3 ☐
1.7	Sitting quietly after lunch without alcohol	0 ☐	1 ☐	2 ☐	3 ☐
1.8	In a car, while stopped for a few minutes in traffic	0 ☐	1 ☐	2 ☐	3 ☐

Appendix no 5

Second MRI screening form.

This form should be completed and signed immediately before your scan, after removal of all jewellery or other metal objects and (if required by the operator) changing your clothes.

NAME OF PARTICIPANT ..... Date of birth..... Sex: M / F

Please read the following questions CAREFULLY and provide answers. For a very small number of individuals, being scanned can endanger comfort, health or even life. The purpose of these questions is to make sure that you are not such a person.
You have the right to withdraw from the screening and subsequent scanning if you find the questions unacceptably intrusive. The information you provide will be treated as strictly confidential and will held in secure conditions.

Before you are taken through for your scan it is essential that you remove all metal objects including: Watches, Pens, Loose Change, Keys, Hair clips, All Jewellery, brassieres with metal fasteners, metallic cosmetics, Cash/debit cards

Please answer all questions	Circle your answer
1. Are you wearing or carrying any metal items such as those listed above?	YES/NO
2. Have your answers to any of the questions in the initial screening form changed? (The initial screening form must be shown to you before you answer this question.)	YES/NO
3. Have you been fitted with a pacemaker, artificial heart valve, cochlear implant or any other implanted device?	YES/NO
4. Is there any possibility that you might be pregnant?	YES/NO
5. Are you currently feeling unwell (colds, flu etc.) or have you been unwell in the last week?	YES/NO

I have read and understood the questions above and have answered them correctly.

SIGNATURE..... **DATE**.....

FOR STAFF USE:
I certify that the initial screening form and the consent form have been completed by the person named above and I have attached them to this form. The volunteer has been given the standard information sheet about MRI scans, together with any necessary scan-specific information, and has been given an opportunity to ask questions. I am satisfied that the volunteer is adequately informed and understands the content of the consent form. I have taken adequate steps to ensure that the volunteer has no ferro-magnetic metal in or on his/her person and I am satisfied that the scan can proceed.

SIGNATURE..... **NAME (print)** .....