Neuroimaging Approaches to Deconstructing Acupuncture for Chronic Pain

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1. STUDY TEAM ROSTER

1.1. University of Michigan

Richard Harris, PhD	Co-Principal Investigator
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Daniel Harper, PhD	Co-Investigator
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Kathy Scott, RN	Project Manager
Ryan Scott, MPH	Study Coordinator
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Location of Study Site:

Clinic Visits

Chronic Pain and Fatigue Research Center
Lobby M, Domino Farms
24 Frank Lloyd Wright Drive
Ann Arbor, MI

MRI Scans

University of Michigan Hospital and Health Systems
MRI Laboratory
1500 Medical Center Drive
Ann Arbor, MI

1.2. Massachusetts General Hospital

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2. SYNOPSIS

Study Title	Neuroimaging Approaches to Deconstructing Acupuncture for Chronic Pain
Study Sponsor	National Institutes of Health, National Center for Complementary and Alternative Medicine Grant Number: R01AT007550
Study Objective	To evaluate the impact of acupuncture-induced somatosensory afference on altered neurobiology and analgesia in FM.
Study Design	This study design has two components: 1) a cross sectional assessment of brain chemistry, connectivity and response to pain in healthy controls and age- and sex-matched fibromyalgia patients, and 2) a longitudinal assessment of the same fibromyalgia patients randomized to either electro-acupuncture (EA) or mock laser acupuncture (ML).
Study Interventions	Electro acupuncture and mock laser acupuncture. Treatments will be delivered at 2x/week for 4 weeks, for a total of 8 treatments.
Study Duration	The study duration for healthy controls will be 1 week, with a 4 week screening window. The study duration for fibromyalgia patients will be up to 10 weeks: 4- week screening window prior to baseline; 1-week of baseline, 4 week treatment period, and 1 week of follow up.
Sample Size and Population	We will evaluate 40 healthy controls and 80 fibromyalgia patients (n=40 EA and n=40 ML). Demographics will be representative of the population of southeastern Michigan.
Statistical Analyses	Cross-sectional analyses will include a comparison of FM patients with HC with respect to GABA, Glx and other neuroimaging biomarkers (t-tests, linear regression); Longitudinal analysis will study treatment effects on within-patient change in neuroimaging biomarkers and changes in clinical pain (t-tests, linear regression with change as a response, linear mixed model with biomarker as a response); treatment and biomarker predictive effects at baseline on clinically relevant 50% reduction of pain as a binary response (logistic regression); the effects of treatment and biomarker baseline measurements on within-patient change in DMN-insula connectivity; biomarker mediation analysis for the treatment effect. In order to screen neuroimaging biomarkers that respond to treatment (show reduced DMN-insula connectivity), interim testing for futility will be performed. Reasons for missingness will be analyzed using multinomial logistic regression with treatment as one of the covariates. The results of formal missing data imputation by predictive-matching algorithms will be compared to the results of missing data exclusion in a sensitivity analysis.

3. SCHEDULE OF EVALUATIONS

3.1. Fibromyalgia Cohort

	Screening	**Behavioral Session**	**Baseline MRI**	**Treatment Period**	**Behavioral Session**	**Follow-Up MRI**
	Visit 0 *Day -30 to 0*	*Visit 1* *Day 0*	*Visit 2* *Day 1-3*	*Visit 3-10* *Day 4-32*	*Visit 11* *Day 33-40*	*Visit 12* *Day 34-43*
Informed Consent	X					
Tender Point (1990 ACR FM Criteria)	X					
FM Survey Questionnaire (FSQ) Wolfe et al 2011 criteria for FM	X	X			X	
VAS – Pain *(7day recall)*	X					
FM Treatment History	X					
Medical History	X					
Vitals (Height/Weight)	X					
Urine Pregnancy Test	X	X	*a*		X	*a*
HADS	X				X	
PHQ-9 (suicidal ideation)	X	*c*		*c*	X	
Demographics	X					
Socio-demographics	X					
fMRI Screening Form		X			X	
Concomitant Medications	X	X	X	X	X	X
BPI – Severity/Interference	X	X	X		X	X
PROMIS – 29		X			X	
Perceptions of Bodily Sensations		X			X	
Pain Catastrophizing		X			X	
Credibility Scale				X	X	
PainDETECT		X			X	
Desire of Relief (DRF)		X			X	
Expected Relief Scale (ERS)		X				
MASQ (Cognition)		X			X	
Pittsburgh Sleep Quality Index (PSQI)		X			X	
VAS - Present Pain		X	X		X	X
Menstrual questionnaire		X			X	
Pain Assessment/Visual Stimulation Assessment	*b*	X			X	
After Cuff Questionnaire		X	X		X	X

