Randomized Double-Blind Placebo-Controlled Trial: fMRI Assessment of Cranial Electrical Stimulation for Fibromyalgia in Veterans

NCT04115033

October 20, 2022

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Version Date: 10/20/2022

BACKGROUND AND SIGNIFICANCE

Fibromyalgia Prevalence, Function, and Impact

Chronic pain affects more individuals than heart disease, diabetes, and cancer combined, and the subjective nature of its assessment places it as a primary driver for the opioid epidemic, given the difficulty in discerning incompletely treated physical pain from substance use disorder and drug-seeking behavior.[1] In 2016, 63,632 drug overdose deaths occurred in the United States (a 21.5% increase from 2015), and opioids were found to be the main driver of these deaths (66.4% of all 2016 drug overdose deaths).[3] Over 50% of veterans treated at VA hospitals suffer from chronic pain, and 63% of those who receive opioids for pain are also deemed high-risk patients with co-morbid psychiatric conditions.[4] Thus, non-pharmacologic alternatives to opioids and other drugs are desperately needed for chronic pain treatment in order to slow the opioid epidemic, and this is particularly important in the veteran population where the risk of suicide and opioid use disorder is higher.

Many chronic pain syndromes are difficult to treat, but one that is notoriously difficult and relies primarily on clinical judgement and diagnosis is fibromyalgia. Fibromyalgia is a chronic pain syndrome that consists of chronic widespread pain, decreased physical function, fatigue, psychoemotional and sleep disturbances, and various somatic complaints affecting approximately 2-3% of the general population in the Americas (~8 million people in the U.S.).[5] This syndrome is not only devastating to the patient, but also represents an economic burden to society due to disability and increased healthcare utilization. It is estimated that fibromyalgia costs the U.S. population over $20 billion/year in lost wages and disability.[6,7] Although the pathophysiologic mechanisms leading to development of the disease are not well-established, there is sufficient evidence to support the idea that fibromyalgia is a disorder of autonomic nervous system dysfunction[8] and central (i.e. brain and spinal cord) pain processing mechanisms.[9] A reliable clinical biomarker to guide fibromyalgia diagnosis and treatment is necessary to better serve these patients. The current proposal addresses this gap by developing rs-fcMRI as a novel and reliable biomarker of pain severity and underlying neural circuitry in chronic pain.

Fibromyalgia affects women at a higher rate than men (estimated anywhere from 7:1 to 9:1) in the general population.[10] However, in the veteran population, a large number of males are also affected by fibromyalgia, according to data acquired at our institution (**Fig 1**). In veterans deployed to Iraq and Afghanistan, the prevalence of fibromyalgia and other chronic multi-symptom illnesses is twice as high as in the general veteran population.[11-15] This makes the syndrome particularly relevant to our contemporary veterans returning from Operation Enduring Freedom, Operation Iraqi Freedom, and Operation New Dawn. A better understanding of fibromyalgia and its treatments is necessary to prevent long-term morbidity and suffering in these young veterans. Currently

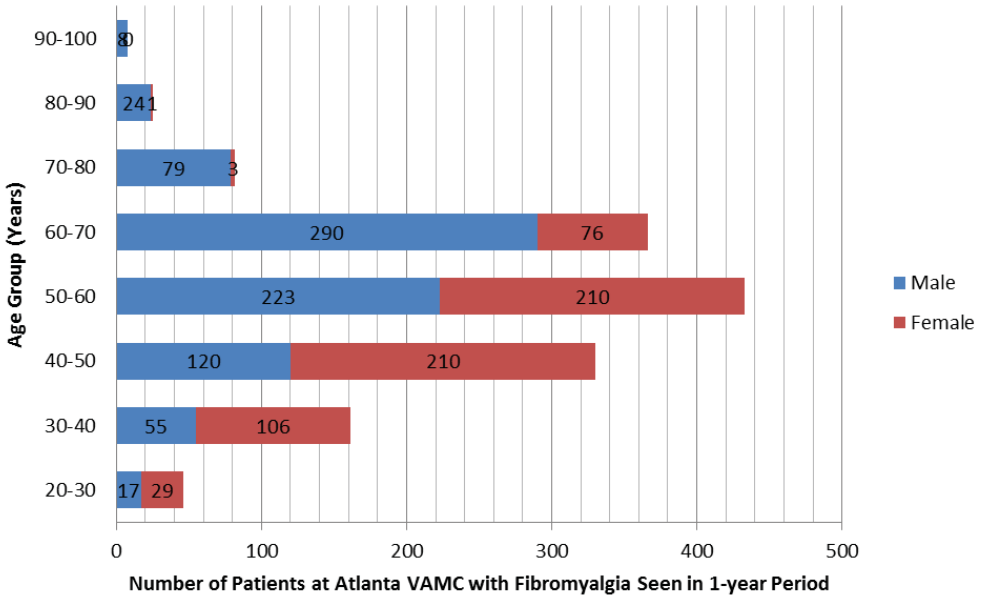


Figure 1. Prevalence of Fibromyalgia in Atlanta VAHCS (2014-2015). A total of 1,523 veterans with fibromyalgia were evaluated at the Atlanta VA in a one-year period. Both male and female veterans are affected in relatively equal numbers, though the gender distribution varies with age. A larger number of males are affected in the elderly veteran population, with a ~ 2:1 male to female ratio in the 20-50 age range at the Atlanta VA. This figure demonstrates our ability to recruit both males and females aged 20-60 years from our underlying population.

available therapies often lead to intolerable side effects,[16] and non-pharmacologic and complementary therapies are therefore often used as first-line treatments.[17]

Innovation and Significance: The present proposal addresses several gaps in fibromyalgia research by **1)** investigating the clinical effect of cranial electrical stimulation (CES) using a FDA-cleared, non-invasive, non-pharmacologic treatment for fibromyalgia and chronic pain **2)** defining underlying neural substrates of fibromyalgia clinical pain and treatment response, and **3)** applying rs-fcMRI as a predictor of treatment response. The evaluation of non-pharmacologic treatments for pain conditions such as fibromyalgia is of utmost importance in the current setting of the opioid epidemic and chronic pain as a risk factor for suicide,[18,19] which is substantially increased among the veteran population. The lack of adequate clinical biomarkers for pain is a recognized knowledge gap, leading to NIH-generated funding announcements to explore this area. Using neuroimaging at multiple timepoints to assess treatment response is a novel approach to fibromyalgia evaluation, as is the utilization of auricular CES as a treatment. Discoveries from this project related to fibromyalgia in veterans may have broader applications to the general chronic pain patient population. Further, this proposal directly fits in the Rehabilitation Research and Development (RR&D) Priority areas targeting "injuries, disorders and diseases with the potential to cause long term impairment and disability in the Veteran population; rehabilitation interventions and techniques designed to maximize motor, sensory, and psychological recovery; and endpoints that include functional outcomes of study subjects."

Auricular CES as a Treatment

CES is an FDA cleared treatment for insomnia, depression, anxiety, and pain that consists of pulsed, alternating microcurrent applied via electrodes placed on the earlobes. The mechanisms are not fully understood, though prior neuroimaging studies have explored the direct effect of CES on network connectivity. Feusner et al. found that CES is associated with cortical deactivation for 0.5 Hz and 100 Hz frequencies in bilateral frontal, parietal and posterior midline regions and postulated that current intensity may be less critical than frequency of stimulation in relation to cortical deactivation.[20] Their group found significant effects on some but not all nodes of the default mode network (**Table 1**). The authors suggest that based on this data CES may affect resting state functional connectivity and further exploration of the longer-term effects of daily treatment in relation to clinical improvement is needed, as well as how brain deactivation relates to previously observed decreases in electroencephalogram (EEG) frequencies[21] to further understand the therapeutic mechanism of action.

Table 1. Regions of altered functional connectivity associated with CES stimulation at 100 Hz between the bilateral posterior cingulate gyrus (seed region) and other regions within the default mode network.

Default mode network region	Z score	x, y, z
Increased connectivity		
Left planum temporale	3.87	−52, −34, 14
Right postcentral gyrus	3.56	66, −14, 14
Left supramarginal gyrus, anterior	3.48	−68, −26, 24
Left postcentral gyrus	3.4	−68, −22, 24
Right supramarginal gyrus, anterior	2.89	58, −26, 32
Decreased connectivity		
Left supramarginal gyrus, posterior	3.34	−42, −44, 34
Left angular gyrus	3.18	−38, −58, 40
Left lateral occipital cortex, superior	2.59	−48, −62, 50

Z scores and MNI coordinates for local maxima (x, y, z) are given. Table adapted from Feusner et al.[20]

There is some preliminary evidence to support the use of cranial electrical stimulation (CES) in the treatment of pain conditions including fibromyalgia.[2,22] A 2001 study of 60 subjects randomized to active or sham CES for 3 weeks of daily 60 minute sessions revealed a 28% improvement in tender point scores, 27% improvement in general pain scores, and no placebo effect.[23] In a 2013 study of 46 individuals with fibromyalgia primarily consisting of well-educated Caucasian females (>93% female), 17 participants were randomized to active CES, 14 to sham, and 15 controls to usual care. Participants in the active CES and sham groups were instructed to use the Alpha-Stim CES device for 60 continuous minutes each day for 8 weeks. Participants in the CES device group received devices that were active and preset at the factory to provide maximum of 60 minutes of modified square-wave biphasic stimulation at 0.5 Hz and 100 μA, the lowest setting that has been used in previous studies with patients with FM and below the level of perception. Participants in the sham device group

received sham devices that were identical to the active device, but did not deliver any electrical stimulation. Device use was monitored by asking participants to document at what time and for how long the device was used each day. Results of the study indicated that the active CES reduced average pain (p = .023), fatigue (p = .071), and sleep disturbance (p = .001) to a greater degree than the sham device or usual care alone over time. Participants using active CES also had improved functional status versus the other groups over time (p = .028).[22]

