**An investigation of the effectiveness of a mindfulness meditation on sleep quality in patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)**

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**Background:** Unrefreshing sleep is the hallmark of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), affecting up to 95% of patients. Sleep is defined by coordinated cortical and cardiac oscillations reflecting communication between the central (CNS) and autonomic nervous systems (ANS), which can be modulated by mindfulness meditation. There is a growing interest in the effectiveness of mindfulness meditation for sleep disturbed populations. Mindfulness meditation may help improve sleep quality in ME/CFS patients. This study is designed to evaluate the effect of an 8-week mindfulness meditation on sleep quality in patients with ME/CFS and understand the underlying neurobiological process of meditation in patients with ME/CFS using self-report questionnaires and neuroimaging measures.

**Methods and Analysis:** 45 participants from 18-65 years-old diagnosed with ME/CFS will participate in an 8-week mindfulness meditation. At baseline and post-intervention, participants will complete 6 self-report questionnaires, a sleep diary, a meditation journal, and a 30-minute Magnetic Resonance Imaging (MRI) brain scan. The MRI scan session will include multiple imaging modalities, such as, structural MRI, and resting-state fMRI (rsfMRI) with simultaneous pulse oximetry data recording. A pulse oximeter is placed on the participant's finger. The high frequency (0.15 - 0.4Hz) heart rate variability (HF-HRV) of 10 beats will be calculated at each rsfMRI volume using a sliding window method so that its temporal resolution matches with rsfMRI data. We will use HF-HRV as a measure of parasympathetic outflows. Heart rate will be also measured outside of the scanner using Apple watches.

**Ethics and study registry:** This study was reviewed and approved by the University of the Sunshine Coast Ethics committee (A231955).

**Dissemination of results:** The results will be disseminated through peer reviewed scientific manuscripts and conferences, as well as to patients through UniSC’s ME/CFS Study website, social media platforms such as UniSC’s Facebook, Instagram, and LinkedIn.

**Introduction**

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)is a debilitating illness that impacts people’s lives significantly. Patients with ME/CFS experience periods of severe fatigue lasting at least six months, as well as post-exertional malaise and cognitive impairments (Arroll et al., 2014). Research has shown that there is a significant relationship between unrefreshing sleep and chronic fatigue syndrome (Gotts et al., 2016). Sleep is a consistent complaint of those suffering from CFS (Gotts et al., 2016). 87–95% of patients with CFS report feeling unrefreshed on waking, despite adequate sleep duration (Gotts et al., 2016, Mohamed et al., 2023). Patients also report experiencing disrupted and fragmented sleep and difficulties in getting to sleep despite feeling tired (Gotts et al., 2016). The effect of sleep is important to consider in CFS because disrupted sleep can cause fatigue, myalgia, and poor concentration. Therefore, sleep disruption may result in a worsening of the effects of fatigue (Gotts et al., 2016). Despite unrefreshing sleep being the most debilitating and distinctive symptom of ME/CFS, the neurobiological underpinnings remain unknown (Russell et al., 2016; Shan et al., 2017).

One review defines sleep by coordinated cortical and cardiac oscillations reflecting communication between the central (CNS) and autonomic nervous systems (ANS) (de Zambotti et al., 2018). The central autonomic nervous (CAN) system controls body functions and physiology and involves wide-spread brain areas operating in a network-based approach, from the neocortex to the brainstem, as well as the cerebellum (Macey et al., 2016). The two branches of the ANS, sympathetic (SANS) and parasympathetic nervous system (PANS), regulate visceral functions to maintain the homeostatic milieu of the body and to render the body able to react and to adapt to external and internal stressor stimuli. These systems primarily work unconsciously in opposite ways to regulate many functions and parts of the body. The SANS governs the "fight or flight" response while the PANS controls the "rest and digest" response (Alshak, 2019). The parasympathetic nervous system is predominant during sleep (Zoccoli & Amici, 2020), with an increase of parasympathetic activity associated with a reduction in sympathetic activity. In patients with sleep disorders, the physiological reduction in sympathetic activity is prevented. In these subjects, sympathetic activity is high during sleep, and it remains high when they are awake (Zoccoli & Amici, 2020). Symptoms of autonomic dysfunction are one of the characteristic features in CFS. In particular, research suggests that PANS is impaired in CFS (Yamaguti et al., 2013).