Conditioned Pain Modulation (CPM)	*b*	X			X	
Neuroimaging ♦ Structural MRI ♦ Resting fcMRI ♦ Evoked Pain Test ♦ CPM (cuff) ^{1}H-MRS ♦ Visual Stimulation			X			X
Randomization				X		
Treatment (EA / ML)				X		
Needling Sensations (MASS)				X		
Fibromyalgia Impact Questionnaire (FIQ-R)	X	X		X	X	
Harvard Food Diary (FFQ)		X				
Adverse Events		X	X	X	X	X
Debriefing						X

[a] *If not performed at behavioral session*
[b] *Familiarization Only*
[c] *Additional Monitoring for PHQ9 scores (10 – 14) – baseline behavioral and Treatment #3*

3.2. Controls

	Screening	**Behavioral Session**	**Baseline MRI**
	Visit 0 *Day -30 to -0*	*Visit 1* *Day 0*	*Visit 2* *Day 1-3*
Informed Consent	X		
Tender Point (1990 ACR FM Criteria)	X		
FM Survey Questionnaire (FSQ) Wolfe et al 2011 criteria for FM	X	X	
VAS – Pain *(7-day recall)*	X		
Medical History	X		
Vitals (Height/Weight)	X		
Urine Pregnancy Test	X	X	*a*
HADS	X		
PHQ-9 (suicidal ideation)	X	*c*	
Demographics	X		
Socio-demographics	X		
fMRI Screening Form		X	
Concomitant Medications	X	X	X
BPI – Severity/Interference	X	X	X
PROMIS – 29		X	
Perceptions of Bodily Sensations		X	
Pain Catastrophizing		X	
Credibility Scale			
PainDETECT		X	
Desire of Relief (DRF)			
Expected Relief Scale (ERS)			
MASQ (Cognition)		X	
Pittsburgh Sleep Quality Index (PSQI)		X	
VAS - Present Pain		X	X
Menstrual questionnaire		X	
Harvard Food Diary (FFQ)		X	
Pain Assessment/Visual Stimulation Assessment	*b*	X	
After Cuff Questionnaire		X	X

Conditioned Pain Modulation (CPM)	*b*	X	
Neuroimaging ♦ Structural MRI ♦ Resting fcMRI ♦ Evoked Pain Test ♦ CPM (cuff) ^{1}H-MRS ♦ Visual Stimulation Assessment			X
Randomization			
Treatment (EA / ML)			
Needling Sensations (MASS)			
Adverse Events		X	X
Debriefing			

[a] *If not performed at behavioral session*
[b] *Familiarization Only*
[c] *Additional Monitoring for PHQ9 scores (10 – 14) – baseline behavioral*

4. TABLE OF CONTENTS

5. OBJECTIVES

Several recent meta-analyses of acupuncture for chronic pain have shown that verum acupuncture is not superior to sham/placebo acupuncture [1, 2]. However, these same meta-analyses have also noted significant heterogeneity in acupuncture methods across trials, which complicates combining results. For example, while electro-acupuncture (EA) was superior to sham acupuncture in relieving pain for fibromyalgia (FM), manual acupuncture (MA) was not [1]. In terms of physiologic action, EA and MA are very different procedures. They provide somatosensory afference over very different durations of time, effect different peripheral targets, induce different psychophysical perceptions [3], and manifest in different brain response [3, 4]. Somatosensory afference may be a key variable for acupuncture analgesia as some sham needling devices (e.g. the Streitberger needle [5]) have been criticized as they also produce somatosensation [6]. Moreover, somatosensation may be a particularly important variable for FM, as this chronic pain disorder is known to display altered sensory and nociceptive amplification [7]. In fact, our recent neuroimaging studies on FM have demonstrated enhanced excitatory and reduced inhibitory neurotransmitter levels in the insula [8, 9], as well as increased resting, or intrinsic connectivity between the insula and the brain's default mode network (DMN) [10]. Thus, we hypothesize that EA has been more effective for FM because it functions as a desensitization therapy, which when applied repeatedly over multiple treatment sessions, gradually habituates the nervous system to continuing endogenous pain and somatosensory signaling.