The effects of alpha-stim are thought to be mediated through a direct action on the brain, and therefore this same research group examined fMRI outcomes in 6 subjects from the active and sham groups and found decreased BOLD signal in the posterior cingulate gyrus (p = 0.034), cingulate gyrus (p = 0.001), anterior cingulate (p = 0.0056) and thalamus (p = 0.031) from baseline to week 8 in the active vs. sham CES group (**Fig 2**). An increase was observed in the insula (p = 0.044) and the prefrontal cortex (p = 0.0003) from baseline to week 8 in the sham vs. active CES group. No significant differences were found in the somatosensory cortices or amygdala between the two groups.[2]

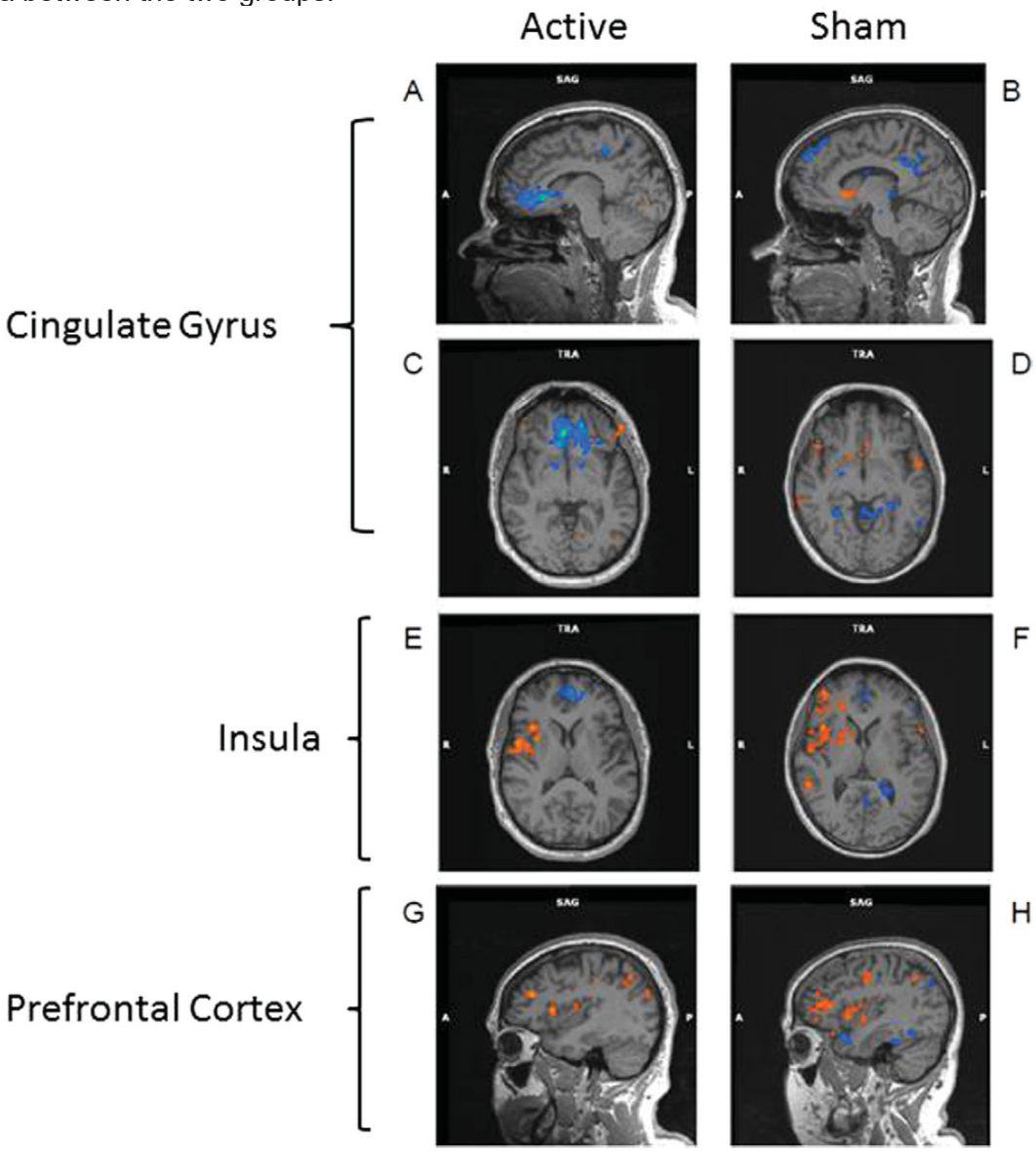


Figure 2. fMRI data analysis (Excerpt from Taylor et al.) Mean representative images of changes in BOLD activation between the active device group versus the sham device group from baseline (pre-intervention) to week 8 (post-intervention), showing increases in activity (orange) and decreases in activity (blue) in the (A–D) cingulate gyrus, (E and F) insula, and (G and H) prefrontal cortex. [2]

While the study by Taylor et al. has evaluated neuroimaging in a small sample (6 subjects in active and sham groups) of primarily female fibromyalgia patients and has shown a positive effect of CES for clinical outcomes as well, CES has not been evaluated in a veteran population nor has it been adequately evaluated in males with fibromyalgia. A VA-funded systematic review of CES published in 2018 concluded that

evidence is insufficient for CES to have clinically important effects on fibromyalgia given that most trials had small sample sizes, short durations, and high risk of bias due to inadequate blinding.[24] However, the review suggests that CES does not cause serious side effects and there is low-strength evidence to suggest modest benefit in patients with anxiety and depression. Therefore, further research is warranted regarding the use of this low-risk device, particularly in fibromyalgia. Using the Taylor et al. study as preliminary data, we propose a study to evaluate the effects of CES in a double-blind, randomized, sham-controlled trial of active vs. sham CES in 50 male and female veterans diagnosed with fibromyalgia and aim to explore the neural substrates of CES-induced analgesia using rs-fcMRI in all 50 subjects.

Neuroimaging as a Biomarker

Demonstrating neural changes as they relate to pain treatment is important to the field of fibromyalgia research, as these changes can provide a biomarker against which treatment success can be bench-marked and lead to the discovery of new treatment targets. Currently, validated tools for analgesic response are primarily subjective. The visual analogues scale, numerical rating scale, and DVPRS (Defense and Veterans Pain Rating Scale) are all validated, but subjective measures based on patient-reported outcome measures that can be affected by malingering and secondary gain.[25] The subjective nature of pain and its assessment is a likely driver for the current opioid epidemic, in which physicians are unable to distinguish opioid-seeking related to poorly controlled pain from opioid-seeking related to substance use disorder. Chronic pain is distinguished from acute pain primarily by the duration of disease, but is in reality a much more complex state that involves not only physical pain but also psychological distress, despair and anxiety.[26] Because chronic pain is complex and often hard to objectively define, neuroimaging has been used in research to more objectively assess pain states, and has even been employed in litigation cases regarding pain.[27,28]

Functional magnetic resonance imaging (fMRI), positron emission tomography (PET) and other neuroimaging techniques are now increasingly used to study the neural bases of pain.[29,30] Several regions in the human brain have been identified as important for the perception of pain (**Fig 3**). The perception of pain includes activation, at times bilaterally, of: 1) the primary and secondary somatosensory cortices 2) insula, anterior cingulate cortex (ACC) and hippocampus and 3) prefrontal cortices (PFCs), as well as alterations in: subcortical areas (brainstem and periaqueductal grey [PAG], hypothalamus, amygdala and cerebellum) during painful experiences.[29] Brain activity in response to pain can be localized using a technology called Blood Oxygen Level Dependent (BOLD) fMRI. The BOLD fMRI signal relies on local blood flow and metabolic changes in response to changes in neural activation. These localized changes in neural activation are then spatially mapped on the patient's brain, and anatomically identified by an experienced neuroimaging scientist, such as the **applicant's primary mentor, Dr. Crosson.** "Seeds," which are pre-determined neuroanatomical areas of importance or regions of interest (ROIs) for the study can be localized and BOLD signal from these seeds correlated with the whole brain analysis to identify temporal correlations with other potentially important regions.

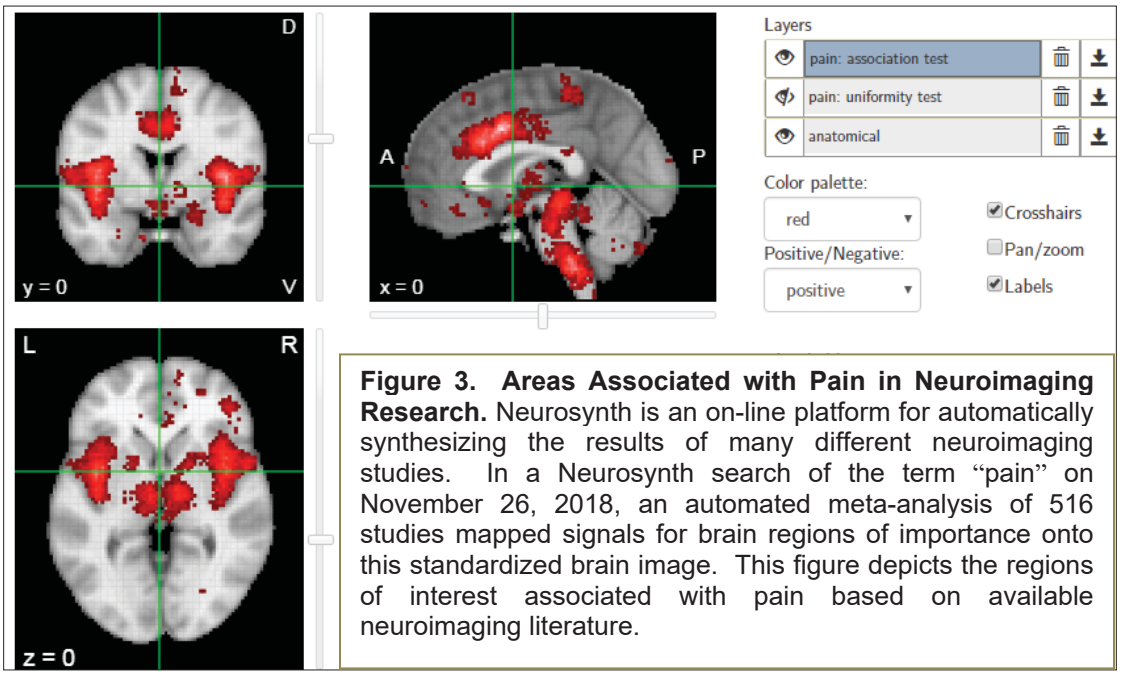


Figure 3. Areas Associated with Pain in Neuroimaging Research. Neurosynth is an on-line platform for automatically synthesizing the results of many different neuroimaging studies. In a Neurosynth search of the term "pain" on November 26, 2018, an automated meta-analysis of 516 studies mapped signals for brain regions of importance onto this standardized brain image. This figure depicts the regions of interest associated with pain based on available neuroimaging literature.