When we sleep, a change occurs in our blood flow that facilitates the movement of a watery liquid called cerebrospinal fluid (CSF) (Fultz et al., 2019). CSF washes through our brain and removes chemical waste which may have accumulated in our brain (Fultz et al., 2019). It has been suggested from Fultz and colleagues that the fundamental purpose of sleep is to act like a garbage disposal for the brain. This allows the brain to function efficiently the next day when we wake up from sleep (Komaroff, 2021). Good sleep is pivotal to physical and mental wellbeing (Buysse, 2014; de Zambotti et al., 2018). Sleep deprivation may impact a variety of factors, including reducing immune function (e.g., lowering cytokine levels), changing hormone secretion (e.g., increase in cortisol levels), and triggering adverse psychological changes (e.g., increase in anxiety and depression symptoms) (Guerra et al., 2020). Research suggests a significant link between sleep quality and mental health (Evans et al., 2021), for example, the potential relationship between sleep problems and suicidal thoughts during the COVID-19 pandemic (Merikanto et al., 2022). Conversely, high sleep quality contributes significantly to improving general population health and reducing health care costs (Buysse, 2014). High sleep quality helps preventing or mitigating health issues that would otherwise require medical treatment, hospitalizations, or long-term care, such as, preventing chronic health conditions including heart disease and stroke, diabetes, obesity (Shankar, Syamala & Kalidindi, 2010), improving mental health (Scott et al., 2021), reducing risk of injuries (Huang & Ihm, 2021), enhancing immune system function (Besedovsky, Lange & Born, 2012), reducing healthcare utilization (Daley et al.,2009), delaying aging and chronic illness (Feliciano, Walden & Okun, 2022), reducing dementia and cognitive decline (Porter, Buxton & Avidan, 2015).

There is a pressing unmet need to improve sleep quality in patients with ME/CFS (Gotts et al., 2016). A review study from Albakri, Drotos, and Meertens (2021) suggests that sleep interventions such as sleep education, relaxation techniques, physical exercise, aromatherapy, massage, psychotherapy, and environmental interventions showed promising but inconsistent or limited results. [Cognitive behavioural therapy](https://www.sciencedirect.com/topics/neuroscience/cognitive-behavioral-therapy) (CBT) and [graded exercise therapy](https://www.sciencedirect.com/topics/psychology/graded-exercise-therapy) (GET) are recommended [evidence based treatments](https://www.sciencedirect.com/topics/psychology/evidence-based-treatment) for CFS, with research supporting their effectiveness in reducing fatigue and functional impairment. However, little research has focussed on the effect of these treatments on sleep, despite high reported sleep disturbance in CFS (Russell et al., 2017). Overall, studies investigating effective interventions in improving sleep quality in ME/CFS participants still remain scarce (Russell et al., 2017).