Resting functional connectivity magnetic resonance imaging (rs-fcMRI) is a type of BOLD fMRI that examines the intrinsic connections in the brain when no external stimuli are applied. The "resting state" evaluates the brain while the patient is lying at rest, but still awake, thus enabling researchers to investigate chronic, clinical

pain at rest, as opposed to evoked, experimentally-applied pain. The **applicant's co-mentor, Dr. Napadow**, and others have observed altered cross-network connectivity between seeds of the somatosensory, salience, and default mode network(s) in chronic pain patients, including those with fibromyalgia, using rs-fcMRI.[31-34] Activity within the cingulo-frontal cortex, thalamus and PAG has been shown to be involved in distraction from pain, as well.[35,36] With changes in clinical pain and pain processing, rs-fcMRI may identify unexpected regions that respond to CES in fibromyalgia patients. Thus, identifying neural plasticity related to clinical pain and treatment outcomes provides valuable information in both the assessment of pain as well as targeting treatment. Furthermore, evaluating brain reorganization during different stages of recovery following treatment (short and long-term) could also provide valuable clinical information for enhancing the effectiveness of treatment outcome.

Several studies have brought attention to the potential for neuroimaging to be used as a biomarker for pain treatment outcomes and serve as the basis for the current proposal's use of rs-fcMRI to evaluate aberrant network connectivity in fibromyalgia. In a study by Harris, Napadow, et al., a reduction in clinical fibromyalgia pain was found to be associated with reductions in brain connectivity from the posterior insula to DMN regions during treatment with pregabalin but not placebo, and baseline neuroimaging values were able to predict analgesic response to pregabalin but not placebo.[37] Another pharmacological treatment study revealed reductions in clinical pain associated with decreased functional connectivity between pain regions such as the rostral part of the ACC and the insular cortex, as well as the PAG and the insular cortex for fibromyalgia patients treated with milnacipran, but not placebo.[38] Importantly, those with lower baseline functional connectivity had the greatest reduction in clinical pain, indicating the potential predictive value of rs-fcMRI as a biomarker.[38] More recently, Napadow and colleagues have published a paper using rs-fcMRI as one of 3 multimodal metrics to predict clinical pain intensity with greater than 90% success.[39]

Napadow and colleagues also explored the ability of a non-pharmacologic treatment (acupuncture) to modulate resting state connectivity in DMN and SMN, showing that true acupuncture but not sham increased DMN connectivity with pain, affective, and memory-related brain regions, and that increased DMN connectivity with the hippocampal formation was negatively correlated with increased sympathetic related heart rate variability while positively correlated with the parasympathetic related metric; the authors theorize that this modulation and sympathovagal response may help to explain acupuncture's therapeutic effects.[40] Another study by Napadow et al. correlated reductions in clinical pain related to reductions in intrinsic DMN to insula connectivity in 17 fibromyalgia patients undergoing nonpharmacologic acupuncture treatment, suggesting that intrinsic brain connectivity can be used as a biomarker of spontaneous chronic pain in fibromyalgia.[41] These studies utilizing a non-pharmacologic treatment to alter rs-fcMRI through a possible sympathetically-mediated pathway serve as a basis for the present investigation. Thus, the experience of pain and its treatment involves complex interactions and diverse brain regions, necessitating further study.

As evidenced by some of the already cited studies above, neuroimaging and rs-fcMRI can be applied more broadly to pain syndromes other than fibromyalgia, as well The use of rs-fcMRI is superior to assessing clinical pain scores alone; while patient reported outcomes are important, they are subject to malingering and secondary gain. Furthermore, rs-fcMRI can evaluate the neural correlates and mechanisms for the development of chronic pain. Though this field of research is still evolving, rs-fcMRI research can lead to an improved understanding of how brain changes reflect or maintain pain. Understanding mechanisms for the development of chronic pain and identifying neural correlates of pain and pain relief through the proposed study may help to differentiate between pain syndromes such as fibromyalgia, target treatment response in a personalized way, and lead to a set of reliable neuroimaging biomarkers to assess pain and treatment.[42]

PRELIMINARY DATA & FEASIBILITY

In our CDA-1 feasibility study, were able to obtain baseline and follow-up rs-fcMRI as well as multiple functional outcomes at baseline, 4, 8 and 12 weeks following the completion of treatment in 21 subjects over the course of 2 years in an open-label trial evaluating an auricular stimulator for pain. All subjects assigned to the "treatment" group completed the study, suggesting that the addition of a sham control will increase participant follow-through and study completion.

The proposed CDA-2, a randomized, sham-controlled trial of auricular CES, will evaluate **1)** the clinical utility of CES for fibromyalgia as compared to placebo control, **2)** long-term CES-related neural changes visualized on rs-fcMRI and **3)** the ability of rs-fcMRI to predict CES treatment response. Should CES prove an effective treatment for fibromyalgia, the device could easily be implemented in the VA pain clinic through an existing VA contract, providing a safe and viable alternative to opioids and other pharmacologic therapies. Results from this study will also help to develop and refine rs-fcMRI as a clinical biomarker for pain assessment.

Tests of a Priori Hypotheses

Aim 1. Primary outcome (improvements in clinical pain and function).

We will investigate whether pain and functional improvements occur with the application of CES, as the ultimate goal of reducing pain is to improve function. Clinical pain, as measured by the DVPRS, will be our primary outcome measure of interest. Dependent variables for the evaluation of functional improvements with CES include PROMIS measures, the arm curl, 30-s chair stand and handgrip strength tests at 1 and 12 weeks follow-up after the completion of the 4-week treatment period. Patient-reported changes in pain will be evaluated using **1)** DVPRS severity scores and **2)** analgesic consumption before and after treatment (1 week) and at long-term follow-up (12 weeks) following completion of sham or active CES for each group. The primary hypothesis is that active CES will show significant improvements post-treatment relative to pre-treatment in multiple domains as compared to sham CES. To test this hypothesis, pairwise repeated measures comparisons between post- and pre-treatment DVPRS, analgesic consumption and functional assessments will be performed within each group, FWE corrected to $p<0.05$. Similar analyses will be conducted at 12 weeks follow-up. Data from each time point can be considered its own family of comparisons for this purpose. Further, analysis of sample characteristics for the groups (active vs. sham CES) will be conducted to assess comparability of the samples. Categorical variables such as sex and biobehavioral data will be assessed using Fisher's exact test, but continuous variables such as age will be assessed using two-tailed t-tests. All reported p-values will be 2-tailed and considered significant at the 0.05 level, FWE corrected. Data collected and analyzed regarding functional changes related to CES treatment will be assessed for new hypotheses.

We will use a 2-groups (active vs. sham CES) x 3 times (pre- and post- treatment at 1, and 12 weeks follow-up) analysis of the variance to compare outcome for the groups over time. We will employ a linear mixed effects (LME) modeling framework to fit the ANOVA model to the data. LME's are more robust than traditional ANOVA methods when there is a possibility for imbalance in the effects due to attrition. Missing data resulting from dropout and other losses to follow-up can be addressed more easily in LMEs and give a stronger estimate of the fixed group effect while controlling for time as a random effect in the model.

Aim 2. Secondary outcome (rs-fcMRI as a biomarker of treatment outcomes)

The secondary outcome measure is connectivity between SMN and DMN, though we will also explore connectivity to the SN based on our preliminary data. We hypothesize that active CES will increase connectivity between the seed and areas of the DMN based on prior mechanistic research using CES (**Table 1**). [20] We also expect a decrease in BOLD signal in the PCC, insula, thalamus, and other areas related to pain based on prior data.[2] We expect that these changes will also be present 12 weeks following treatment, suggesting a mechanism for long-term treatment effects. We do not expect to see similarly significant changes for the sham CES placebo control group. Each of the two correlations will be FWE corrected to $p<.05$ for two comparisons for each group. Generally, we expect no group differences at pre-treatment, but expect that between-group differences will emerge for networks specified a priori as well as exploratory analyses at the post treatment scans.[43]

Seed-voxel Functional Connectivity Approach (Hypothesis-Driven Analysis): Seed-based resting connectivity analyses between relevant areas based on our a priori hypotheses will be performed. The seeds for this analysis were chosen based on our preliminary data, as well as the literature on fibromyalgia, pain, and CES. [2,20,30-34,41,44,45] These seeds come from three networks: DMN, SMN, and SN. **Table 2** identifies the seeds by network. Each seed will be correlated with all other seeds at the individual subject level using a Pearson correlation. Correlations will be converted to Z scores to perform for group analyses. Changes in network connectivity will be tested using a 2 groups (active vs sham) by 2 times (pre-treatment vs post-treatment) analysis of variance (ANOVA). For each analysis, we will first test for the expected interaction term ($\alpha < .05$, false discovery rate (FDR) corrected for number of tests within or between networks), followed by pre- vs post-treatment pairwise comparisons for each group to confirm the expected pattern of change ($\alpha < 0.05$, FDR corrected); we will also test baseline vs. follow-up to determine persistence of change.

Table 2. DMN, SMN, and SN seeds chosen for analysis based on a priori hypotheses.

DMN seeds (x,y,z)	SMN seeds (x,y,z)	SN seeds (x,y,z)
mPFC[31,46]	Right Putamen[34,41]	Right dlPFC[31]
Right PCC[44]	Left M1[44]	Left anterior insula[31]
Left PCC[20,46]	Right M1[44]	Right anterior insula[31]
Precuneus[2]	Right S1-Hand[47,48]	Left posterior insula[34]

	Left S1-Hand[47,48]	Right posterior insula[32]
	Thalamus[2]	dACC[49]
		Right TPJ[31]

Each seed is presented with references to prior literature supporting its testing in pain syndromes.

The seeds will be spherical, 1-cm diameter and centered on the MNI peak coordinates of regions of activity defined from prior published studies and preliminary data. The same seeds will be eroded to include only gray matter voxels using the Johns Hopkins University-International Consortium of Brain Mapping white-matter atlas.[50] We will then correlate the averaged time series from the seed regions using AFNI. The resultant cross-correlation coefficients will be converted to z-scores, which will act as the dependent variables for the primary outcome analysis with rs-fcMRI. The average time series from these distinct ROIs is used as a regressor in a whole brain GLM to find which other regions contain correlated time series. Thus, a limited number of seeds has been carefully chosen based on our preliminary data, previous studies, and known pain pathways. All group analyses will use AFNI. We will further perform a linear regression with z-scores from rs-fcMRI correlations from seed voxels and baseline pain levels (DVPRS) in order to evaluate links between baseline resting connectivity and base-line individual differences in pain sensitivity.

Hierarchical covariate-adjusted ICA (hc-ICA) Approach (Exploratory Analysis): The above approach is a granular comparison of hypothesized changes in node pairs. Hence, it is complemented by the following exploratory network-based approach that will allow us both to assess network level changes in function. The hc-ICA, implemented by the HINT matlab toolbox,[51] is a hierarchical model where the first-level model of hc-ICA decomposes a subject's fMRI signals into a product of subject-specific functional networks and subject-specific temporal mixing matrix to capture between-subject variabilities in the spatio-temporal processes in the functional networks. The second-level model of hc-ICA models subject-specific networks in terms of population-level networks, effects from subjects' covariates and between-subject random variabilities. Compared to alternative methods such as dual regression ICA, hc-ICA has been shown to provide more accurate estimates of the brain functional networks on both the population- and individual-level and provide more reliable and powerful statistical tests for evaluating group differences in brain functional networks by directly modeling covariate effects in ICA decomposition.[43] We plan to apply the hc-ICA to fMRI data to extract brain functional networks. We will include treatment group and DVPRS as the primary covariates in the hc-ICA and include age, sex, ethnicity, and other potential confounding variables. This will allow us to more accurately assess treatment group differences in the networks while controlling for potential confounding factors. Among the ICA-extracted brain networks, we will focus on DMN as our primary network and evaluate medial visual network (MVN) as a negative control, as we expect CES to have no effect on this primary visual sensory network. Based on hypothesis testing in hc-ICA, we will evaluate whether the brain intrinsic connectivity/network covaries with clinical pain and how networks change following CES in active and sham groups. The results will be threshold at $p < 0.05$, cluster-corrected for multiple comparisons. We will also conduct exploratory analyses on other brain functional networks extracted by hc-ICA (corrected for multiple comparisons), which will provide an unbiased data-driven analysis to generate new hypotheses for future studies and grant submissions.

Aim 3. Exploratory outcome (rs-fcMRI as a predictor of treatment response)

Our exploratory outcome measure utilizes rs-fcMRI as a predictor of treatment response. Following completion of treatment and follow-up, we will separate treatment responders and non-responders into two groups. A positive response will be defined by a decrease in clinical pain of at least 2 points (defined by prior studies as the minimally clinically important difference)[52] at 12 weeks. Using the hc-ICA exploratory analysis described above, we will extract brain functional networks from baseline rs-fcMRI data in the active CES treatment group. We will include treatment response and DVPRS as the primary covariates. Based on hypothesis testing in hc-ICA, we will evaluate whether treatment response covaries with baseline brain connectivity and how baseline connectivity may differentiate CES responders from non-responders. We will similarly analyze sham placebo responders and non-responders, and compare the two analyses for differences, as baseline connectivity in individuals with fibromyalgia may also be predictive of a placebo response. The results will be threshold at $p < 0.05$, cluster-corrected for multiple comparisons.

After all analyses have been performed, we will use correlation coefficients to investigate the predictive ability of baseline resting SMN-DMN connectivity to predict post-CES changes in pain levels. Baseline connectivity will be extracted as z-scores from imaging data. Correlations will be assessed using *DensParCorr*, a statistical package developed by collaborator, Dr. Ying Guo, that is designed to better eliminate false positive

correlations.[53] We will also use a linear regression model to explore any association between treatment-modulated clinical pain and the change in resting brain connectivity.

Thus, should active CES show no significant clinical improvement relative to sham CES in group analyses, but clinically significant improvements among a sub-group of responders (decrease in pain score by 2 points at 12 weeks), we can determine the neural correlates that separate responders from non-responders and evaluate the predictive value of baseline rs-fcMRI for treatment response. This is distinguished from Aim 2, which evaluates correlations between changes in clinical pain and changes in rs-fcMRI network connectivity between groups. Thus, the aims are independent of each-other.

RESEARCH DESIGN AND METHODS

Overview

This project is designed as a double-blind, randomized, sham placebo-controlled trial to determine the clinical utility of auricular CES in veterans with fibromyalgia**,** the short and long-term effects of CES on fibromyalgia-related altered network connectivity using rs-fcMRI as a biomarker, and the ability of baseline rs-fcMRI to predict treatment outcomes. Eligibility criteria include:

- Age 20-60 years old (limit set to minimize brain structural changes due to aging).
- Diagnosis of fibromyalgia by the American College of Rheumatology 2016 criteria.[54]
- Right-handedness, in order to provide consistency in brain structure and function.
- Pain score of 4 or greater on DVPRS in the 3 months prior to enrollment.
- Stable medication use related to FM for at least 4 weeks prior to enrollment.
- Ability to safely tolerate MRI

Exclusion criteria include pregnancy; history of seizures or neurologic conditions that alter the brain; claustrophobia, MRI-incompatible implants, or other conditions incompatible with MRI; and history of uncontrolled psychiatric illness or autoimmune disease that leads to pain and can better explain symptoms.

Subjects will be randomized to sham or active CES in addition to standard therapy (n=25 per group, 50 total subjects, see “Sample Size” below). Subjects will be block randomized, stratified for sex. Baseline assessments and rs-fcMRI will be obtained prior to initiation of the intervention. The intervention (both sham and active CES) will occur over the course of 12 weeks, and subjects will be assessed at 4 and 12 weeks post-treatment initiation. Follow-up rs-fcMRI will also be obtained at the 4 and 12 week timepoints during the 12 week course of treatment, to assess for short- and long-term changes in connectivity. Each participant will receive $100 on completion of the study. Text messages may be used by the study team to communicate with subjects who have consented to participate. All text messages will be sent using an encrypted, VA-issued study iPhone. A summary of study phases is presented in **Table 3.**

Table 3. Research Design

	Time	Research Activity
Phase 1	Week 1	Recruitment, Screening, Enrollment
Phase 2	Week 2-3	Baseline Assessments, rs-fcMRI
Phase 3	Weeks 4-7	Intervention
Phase 4	Week 8-9	Acute follow-up, rs-fcMRI (1 wk post)
Phase 5	Week 20-21	Long-term follow-up, rs-fcMRI (12 wks post)

Each Cohort (1-5), consisting of 10 subjects randomized to either inactive sham or active CES, will proceed through Phases 1-5 as described in Table 2 over the course of the 5-year study period.

Sample Size

Three outcomes are used in our sample size calculations: DVPRS (**Aim 1, clinical pain**), 30 second chair stand test (**Aim 1, function**), and DMN-SMN connectivity (**Aim 2, rs-fcMRI**). All power calculations are based on preliminary data presented above. Clinical pain changes using DVPRS is chosen as our primary outcome of interest. The 30-second chair stand test (30sCST) is chosen as our representative functional outcome, since it exhibited the smallest between group change. DMN-SMN connectivity is chosen as our secondary outcome of interest as a neuroimaging biomarker for clinical pain and treatment response. Sample size analyses were conducted assuming a significance of 1% and 80% power (2-sample, 1-sided).

The primary hypothesis (**Aim 1**) is that there will be improvements in clinical pain and function for the active CES group as compared to sham placebo CES treatment. The secondary hypothesis (**Aim 2**) is that increased DMN-SMN connectivity (L-PCC to L-S1M1) will be observed for the CES treatment group relative to the placebo control group. For the PI’s CDA-1, we recruited 20 subjects over the period of 1.5 years without

offering compensation, out of over 1,500 veterans with fibromyalgia seen annually at the Atlanta VAHCS (**Fig 1**), thus demonstrating the feasibility of recruitment of veterans with fibromyalgia for a CES/rs-fcMRI study. Additionally, 12 subjects completed treatment in the 1.5 year period. Ten subjects completed the study. Two subjects were lost to follow-up (17%), and both were in the control group. All active treatment subjects completed 12-week follow-up. Thus, we would expect a more evenly distributed and decreased drop-out rate when a sham, placebo device is used and compensation is offered. Sample sizes required for our clinical outcomes are shown in **Table 4**, but we will power our study to our connectivity outcome, which requires a larger sample.