Meditation is considered by a large body of research to be a non-pharmacological intervention able to provide health related benefits (Ferrarelli et al., 2013), including improving sleep quality in adults (Barrett et al., 2020; Guerra et al., 2020). There is evidence for meditation’s efficacy in improving sleep quality among cancer patients (Rao et al., 2017; Zeichner et al., 2017) and fibromyalgia patients (Amutio et al., 2018; Guerra et al., 2020). Meditation interventions can result in increased central and autonomic nervous system interaction (Tang et al., 2009). Tang and colleagues measured the physiological and brain changes at rest before, during, and after 5 days of meditation training and relaxation training on 86 Chinese undergraduates. During and after training, the meditation training group showed significantly better physiological reactions in heart rate, respiratory amplitude and rate, and skin conductance response (SCR) than the relaxation control. Differences in heart rate variability (HRV) and EEG power suggested greater involvement of the autonomic nervous system (ANS) in the meditation training group during and after training. Imaging data demonstrated stronger subgenual and adjacent ventral anterior cingulate cortex (ACC) activity in the meditation training group. Frontal midline ACC theta was correlated with high-frequency HRV, suggesting control by the ACC over parasympathetic activity. These results indicate that after 5 days of training, the meditation training group showed better regulation of the ANS by a ventral midfrontal brain system than does the relaxation group. Peng and colleagues’ study suggests a mutual activation of parasympathetic and sympathetic systems as there is variability in HR during meditation. The increased variation in HR was due to an increase of activity in ANS (Peng et al., 1999). During meditation, the increase in parasympathetic activity of the brain leads to a decrease in HR, BP, and respiratory rate (RR) (Jevning et al., 1992; Tang et al., 2009). Due to decrease in HR and RR, the paragigantocellular nucleus of the medulla decreases innervations of the locus coeruleus which produces and distributes norepinephrine (NE) (Foote & Morrison, 1987). This reduction in NE reduces stimulation of hypothalamic paraventricular nucleus, which helps decrease corticotrophin-releasing hormones and would subsequently decrease cortisol levels (Jevning et al., 1978; Sudsuang et al., 1991; Walton et al., 1995). Various studies have found associations between sleep and cortisol (Eek et al., 2012; Chicoine et al., 2013; Hirotsu et al., 2015); with higher cortisol levels and poor sleep highly correlated. Furthermore, activity in the anterior cingulate cortex (ACC) is increased during meditation practice (Tang et al., 2009). As discussed above, symptoms of autonomic dysfunction are one of the characteristic features in CFS. Research suggests that PANS is impaired in CFS (Yamaguti et al., 2013). Therefore, meditation might be beneficial to improve sleep quality in patients with ME/CFS.

Modern neuroimaging methods provide important insights into sleep habits (Duyn, 2012). Functional MRI (fMRI) studies have identified dorsal and ventral ACC involvement in autonomic control. High-frequency HRV is associated with the parasympathetic system of the ANS and ventral ACC activation correlated significantly with high-frequency HRV (Tang et al., 2009). A recent study suggests that poor sleep quality is adversely associated with HRV, HR and BP (Sajjadieh et al., 2020). Chronic fatigue syndrome (CFS) and fibromyalgia (FMS) are medically unexplained illnesses, with 2–3 times higher prevalence for females than males (Zambolin et al., 2022). Neuendorf and colleagues reviewed 112 research studies testing a variety of different mind-body interventions including meditation, and found that meditation could be considered as a treatment option for patients with insomnia or sleep disturbance. Participants were diverse, including the elderly, women of menopausal, perimenopausal, and postmenopausal age, stressed working adults, medical and college students, veterans, inmates, cancer patients and survivors, people with insomnia, chronic pain, fibromyalgia, posttraumatic stress disorder, tinnitus, and Guillain-Barre syndrome, and patients undergoing hemodialysis and organ transplant (Neuendorf et al., 2015). Another pilot study from Arroll and colleagues conducted a specialized online symptoms management program (N=19) and a control intervention based on an online meditation website (N=9) for ME/CFS patients (Arroll et al., 2014). Sleeping problems improved across all groups, suggesting that meditation may improve sleep quality in patients with ME/CFS, though the small sample size notably increases the risk of false positive results. Furthermore, the neurobiological underpinnings by which meditation improves sleep quality in patients with ME/CFS remains unexplored. Therefore, we propose to assess the effectiveness of meditation in enhancing sleep quality in patients with ME/CFS and a sample size (N = 45) determined through power analysis. Additionally, we intend to investigate the neurobiological underpinnings of unrefreshing sleep in ME/CFS patients using MRI scans. Even though evidence is still emerging, initial findings indicate the positive effects of meditation on many aspects related to sleep quality improvement, such as, reducing stress and anxiety, improving sleep onset, enhancing sleep duration, reducing insomnia symptoms, improving sleep quality, enhancing regulation of the autonomic nervous system, alleviating pain and discomfort, balancing hormones related to sleep including melatonin, **serotonin and gamma-aminobutyric acid (GABA),** improving sleep disorders, and Increasing mind-body awareness (Guerra et al., 2020; Rusch et al., 2019).