Based on our preliminary data, the mean (SD) of the post-treatment change in L-S1M1 to L-PCC connectivity is 0.041 (0.079) for the treatment group and -0.026 (0.049) for the standard treatment group; the observed between-group difference effect size is 1.03. We would need 20 subjects in each group to achieve 80% power to detect the difference between the CES group and the standard treatment group in their post-treatment change in connectivity for L-S1M1 to L-PCC at the significance level of 0.01 using a two-sided t-test, assuming the between-group difference effect size is 1.03 as observed in the pilot data. Though we observed a 17% attrition rate for 12 subjects in the CDA-1 (all completed their follow-up MRI, but two were lost to follow-up at 8 weeks and 12 weeks), in order to maintain a conservative estimate for sample size calculations, we will assume a 20% attrition rate. If we expect a 20% attrition at post-treatment visit, we will then need to recruit 20/0.8 = **25 subjects per group.**

Table 3. Sample Size Calculations

	Control		**Intervention**		**N per group**
	Mean Change	***SD***	***Mean Change***	***SD***	
Clinical pain (DVPRS)	0.375	1.493	-1.833	2.229	10
Function (30sCST)	-0.250	1.500	3.000	4.980	14
Rs-fcMRI (S1M1 to PCC)	-0.026	0.049	0.041	0.079	20

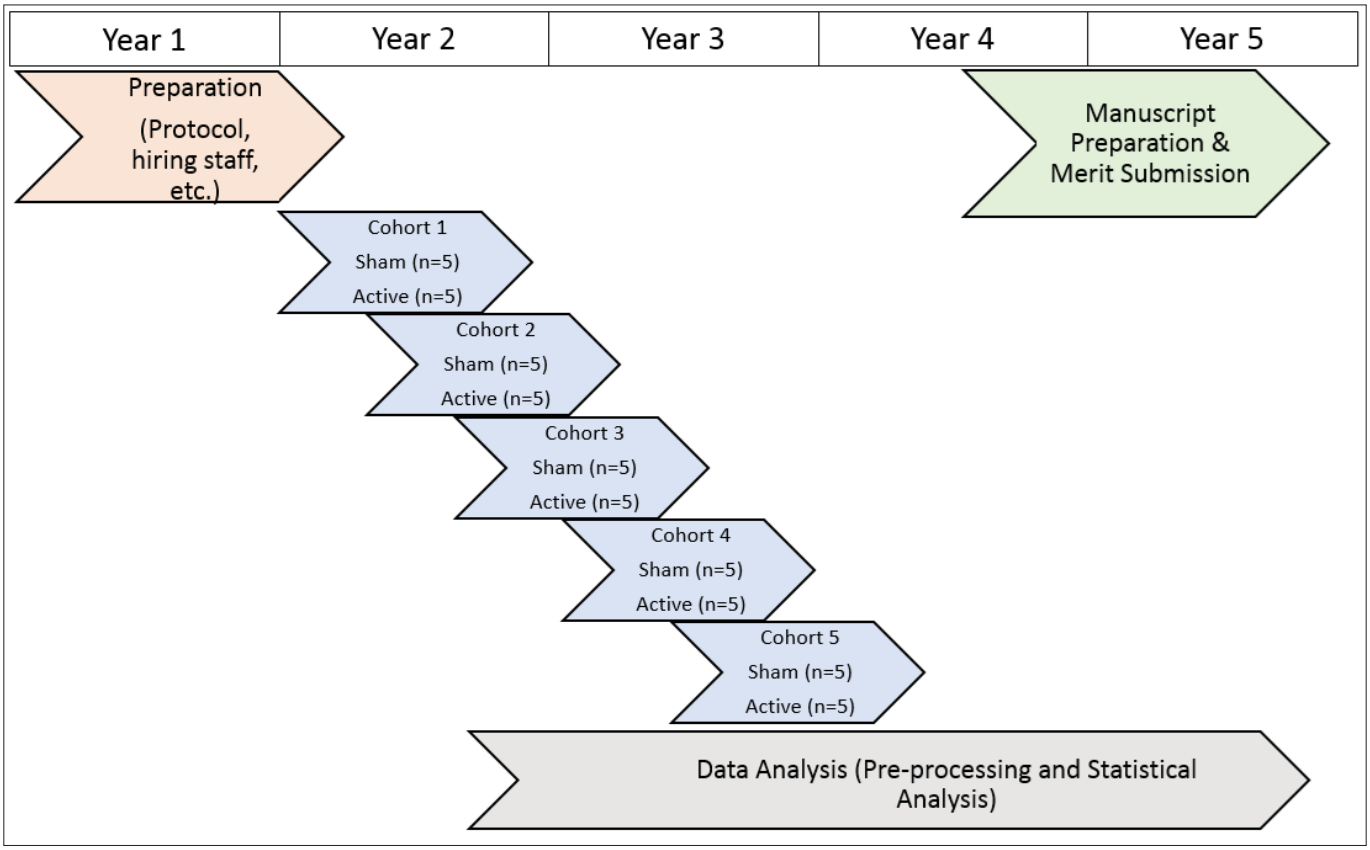
Procedures

All study protocols will be approved by the Emory University/VA institutional review board (IRB) and VA R&D committee. A summary of the study protocol and timeline is provided in **Figure 9**. Patients will be pre-screened using chart review of patients at the Atlanta VAMC and then invited via a phone call for a face-to-face screening session. At the screening session, an informed consent will be signed in accordance with ethical principles from the Declaration of Helsinki and the Ethical Committee at Karolinska Institutet. Then, the study physician (A.W.) will make assessments according to the stated inclusion and exclusion criteria. Subjects who meet study criteria will return for baseline assessments including rs-fcMRI, collection of biobehavioural information such as cognitive and psychological assessments, eating, sleeping and drinking habits, PROMIS measures including “physical function,” “anxiety,” “depression,” “fatigue,” “sleep disturbance,” “social function,” “pain interference,” “global health”, arm curl, 30s chair stand, handgrip strength tests, Defense and Veterans Pain Rating Scale (DVPRS) and documented baseline analgesic consumption.

To decrease the amount of face-to-face interaction with our participants, the study will be using a VA approved audio (telephone) or video communication (*insert type here*) to screen and consent participants. Recordings of the audio or visual communication will not be permitted. If the participant meets initial eligibility criteria, we will mail or email through VA Outlook using Azure encryption two unsigned copies of the ICF/HIPAA. We will ensure the individual has enough time after receiving the document to read it before scheduled phone/video call. Trained staff will perform consenting process including speaking with the individual to discuss the study and highlighting each section of the consent form, allowing the participant an opportunity to ask questions before providing consent, and giving the participant enough time to consider being in the study. Study team will inform the individual that if they would like to take more time to consider the study, another telephone call can be scheduled. If the individual would like to participate, the participant will sign and date the document and return it to study team via mail (phone) or via email to your VA Outlook email address (video). Study team will write a “Note to File” that documents everything about the interaction including: 1.

When and how the consent form was sent 2. When the video/telephone call was made 3. What was discussed during the call 4. When the signed consent form was received 5. When the signed consent form was signed by the person obtaining consent 6. When a copy of the consent form signed by both subject and study team was given to the participant and 7. A description of why signature dates are different (if applicable). Once the study team receives the signed consent form, the person obtaining consent should sign the form and date it for the day it was signed. Study procedures will begin once the signed copy is received. A copy of the fully signed consent form will be given to the participant via mail or in person at next scheduled visit.

Figure 9. Timeline for executing tasks over CDA-2 period



Stratification based on sex will be performed to account for differential pain perception and neurological responses to pain. Both men and women are included because despite the preponderance of women with fibromyalgia in the general population, there are more male veterans within the V.A. setting (**Fig 1**). Subjects will be stratified based on sex and block-randomized to either sham or active CES device using pre-set settings at the manufacturer (blinding both the researchers as well as the participants), then assessed for changes in pain and function at 4 and 12 weeks post-treatment initiation. The 4 week time-point is chosen as the acute phase following treatment initiation. However, the 12 week time point is chosen to evaluate longer-term neural changes as an indicator of neural plasticity. Participants will be instructed in device use by Dr. Kalangara, a board certified pain physician who will be blinded to active and sham groups. The PI, Dr. Woodbury, is a pain physician qualified to perform the pain and functional assessments and will perform these, similarly blinded to active and sham CES groups. Randomization will be performed at the manufacturer, who will assign each device a study number and keep a record of whether they are receiving active or sham CES, thus maintaining blinding of the subject, the study physician (Dr. Kalangara), and the P.I. (Dr. Woodbury) until the subject has completed the follow-up period and data analyses can be performed. At the end of the study period, subjects, study physician, and P.I. will be asked to evaluate whether they believed the participant received treatment, sham, or “don’t know”. The research coordinator will have access to the list of study numbers from the device manufacturer in order to un-blind for study analysis. The devices will be returned to the manufacturer and assessed for fidelity (to ensure that active devices remained active throughout the study period, and sham devices did not deliver active stimulation during the intervention period).

Intervention

CES will be applied using Alpha-Stim, loaned by the device manufacturer for research purposes. Randomization assignment will be established prior to the start of the study by the manufacturer of the Alpha-Stim device through the assignment of unique numbers (active or sham) to devices. Devices will be assigned to subjects in the order listed on the device log. Baseline measures will be taken prior to start of treatment period and again at the endpoints of the study (4 weeks, 12 weeks). No change will be made in the medical management of the patient during the study. Participants in the active CES and sham groups will be instructed to use the Alpha-Stim CES device for 60 continuous minutes each day for 8 weeks. Participants in the CES device group will receive devices that are active and preset at the factory to provide maximum of 60 minutes of modified square-wave biphasic stimulation at 0.5 Hz and 100 μA, the lowest setting that has been used in previous studies with patients with fibromyalgia and below the level of perception. Participants in the sham device group will receive sham devices that are identical to the active device, but do not deliver any electrical stimulation. Device use will be monitored by asking participants to document at what time and for how long the device was used each day.

Resting State Functional Connectivity Magnetic Resonance Imaging (rs-fcMRI):

The design for our MRI acquisition protocol and data analysis will be performed in direct collaboration with Dr. Venkatagiri Krishnamurthy, a VA-funded investigator and CVNR Neuroimaging Core member with expertise in MR-physics and neurophysiology. Baseline scans will be collected within 1 week prior to commencement of treatment and within 1 week of the final treatment to evaluate changes in resting state connectivity, using metrics our group has previously associated with chronic pain severity. BOLD rs-fcMRI images will be acquired on a 3T Siemens Prisma scanner with a 32-channel phased array head coil using a gradient echoplanar imaging (EPI) sequence with following MR parameters: FOV = 220 mm; TR/TE = 2000/25 msec, multiband-acceleration factor =3 ; flip angle = 60˚; 110 x 110 matrix size; slice thickness = 2mm; GRAPPA factor = 2; Partial fourier of 6/8; 34 phase-encode reference lines, seventy-two interleaved axial slices covering the whole brain, roughly 320 scan volumes to yield eight minutes of resting state fMRI data for stable estimation of connectivity networks. T2/FLAIR scans also will be performed to evaluate any white matter disease that may occur in older veterans using validated evaluations for leukoaraiosis. Those with significant leukoaraiosis based on methodology established by Junque et al. will be excluded due to the potential effects of white matter disease on resting state connectivity metrics.[55,56]

Prior to scanning, subjects will be asked to rate the intensity of their fibromyalgia pain as part of the DVPRS, a validated measure of pain for military and veteran populations.[57] In the scanner, foam cushions will be used to reduce head movement and headphones to dampen scanner noise. Patients will be asked to keep their eyes open during the resting state scan. Physiological data will be collected simultaneously to the fMRI data (time-locked), as cardio-respiratory fluctuations are known to artifactually influence fMRI intrinsic connectivity estimation within several brain networks.[58,59] Cardiac data will be acquired using an MR-compatible infrared pulse oximeter attached to the right middle finger and supplemented at times by an MRI compatible 4-lead ecg. Respiratory rate data will be acquired using an MR-compatible respiratory rate monitor and plethysmograph. De-noising will be performed using validated tools (described below). The basis for analyses of rs-fcMRI findings used in the present study is adapted from prior studies by Napadow and Hemington et al. using rs-fcMRI to examine intrinsic brain connectivity in fibromyalgia patients before and after treatment with pregabalin and acupuncture, as previously noted.[31,37,45,60] Data will be preprocessed and analyzed using the validated FSL (FMRIB's Software Library) package and cardiorespiratory physiologic artifacts will be mitigated using validated software packages. Further, artifacts related to subject motion will be minimized in rs-fcMRI time series using the validated ICA-FIX tool. This algorithm is a data-driven method to identify and reduce motion-related artifacts (ICA components) from fMRI data for structured noise removal.[61] Though we have previously used ICA-FIX successfully, we will continue to collect cardiac and respiratory data in case ICA-FIX is insufficient for denoising in our sample population and RETROICOR or another denoising tool becomes necessary.

Co-registration of the functional images to a common template space will be accomplished in two steps. First, a boundary-based registration between the functional image and high resolution T1-MPRAGE (magnetization prepared rapid acquisition gradient echo) will bring the functional and structural data into co-registration. Second, the high-resolution T1-MPRAGE will be co-registered to standard Montreal Neurological Institute (MNI) space using non-linear co-registration algorithms (FNIRT, FSL). The spatially normalized functional data will be temporally filtered using high-pass temporal filtering (f = 0.008 Hz) and smoothed using a Gaussian kernel (FWHM of 6 mm) to enhance the functional signal-to-noise ratio. In order to quantify brain

connectivity, rs-fcMRI data will be analyzed with both a seed-voxel and ICA approach. These approaches are complementary, as they quantify intrinsic brain connectivity on a network level (dual regression pICA) and a more specific regional level (seed-voxel).

DATA SECURITY AND MONITORING PLAN

Only personnel who are qualified and IRB approved and trained for maintaining the privacy of each participant will be permitted to view records. Each participant will be assigned a unique study ID that will be used on all study documents. All identified patient data will be maintained on a secure VA research server. All participant study documents which are identified by the participant's unique study ID will be kept in locked filing cabinets in a locked research office accessible by study personnel only. Data collected on computers during assessment will be de-identified. These data will be exported as a text file and transferred to the secure VA network using a VA-issued USB drive.

Data will be monitored by Dr.Woodbury for quality control and any data questions will be discussed during study staff meetings. Patients from both groups will be followed-up weekly during the 4-week treatment intervention period. Patients will also be given the clinic phone number and the P.I.'s cell phone number to contact if potential issues arise during treatment. If complications occur, the procedure will be aborted and the patient would not be included in the study. The practitioners are well-trained in resuscitation techniques since both Dr. Kalangara and Dr. Woodbury are anesthesiologists with ACLS training. Phone reminders and telephone contact will be made on a regular basis in order to encourage follow-up and compliance. Compliance can be easily assessed as described in the research protocol, since device removal is apparent and the device cannot be reinserted except by a skilled practitioner. Follow-up by phone will also be performed following the final fMRI to assess for any potential delayed reactions related to the device or to the MRI exposure. Data will be monitored by collaborator Dr. Jerry Kalangara during the treatment period. Dr. Anna Woodbury, P.I., will be blinded to treatment and control groups for fMRI acquisition and data analysis. All analysis will be done on VA approved servers using a data usage agreement with approved entities. All data security will follow local policy and procedures as outlined through Emory IRB and Atlanta VAHCS R&D.

Adverse event reporting

In the case of a reportable event (Adverse Event, Serious Adverse Event, Unexpected Problem), the Principal Investigator will follow Atlanta VAHCS and Emory IRB reporting requirements.

POTENTIAL PITFALLS, LIMITATIONS, AND ALTERNATIVE STRATEGIES

Pitfalls

Potential pitfalls include **1)** the possibility of adverse events **2)** inadequate patient recruitment and possible non-compliance with the treatment modalities **3)** potential for no correlation/connectivity in ROIs **4)** potential effects of white matter disease on connectivity, and **5)** possibility of bias and inadequate blinding should subjects be able to determine sham from active CES treatment.

Alternative Strategies

<u>These potential pitfalls can be addressed as follows:</u>

1) Adverse events are unlikely, but may include dizziness or drowsiness on initial application. If syncope occurs, the practitioners are well-trained in resuscitation techniques since both Dr. Kalangara and Dr. Woodbury are anesthesiologists with ACLS training. In this case, the procedure would be aborted and the patient would not be included in the study. Participants will be asked to use the device at home, preferably before bed time, in case of drowsiness.

2) Though our experiences has shown our participants are highly motivated, given the duration of the study, recruitment and drop-out may be an issue. Recruitment is feasible based on the preliminary data presented above, the number of cases seen at the Atlanta VAMC, and our demonstration of ability to fully recruit 20 subjects within a 1.5 year period even with no compensation offered. We anticipate that loss to follow-up will decrease with a monetary incentive to complete the study. **Dr. Rauch, Dr. Woodbury's co-mentor, directs the Emory Veterans Program**, which can serve as an additional base for recruitment, if needed. Since the scanner at Emory University is a 5-minute drive from the VAMC pain clinic, it is reasonable for the patients to

acquire their scans before or after appointments at the Atlanta VA. Most of our subjects are highly motivated to progress research in their condition and to explore modalities that may improve their pain and function. However, it is possible that some patients would remove the device if they find it uncomfortable and would be dropped from the study due to inadequate treatment duration. Analysis will be based on intent-to-treat. Phone reminders and telephone contact will be made on a regular basis to encourage continued participation. Patients will be interviewed for compliance and checked at weekly follow-ups. Subjects will also be offered compensation only after completion of the follow-up period. Should the patient drop-out rate be too high, this may necessitate the recruitment of greater numbers of patients. Though our preference is to report and analyze only the data that is actually collected, a multiple imputation technique to handle missing data using a Mixed Effects models approach may be considered if missingness is a significant problem.

3) We have made certain assumptions about our rs-fcMRI data and purposely used well-tested analyses in order to yield the highest power for a limited sample size. Although we have cited evidence to support investigating the specific regions of the SN, DMN, and SMN identified, it is possible that these areas will not be correlated in our study. Therefore, ancillary analysis of more traditional structures will also be performed, including placement of seeds in the ventral posterior medial nucleus of the thalamus. We will also plan to perform unbiased data-driven rs-fcMRI analyses using independent component analysis (hc-ICA approach, discussed above) for new hypothesis generation from this pilot study in case the stated aims of the hypotheses are not supported. It is possible that results will be discordant with previously published data regarding fibromyalgia and pain. In previously published literature regarding transcranial magnetic stimulation, for example, both concordant and discordant results are present. Given the novelty of the investigation using rs-fcMRI to evaluate auricular CES, it is entirely possible that the mechanism of altered cross-network connectivity differs from previously published studies.[62] However, the results should still help to elucidate the neural substrates of fibromyalgia and CES-induced neural plasticity.

4) White matter disease has the potential to effect connectivity. In order to minimize these structural changes from aging, we have limited our age range to veterans <60 years old. Further, any veterans who are found to have significant leukoaraiosis on T2/FLAIR will be excluded from the study and their primary care provider will be contacted for further evaluation of white matter disease.