Sleep studies have historically used both subjective and objective instruments to determine sleep quality in ME/CFS. Subjective measurements include self-report measures such as sleep diaries and scales using Pittsburgh Sleep Quality Index (PSQI) (Tobback et al, 2016). To measure sleep quality objectively, studies have used electroencephalography (EEG), polysomnography (PSG), and/or actigraphy (Tobback et al, 2016). EEG is used to record the electrical patterns of brain activity during sleep and can be determine sleep stages as well as cycle functions (Campbell, 2009). PSG has remained the most frequently used technique to evaluate the sleep disturbances in ME/CFS (Rundo & Downey III, 2019), and it measures physiological functions including brain waves, blood oxygen level, heart rate and breathing, eye and leg movements (Rundo & Downey III, 2019). Using EEG and PSG, the two sleep cycles of non-rapid eye movement and rapid eye movement (REM) sleep can be studied (Harding & Feldman, 2008), with non-rapid eye movement being further divided into three distinctive stages (i.e. Stage 1, Stage 2, Stage 3) (Harding & Feldman, 2008). However, both PSG and EEG require the patient to be at a sleep laboratory or hospital facility. Recent studies have started to use Actigraphy watch to investigate sleep quality in ME/CFS patients (Russell et all., 2016), due to their ease of use. In addition, some studies have investigated the correlation between the observed sleep patterns between subjective and objective measures in ME/CFS (Creti et al., 2010). A meta-analysis from Mohamed et al., 2023 revealed that patients tend to report longer sleep onset latency in subjective sleep measures but not in objective measure using the actigraphy watch, and this was predictive of the next-day's fatigue level in ME/CFS, suggesting the sleep perception and negative mood on waking might be the key elements for the unrefreshing sleep (Russell et al., 2016). There are still many unanswered questions regarding the pathogenesis and nature of sleep disturbance and unrefreshed sleep in CFS/ME. Sleep disturbance may precipitate CFS/ME, may alter or complicate its course by worsening fatigue, pain, or mood, or may represent an independent factor unrelated to fatigue itself. With the development of new standardized criteria for diagnosing CFS/ME, more homogenous patient samples and comparability across studies will be afforded for future research. For many years, several techniques have been developed for the assessment of ANS. These include using (1) dosage of plasmatic and urinary catecholamines (Dimsdale et al., 1995), (2) muscle sympathetic nerve activity (Saul et al., 1990), (3) analysis of heart rate variability (HRV), which is a non-invasive tool able to provide reliable information on sympathetic and parasympathetic oscillations of the heart period and arterial pressure time series (Kasamaki et al., 2013), and (4) entropy-derived measures and symbolic analysis of heart period time series (Montano & Tobaldini, 2016). HRV can be a measure of our autonomic nervous system and the balance between our parasympathetic and sympathetic branches. The parasympathetic branch is our “Rest & Digest” and correlates with a high frequency HRV (HF-HRV). The sympathetic branch is our “Fight or Flight” and correlates with a low frequency HRV (LF-HRV) (Tang et al., 2009).