5) The active CES device and sham device are indistinguishable in terms of placement and exterior appearance. However, the sham device will not deliver electrical stimulation. To ensure adequate blinding, subjects will be surveyed at end of study to determine whether they *believe* they received active treatment.

Potential Limitations

1) Possibility of Active Placebo: Pressure of the ear is similar to auricular acupressure and may result in some effects on pain and the limbic system, thus serving as an active placebo. However, should no difference be found between sham and active CES, it would support the hypothesis that auricular acupuncture (or another modality similar to sham CES) could provide similar results to CES delivered via alpha-stim.

2) Possibility of Non-CES Therapies Confounding: In the interest of study participant comfort and compliance, these subjects suffering from chronic pain will be allowed to continue their previously initiated pain medication regimen, unless an increase or decrease in analgesic consumption is indicated by the patient's pain and functional measures. In prior studies, pregabalin decreased insula-DMN connectivity, correspondent with decreasing pain scores.[37] Thus, these pharmaceutical agents act as confounders in their ability to alter cross-network connectivity, though they may do so in a different manner than our neuromodulatory technique. Because of the possibility of confounding, analgesic consumption will be carefully monitored and documented throughout the study. Documentation of cognitive behavioral therapy, acupuncture, and other techniques will also be performed, as these non-pharmacological techniques may exhibit synergistic effects with CES to induce neural plasticity and offer another avenue for further research and exploration.

Future Directions

My primary goal is to obtain proficiency in using rs-fcMRI techniques for the study of chronic pain supported by the CVNR Neuroimaging Core so that I will have an additional, advanced tool for evaluating chronic pain patients and pain treatments in future studies. This tool is becoming accepted within the field of pain research as the most objective measure currently available for assessing the experience of pain. Recently, several research funding announcements from the NIH have called for an evaluation of biomarkers for pain, including neuroimaging techniques. Thus, there is a well-known knowledge gap when it comes to adequate biomarkers for the assessment of chronic pain. This especially holds true in the case of fibromyalgia. Current tools are primarily based on self-report and are subject to possible malingering and secondary-gain. Analysis

and interpretation of rs-fcMRI findings is a new skill that I have developed over the course of my CDA-1, and though I have gained an immense amount of knowledge regarding pre-processing steps for rs-fcMRI data, I believe that experience with in-depth data analytic and post-processing techniques are necessary to further my training and increase my ability to communicate and collaborate with other neuroimagers and imaging biostatisticians. When I have obtained more experience with neuroimaging, I intend to further investigate the pathophysiology of fibromyalgia, other pain syndromes and their treatments to better understand the neural correlates of pain and the mechanisms underlying pain and analgesia. If CES provides increased benefit and long-term changes to rs-fcfMRI connectivity, this would support the hypothesis that CES directly modulates a centrally-mediated pain mechanism in fibromyalgia.

When this project is complete, we expect to have better insight into the neural substrates of pain related to fibromyalgia and CES-induced treatment effects. This project has the potential to not only establish CES as a non-pharmacologic treatment for fibromyalgia, but also to further develop rs-fcMRI as a clinical tool to assess pain. Based on the results of the proposed research project, I plan to submit Merit Review proposals:

A) rs-fcMRI as a Biomarker for Treatment Response in Fibromyalgia. In our analysis of rs-fcMRI for CES-induced changes in veterans suffering from fibromyalgia, we may identify differences in rs-fcMRI among treatment responders and non-responders through our Aim 3 exploratory analysis. The ability to identify and separate responders from non-responders using rs-fcMRI will allow us to use pre-treatment rs-fcMRI to gauge whether CES or another neuromodulatory technique (such as transcranial magnetic stimulation) will have a positive effect. Furthermore, rs-fcMRI may help to distinguish or categorize certain sub-types of fibromyalgia, in line with the ideals of personalized, precision medicine.

B) CES-induced Neural Plasticity as a Primer for Biobehavioral Therapies. Cognitive behavioral therapy (CBT) has been shown to induce brain changes related to neural plasticity for a variety of disorders, including social anxiety disorder, chronic fatigue syndrome, and chronic pain. Gray matter volumes in the prefrontal cortex have been specifically implicated in chronic fatigue syndrome and chronic pain, which both share overlap with fibromyalgia syndrome, and CBT has been shown to slow gray matter loss in patients with fibromyalgia and insomnia.[63] Should our results reveal that CES does indeed induce neural plasticity, it would be worthwhile to explore whether CES increases treatment response and neural plasticity related to CBT for chronic pain.

C) CES-induced Gray Matter Changes. Because gray matter (GM) abnormalities have also been identified in subjects with fibromyalgia, we plan to evaluate whether CES rectifies these GM changes in an exploratory analysis using data from the proposed investigation for further hypothesis generation. Using previously reported discrete volumes of interest (VOIs) identified in fibromyalgia subjects,[45,64,65] we will create masks of the VOI's and apply them to our segmented data. Areas previously identified to exhibit significant gray matter reductions in fibromyalgia patients include medial frontal and insular cortices, cingulate and parahippocampal gyri, among others. This has been identified as a high priority area of research by the American Fibromyalgia Society.

D) Evaluation of DMN Stimulation on SMN/SN. Our current proposal evaluates altered connectivity between the DMN and SMN/SN nodes. To evaluate the proposed causal pathway that CES-induced pain relief is accomplished through changes in the DMN resulting in an effect on the SMN or SN, we will propose direct stimulation using rTMS or tDCS on specific nodes of the DMN and evaluate changes in the SMN and SN, as well as in clinical outcomes.

REFERENCES

1. Woodbury A. Opioids for nonmalignant chronic pain. *AMA J Ethics.* 2015;17(3):202-208.
2. Taylor AG, Anderson JG, Riedel SL, Lewis JE, Bourguignon C. A randomized, controlled, double-blind pilot study of the effects of cranial electrical stimulation on activity in brain pain processing regions in individuals with fibromyalgia. *Explore (NY).* 2013;9(1):32-40.
3. Hedegaard H, Warner M, Minino AM. Drug Overdose Deaths in the United States, 1999-2016. *NCHS Data Brief.* 2017(294):1-8.
4. *Opioid Prescribing to High-Risk Veterans Receiving VA Purchased Care.* Washington, D.C. July 31, 2017 2017. 17-01846-316.
5. Heidari F, Afshari M, Moosazadeh M. Prevalence of fibromyalgia in general population and patients, a systematic review and meta-analysis. *Rheumatol Int.* 2017;37(9):1527-1539.

6. Berger A, Sadosky A, Dukes EM, Edelsberg J, Zlateva G, Oster G. Patterns of healthcare utilization and cost in patients with newly diagnosed fibromyalgia. *Am J Manag Care.* 2010;16(5 Suppl):S126-137.
7. Quintner J, Cohen M. Economic cost and epidemiological characteristics of fibromyalgia. *J Rheumatol.* 2004;31(11):2311; author reply 2311.
8. Martinez-Lavin M, Hermosillo AG. Dysautonomia in Gulf War syndrome and in fibromyalgia. *Am J Med.* 2005;118(4):446.
9. Petersel DL, Dror V, Cheung R. Central amplification and fibromyalgia: disorder of pain processing. *J Neurosci Res.* 2011;89(1):29-34.
10. Hawkins RA. Fibromyalgia: a clinical update. *J Am Osteopath Assoc.* 2013;113(9):680-689.
11. Mohanty AF, McAndrew LM, Helmer D, Samore MH, Gundlapalli AV. Chronic Multisymptom Illness Among Iraq/Afghanistan-Deployed US Veterans and Their Healthcare Utilization Within the Veterans Health Administration. *J Gen Intern Med.* 2018;33(9):1419-1422.
12. DeBeer BB, Davidson D, Meyer EC, Kimbrel NA, Gulliver SB, Morissette SB. The Association Between Toxic Exposures and Chronic Multisymptom Illness in Veterans of the Wars of Iraq and Afghanistan. *J Occup Environ Med.* 2017;59(1):54-60.
13. McAndrew LM, Helmer DA, Phillips LA, Chandler HK, Ray K, Quigley KS. Iraq and Afghanistan Veterans report symptoms consistent with chronic multisymptom illness one year after deployment. *J Rehabil Res Dev.* 2016;53(1):59-70.
14. Mohanty AF, Muthukutty A, Carter ME, et al. Chronic multisymptom illness among female Veterans deployed to Iraq and Afghanistan. *Med Care.* 2015;53(4 Suppl 1):S143-148.
15. Smith TC, Powell TM, Jacobson IG, et al. Chronic multisymptom illness: a comparison of Iraq and Afghanistan deployers with veterans of the 1991 Gulf War. *Am J Epidemiol.* 2014;180(12):1176-1187.
16. Oberholzer B, Hoigne R, Hartmann K, et al. [Incidence of drug side effects by symptoms and syndromes. From the experiences of the Comprehensive Hospital Drug Monitoring and the Swiss Drug Side Effect Center. As an example: allergic and pseudo-allergic reactions with mild analgesics and NSAID]. *Ther Umsch.* 1993;50(1):13-19.
17. Perry R, Leach V, Davies P, Penfold C, Ness A, Churchill R. An overview of systematic reviews of complementary and alternative therapies for fibromyalgia using both AMSTAR and ROBIS as quality assessment tools. *Syst Rev.* 2017;6(1):97.
18. Hassett AL, Aquino JK, Ilgen MA. The risk of suicide mortality in chronic pain patients. *Curr Pain Headache Rep.* 2014;18(8):436.
19. Stenager E, Christiansen E, Handberg G, Jensen B. Suicide attempts in chronic pain patients. A register-based study. *Scand J Pain.* 2014;5(1):4-7.
20. Feusner JD, Madsen S, Moody TD, et al. Effects of cranial electrotherapy stimulation on resting state brain activity. *Brain Behav.* 2012;2(3):211-220.
21. Schroeder MJ, Barr RE. Quantitative analysis of the electroencephalogram during cranial electrotherapy stimulation. *Clin Neurophysiol.* 2001;112(11):2075-2083.
22. Taylor AG, Anderson JG, Riedel SL, Lewis JE, Kinser PA, Bourguignon C. Cranial electrical stimulation improves symptoms and functional status in individuals with fibromyalgia. *Pain Manag Nurs.* 2013;14(4):327-335.
23. Lichtbroun AS, Raicer MM, Smith RB. The treatment of fibromyalgia with cranial electrotherapy stimulation. *J Clin Rheumatol.* 2001;7(2):72-78; discussion 78.
24. Shekelle PG, Cook IA, Miake-Lye IM, Booth MS, Beroes JM, Mak S. Benefits and Harms of Cranial Electrical Stimulation for Chronic Painful Conditions, Depression, Anxiety, and Insomnia: A Systematic Review. *Ann Intern Med.* 2018;168(6):414-421.
25. Tuck NL, Johnson MH, Bean DJ. You'd Better Believe It: The Conceptual and Practical Challenges of Assessing Malingering in Patients With Chronic Pain. *J Pain.* 2018.
26. Morley S. Psychology of pain. *Br J Anaesth.* 2008;101(1):25-31.

27. Chang L, Cooper MS, Clark VP. Imaging biomarkers and the role of neuroinflammation in neuropathic pain. *J Neuroimmune Pharmacol.* 2013;8(3):448-451.
28. Somers B. Neuroimaging Evidence: A Solution to the Problem of Proving Pain and Suffering? *Seattle University Law Review.* 2016;39(4):1391-1410.
29. Lee MC, Tracey I. Imaging pain: a potent means for investigating pain mechanisms in patients. *Br J Anaesth.* 2013;111(1):64-72.
30. Napadow V, Harris RE. What has functional connectivity and chemical neuroimaging in fibromyalgia taught us about the mechanisms and management of 'centralized' pain? *Arthritis Res Ther.* 2014;16(5):425.
31. Hemington KS, Wu Q, Kucyi A, Inman RD, Davis KD. Abnormal cross-network functional connectivity in chronic pain and its association with clinical symptoms. *Brain Struct Funct.* 2016;221(8):4203-4219.
32. Ichesco E, Schmidt-Wilcke T, Bhavsar R, et al. Altered resting state connectivity of the insular cortex in individuals with fibromyalgia. *J Pain.* 2014;15(8):815-826 e811.
33. Kim J, Loggia ML, Cahalan CM, et al. The somatosensory link in fibromyalgia: functional connectivity of the primary somatosensory cortex is altered by sustained pain and is associated with clinical/autonomic dysfunction. *Arthritis Rheumatol.* 2015;67(5):1395-1405.
34. Napadow V, LaCount L, Park K, As-Sanie S, Clauw DJ, Harris RE. Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. *Arthritis Rheum.* 2010;62(8):2545-2555.
35. Valet M, Sprenger T, Boecker H, et al. Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain--an fMRI analysis. *Pain.* 2004;109(3):399-408.
36. Tracey I, Ploghaus A, Gati JS, et al. Imaging attentional modulation of pain in the periaqueductal gray in humans. *J Neurosci.* 2002;22(7):2748-2752.
37. Harris RE, Napadow V, Huggins JP, et al. Pregabalin rectifies aberrant brain chemistry, connectivity, and functional response in chronic pain patients. *Anesthesiology.* 2013;119(6):1453-1464.
38. Schmidt-Wilcke T, Ichesco E, Hampson JP, et al. Resting state connectivity correlates with drug and placebo response in fibromyalgia patients. *Neuroimage Clin.* 2014;6:252-261.
39. J. Lee IM, J. Kim, M. Loggia, A. Ortiz, C. Jung, S. Chan, J. Gerber, R. Edwards, A. Wasan, V. Schmithorst, C. Berna, J. Kong, T. Kaptchuk, R. Gollub, B. Rosen and V. Napadow Machine learning-based prediction of clinical pain using multimodal neuroimaging and autonomic metrics. *Pain.* 2018;in press.
40. Dhond RP, Yeh C, Park K, Kettner N, Napadow V. Acupuncture modulates resting state connectivity in default and sensorimotor brain networks. *Pain.* 2008;136(3):407-418.
41. Napadow V, Kim J, Clauw DJ, Harris RE. Decreased intrinsic brain connectivity is associated with reduced clinical pain in fibromyalgia. *Arthritis Rheum.* 2012;64(7):2398-2403.
42. Sundermann B, Burgmer M, Pogatzki-Zahn E, et al. Diagnostic classification based on functional connectivity in chronic pain: model optimization in fibromyalgia and rheumatoid arthritis. *Acad Radiol.* 2014;21(3):369-377.
43. Shi R, Guo Y. Investigating Differences in Brain Functional Networks Using Hierarchical Covariate-Adjusted Independent Component Analysis. *Ann Appl Stat.* 2016;10(4):1930-1957.
44. Cifre I, Sitges C, Fraiman D, et al. Disrupted functional connectivity of the pain network in fibromyalgia. *Psychosom Med.* 2012;74(1):55-62.
45. Puiu T, Kairys AE, Pauer L, et al. Association of Alterations in Gray Matter Volume With Reduced Evoked-Pain Connectivity Following Short-Term Administration of Pregabalin in Patients With Fibromyalgia. *Arthritis Rheumatol.* 2016;68(6):1511-1521.
46. Fallon N, Chiu Y, Nurmikko T, Stancak A. Functional Connectivity with the Default Mode Network Is Altered in Fibromyalgia Patients. *PLoS One.* 2016;11(7):e0159198.
47. Bingel U, Lorenz J, Glauche V, et al. Somatotopic organization of human somatosensory cortices for pain: a single trial fMRI study. *Neuroimage.* 2004;23(1):224-232.

48. Mayka MA, Corcos DM, Leurgans SE, Vaillancourt DE. Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. *Neuroimage.* 2006;31(4):1453-1474.
49. Wager TD, Atlas LY, Botvinick MM, et al. Pain in the ACC? *Proc Natl Acad Sci U S A.* 2016;113(18):E2474-2475.
50. Oishi K, Zilles K, Amunts K, et al. Human brain white matter atlas: identification and assignment of common anatomical structures in superficial white matter. *Neuroimage.* 2008;43(3):447-457.
51. Lukemire J, Wang, Y., Verma, A. and Guo, Y. HINT: A Hierarchical Independent Component Analysis Toolbox for Investigating Brain Functional Networks using Neuroimaging Data. *arXiv : http://arxivorg/abs/180307587.* 2018.
52. Mease PJ, Spaeth M, Clauw DJ, et al. Estimation of minimum clinically important difference for pain in fibromyalgia. *Arthritis Care Res (Hoboken).* 2011;63(6):821-826.
53. Wang Y, Kang J, Kemmer PB, Guo Y. An Efficient and Reliable Statistical Method for Estimating Functional Connectivity in Large Scale Brain Networks Using Partial Correlation. *Front Neurosci.* 2016;10:123.
54. Wolfe F, Clauw DJ, Fitzcharles MA, et al. 2016 Revisions to the 2010/2011 fibromyalgia diagnostic criteria. *Semin Arthritis Rheum.* 2016;46(3):319-329.
55. Bartres-Faz D, Clemente IC, Junque C. [White matter changes and cognitive performance in aging]. *Rev Neurol.* 2001;33(4):347-353.
56. Arenaza-Urquijo EM, Bosch B, Sala-Llonch R, et al. Specific anatomic associations between white matter integrity and cognitive reserve in normal and cognitively impaired elders. *Am J Geriatr Psychiatry.* 2011;19(1):33-42.
57. Buckenmaier CC, 3rd, Galloway KT, Polomano RC, McDuffie M, Kwon N, Gallagher RM. Preliminary validation of the Defense and Veterans Pain Rating Scale (DVPRS) in a military population. *Pain Med.* 2013;14(1):110-123.
58. Birn RM, Diamond JB, Smith MA, Bandettini PA. Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI. *Neuroimage.* 2006;31(4):1536-1548.
59. Chang C, Glover GH. Effects of model-based physiological noise correction on default mode network anti-correlations and correlations. *Neuroimage.* 2009;47(4):1448-1459.
60. Zucker NA, Tsodikov A, Mist SD, Cina S, Napadow V, Harris RE. Evoked Pressure Pain Sensitivity Is Associated with Differential Analgesic Response to Verum and Sham Acupuncture in Fibromyalgia. *Pain Med.* 2017;18(8):1582-1592.
61. Feis RA, Smith SM, Filippini N, et al. ICA-based artifact removal diminishes scan site differences in multi-center resting-state fMRI. *Front Neurosci.* 2015;9:395.
62. Fox MD, Halko MA, Eldaief MC, Pascual-Leone A. Measuring and manipulating brain connectivity with resting state functional connectivity magnetic resonance imaging (fcMRI) and transcranial magnetic stimulation (TMS). *Neuroimage.* 2012;62(4):2232-2243.
63. McCrae CS, Mundt JM, Curtis AF, et al. Gray Matter Changes Following Cognitive Behavioral Therapy for Patients With Comorbid Fibromyalgia and Insomnia: A Pilot Study. *J Clin Sleep Med.* 2018;14(9):1595-1603.
64. Staud R, Craggs JG, Perlstein WM, Robinson ME, Price DD. Brain activity associated with slow temporal summation of C-fiber evoked pain in fibromyalgia patients and healthy controls. *Eur J Pain.* 2008;12(8):1078-1089.
65. Shi H, Yuan C, Dai Z, Ma H, Sheng L. Gray matter abnormalities associated with fibromyalgia: A meta-analysis of voxel-based morphometric studies. *Semin Arthritis Rheum.* 2016;46(3):330-337.