The brain and the heart communicate continuously through a number of neurological, hormonal, and biophysical mechanisms that ensure physiological homeostasis as well as complex cardiovascular and systemic responses to external stimuli (Valenza et al., 2017). Some of the key brain regions involved in regulating parasympathetic activity include the brain stem, hypothalamus, nucleus solitarius and sacral spinal cord. The brain stem is the main component of the central autonomic nervous system and is divided into the midbrain (or mesencephalon), pons, and medulla oblongata. The main component in the brainstem responsible for indicating the parasympathetic nervous system's activity and functional network is the nucleus ambiguous, located in the reticular formation of the medulla oblongata, the lowest part of the brainstem. It plays a crucial role in regulating various autonomic functions, including heart rate, digestion, and respiratory rhythm (Gibbons, C. H. (2019). Hölzel et al. (2011) reported the first longitudinal study of grey matter changes following an 8-week-meditation course. One region with enhanced grey matter concentration following the meditation course was in the cerebellar vermis, reaching into a region of the brain stem that included the locus coeruleus, nucleus raphe pontis, pontine tegmentum, and the sensory trigeminal nucleus. The pontine tegmentum, part of the cholinergic system, is implicated in regulating selective attention, wakefulness, learning, reward, and sleep. These regions are well-known to modulate several systems, including the serotonin, dopamine, and norepinephrine systems, as well as play central roles in processes such as mood, arousal, sleep, and appetite.

To the best of our knowledge, currently there is no study investigating the effects of meditation on sleep quality in patients with ME/CFS looking at brain function and the central nervous system (CNS). This will be the first fMRI study that investigates the effectiveness of an 8-week guided meditation on sleep quality in ME/CFS subjects (N=45). Ultimately, we seek to understand the impact of mindfulness meditation on the brain and central parasympathetic activity of CFS patients. Through the use of self-report questionnaires and diaries, we are able to align this data with heart rate variability and imaging shown through the MRIs which include multiple scan modalities, such as, resting-state functional magnetic resonance imaging (rs-fMRI) and structural MRI.

**Methods and analysis**

***Study design***

The investigation of mindfulness meditation on sleep quality in patients with ME/CFS is a cross-sectional study that commenced in 2023 and will be completed in 2026. Participants who express interest will complete a screening questionnaire to confirm eligibility according to inclusion/exclusion criteria. Symptom scores, sleep diaries, heart rate variabilities, and MRIs will be collected after receiving written consent from eligible participants (figure 1).

***Primary outcomes***

**Aim 1**

Investigate if meditation will improve sleep quality in patients with ME/CFS by:

1. Assessing the safety, feasibility and tolerability of a self-administered mindfulness meditation as an intervention for patients with CFS by using a meditation journal.
2. Examining sleep quality changes at baseline and post-intervention by using self-report questionnaires and a sleep diary.
3. Assessing changes in scores on symptom rating scales for conditions comorbid with ME/CFS, including anxiety, depression, fatigue, pain, quality of life, and wellbeing by using self-report questionnaires.

**Aim 2**

Investigate the mechanism or underlying neurobiological process of meditation in patients with ME/CFS by:

1. Examining changes in high frequency heart rate variability (HF-HRV) before and after intervention.
2. Examining changes in connectivity within brain regions related to parasympathetic response before and after intervention.
3. Examining changes in grey matter concentration in brain regions associated with sleep before and after intervention.

***Recruitment and selection of the sample population***

We will recruit 45 adult participants (18-65 years-old) with ME/CFS diagnosed by a medical professional. The risk of an ill-defined ME/CFS patient cohort will be mitigated by using the Canadian Consensus Criteria (CCC).



*Figure 1*

***Inclusion Criteria***

Eligible participants are individuals aged between 18-65 years that (1) meet ME/CFS definition based on Canadian Concensus Criteria (CCC) and have been diagnosed by a medical professional; (2) have little to no prior experience or exposure to meditation practices (considered beginners or novices) and do not have any formal training or understanding of meditation techniques; (3) are able to understand the Research Project Information Sheet (RPIS) and provide written informed consent on the Participant Informed Consent Form (PICF); (4) are proficient in spoken and written English; (5) have access to a computer at home, in a private space to undergo the mindfulness meditation (6) are available to attend the Thompson Institute (TI) for 2 MRI scans; (7) are able to undergo MRI, and tolerate the mindfulness meditation therapy; (8) have basic computer skills and are able to learn and follow new instructions to engage in the technique.

***Exclusion Criteria***

Participants will be ineligible for participation if they (1) have been diagnosed with obstructive sleep apnoea (OSA); (2) are pregnant; (3) have severe intellectual or mental impairment preventing them from fully understanding the study to give consent; (4) have a known neurological disorder; (5) cannot read and communicate in English; (5) were recruited by a relationship with the supervisory team or where a conflict of interest exists; (6) have an alcohol or substance related disorder; (7) have a BMI >35.

**Data collections**

***Symptom questionnaires***

After informed consent has been obtained, the following assessments will be undertaken: (1) Symptom information relevant to establishing Canadian Consensus Criteria ME/CFS classification ([DePaul Symptom Questionnaire](https://uniofsunshinecoast.syd1.qualtrics.com/jfe/form/SV_2t9VLazKFDJsQEB)); (2) The 36-item Short Form Health Survey ([SF36 Survey](https://uniofsunshinecoast.syd1.qualtrics.com/jfe/form/SV_0VRUQakYatrZKLP)); (3) The Hospital Anxiety and Depression Scale ([HADS)](https://uniofsunshinecoast.syd1.qualtrics.com/jfe/form/SV_1BoJPgkQ57zENsp); (4) The Assessment of Quality of Life Instrument (AQoL-8D); (5) The [Pittsburgh Sleep Quality Index](https://uniofsunshinecoast.syd1.qualtrics.com/jfe/form/SV_djmuTm1Cfhatulf) (PSQI); (6) The PERMA Profiler. Participants will also complete (1) The Sleep Diary, to record sleep pattern; (2) The Meditation Journal, to record meditation practices and safety measurements.

***Physiological Measures***

* Before each MRI scan, participants will have their blood pressure (performed supine, seated and standing), heart rate, height and weight measured. The high frequency (0.15 - 0.4Hz) heart rate variability (HF-HRV) will be calculated during rsfMRI using pulse oximeter. The high frequency (0.15 - 0.4Hz) heart rate variability (HF-HRV) of 10 beats will be calculated at each rsfMRI volume using a sliding window method so that its temporal resolution matches with rsfMRI data. We will use HF-HRV as a measure of parasympathetic outflows. Outside of the scanner,the HRV will be measured using Apple watches (Hernando et al., 2018).

***Multimodal MRI***

Brain images are acquired using a 3T MRI scanner with a 64- channel head coil (Skyra, Siemens) at the Thompson Institute (TI), University of the Sunshine Coast (UniSC). One MRI session will be conducted before and after the intervention.

The MRI session will include:

1. Structural MRI (sMRI) using a T1-weighted magnetization prepared rapid gradient-echo sequence (MPRAGE): 208 slices, dimension 256 × 256, voxel size 1 mm × 1 mm × 1 mm, TR/TE 2,200/1.71 ms, flip angle 7◦ .
2. A set of resting-state fMRI (rsfMRI, 192 volumes) using a multiband EPI sequence: 108 slices, dimension 126 × 126, multiband = 4, dimension 138 × 138, 1.6 mm3 isotropic voxel, TR/TE 2,500/42 ms, flip angle 75◦ . Participant will be instructed to keep their eyes open with fixation of a cross for 8 min acquisition. Heart rate will be recorded simultaneously using pulse oximetry from the MRI scanner

***Mindfulness Meditation***

Participants will practice mindfulness meditation 10 minutes a day, 7 days a week for eight weeks. Participants are required to practice mindfulness meditation every day at the same time to ensure consistency following a voice recording instruction presented via a website and record their practices in a journal. If participants cannot make it consistent every day, they can report any deviations to the planned schedule. The mindfulness meditation includes breath work, body scan, visualization, and positive affirmation.

**Data analysis plan**

This study will utilise longitudinal analyses to understand the sleep improvement before and after intervention. Data for each group will be explored using descriptive statistics (mean +/- SD). Differences in gender distribution, age, body mass index (BMI) and questionnaire scores will be analysed by means of chi-squared and one-way ANOVA. The relationship between age, fatigue, diurnal sleepiness will be analysed separately for each group, through Pearson correlation. Statistics will be analysed with SPSS 27.0; P/q values < 0.05 will be considered statistically significant. Cross-sectional and longitudinal analysis of the self-report sleep data, symptom data, and MRI data will be performed using appropriate statistical tests (e.g., Regression, ANOVA, GEE, etc.) to define the neurological basis for any changes observed in sleep or symptoms measures at the post-intervention and or baseline relationships.

***Analysis for heart rate variability***

Statistical data will be analysed using the IBM SPSS Statistics (IBM). Data normality and equal variance will be studied using the Shapiro–Wilk test and Levene's test, respectively. Differences between groups before and after the meditation will be assessed using the independent t-test and the analysis of covariance (ANCOVA), respectively. To assess differences within a group before and after intervention, we will use the paired t-test. All data will be presented as mean ± standard deviation, with a p value < 0.05 considered significant (Vierra, Boonla & Prasertsri, 2022).

***Analysis for brain stem and parasympathetic nervous system before and after meditation***

To conduct statistical analyses, we will use techniques such as paired t-tests or repeated measures ANOVA to identify significant changes in connectivity within these specific brain regions that associated with parasympathetic regulation and correct for multiple comparisons to control for false positives.

***Analysis for grey matter concentration before and after meditation***

For grey matter concentration analysis, we will use voxel-based morphometry (VBM) and focus on differences in grey matter concentration. Additionally, we will conduct statistical analyses to compare grey matter concentration maps before and after the meditation intervention, using techniques such as paired t-tests or repeated measures ANOVA to identify significant changes in grey matter concentration within specific brain regions that associated with sleep, and correct for multiple comparisons to control for false positives (Hölzel et al., 2011).

***Sample size and power analysis***

A clinically meaningful intervention's average standardised target effect size is 0.3 (Rothwell et al., 2018), which corresponds to a small effect. Thus, we calculated that the theoretical sample size of 31 is required to detect an effect size of 0.3 using analysis of variance (ANOVA) with 2 repeated measures, a type I error of 0.05, and a power of 0.95, assuming correlations between repeated measures of 0.5 and nonsphericity correlations of 1 (G\*Power 3.19). The practical sample size of 45 was planned by estimating that 70% of participants would complete the study with acceptable MR image quality based on our previous study (Kennedy et al., 2022).

**Discussion**

***Controlling confounding factors***

ME/CFS is an illness currently defined by consensus criteria and questionnaires completed by patients. Thus, there is a risk of an ill-defined ME/CFS patient cohort. The proposed study will mitigate this problem by following CCC ME/CFS criteria.

***Progress and plan***

This study commenced in 2023. The participant recruitment will commence in August 2025 and to December 2025. We will start preliminary analysis in January 2026 and disseminate preliminary results in August 2026. The final results and data release will be available in December 2026.

***Ethics statement***

This study was reviewed and approved by University of the Sunshine Coast Ethic committee with the approval number A231955.

***Results dissemination***

The data from the study will be stored and archived by the UniSC library research data management plan. The full access of data will be limited to approved investigators only. Anonymised data will be transferred to password-protected computers in the USC computers for processing and analysis. The disseminating results will not contain any personal information, and no participant will be able to be identified in any presentation or publication. The results will be disseminated to researchers through scientific manuscripts and scientific conferences, and to patients through UniSC’s ME/CFS Study website, social media such as UniSC’s Facebook, Instagram, and LinkedIn. Participants may receive an MRI brain image on request; however, we are unable to provide individual results as its significance at the individual level is not yet established. Likewise, other neuroimaging data will not be disclosed to individual participants as they are not relevant to diagnosis or pathological information.

**Ethics statement**

The studies involving human participants were reviewed and approved by University of the Sunshine Coast University with the approval number A231955. The patients/participants provided their written informed consent to participate in this study.

**Author contributions**

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**Conflict of Interest**

The authors report no competing interests.

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