The overall goal of this study is to evaluate the impact of acupuncture-induced somatosensory afference on altered neurobiology and analgesia in FM.

To test our hypotheses, we will incorporate neuroimaging into a randomized, blinded, longitudinal trial in FM using acupuncture interventions with significantly different amounts of somatosensory afference: (1) EA, and (2) mock laser (ML) acupuncture - a relatively new form of sham acupuncture that does not induce any somatosensory afference. Neuroimaging outcomes will probe pain-related brain neurobiology, and will include (1) proton magnetic resonance spectroscopy (^{1}H-MRS) measures of combined glutamate and glutamine (Glx) and γ-aminobutyric acid (GABA), (2) intrinsic resting brain connectivity (resting functional magnetic resonance imaging, fcMRI) in distinct networks associated with somatosensory processing and chronic pain, and (3) fMRI measures for central amplification of evoked pain and evoked pain modulation (i.e. conditioned pain modulation). Healthy controls (HCs) will be evaluated with all measures to determine the degree of altered brain physiology at baseline in our cohort of FM patients.

- **Aim 1. At baseline, characterize the altered somatosensory-related neurocircuitry underlying chronic pain in FM.**
  - **Hypothesis 1:** Using resting fcMRI, FM patients, as compared to HCs, will show greater insula connectivity to both default mode network (DMN, positive correlation) and sensorimotor network (SMN, anti-correlation).
  - **Hypothesis 2:** Using ^{1}H-MRS, FM patients will show pain related increased Glx and reduced levels of GABA within the insula as compared to HCs.
  - **Hypothesis 3:** Using evoked pain fMRI, FM patients will be more sensitive to evoked pressure pain and demonstrate greater insula activation to pain stimuli, compared to HC.

- **Aim 2. Evaluate longitudinal desensitization effects of electro- and mock laser acupuncture interventions on the neurocircuitry underlying chronic pain in FM, and correlate the change in pain with changes in neurocircuitry physiology.**
  - **Hypothesis 1:** EA, which involves greater somatosensory afference, will more readily reduce resting insula connectivity to somatosensory and pain related networks (SMN, DMN) compared

to ML, which will not reduce this resting connectivity. The change in pain will correlate with change in insula connectivity for EA, but not ML.

- **Hypothesis 2:** EA will more readily reduce Glx and increase GABA in the insula as compared to ML, which will not reduce Glx and GABA. The change in pain will correlate with change in insula GABA and Glx for EA but not ML.
- **Hypothesis 3:** EA will more readily reduce pain-evoked fMRI activation in the insula as compared to ML, which will not reduce pain-evoked fMRI activation. The change in pain will correlate with change in insula response to evoked pain for EA but not ML.

- **Aim 3. Evaluate the ability of altered neurocircuitry at baseline to predict clinical response to electro- and mock laser acupuncture**
  - ***Hypothesis 1:*** FM patients with greater baseline DMN-insula positive correlation and SMN-insula anti-correlation will display greater clinical response to EA. There will be no predictive value for ML – which is an inert form of sham acupuncture with no somatosensory afference.
  - ***Hypothesis 2:*** FM patients with greater baseline insular Glx/GABA ratios will display greater clinical response to EA. There will be no predictive value for ML.
  - ***Hypothesis 3:*** FM patients with greater baseline pain-evoked insular activation will display greater clinical response to EA. There will again be no predictive value for ML.

6. BACKGROUND AND RATIONALE

6.1. Background

Acupuncture research for chronic pain is at a crossroads. Effectiveness has been documented for many refractory chronic pain ailments, but the *specific* component of acupuncture therapy is unknown and sham acupuncture procedures, which are commonly used to control for placebo effects, have yielded near equivalent clinical results in many trials [11-15]. We propose that some of the confusion stems from altered somatosensory components across verum acupuncture methods as well as various forms of sham controls. Specifically, while both manual and electro-acupuncture involve needle insertion, the somatosensory afference afforded by these interventions is markedly different. Additionally, "placebo" control procedures such as "minimal acupuncture," which uses real acupuncture needles but with only shallow insertions [6], and "sham acupuncture" with non-insertive devices which have telescoping blunt-tipped needles that simulate insertion by disappearing into the handle (e.g. Streitberger needle [5, 16]), also involve somatosensory afference. This somatosensory afference, coupled with the acupuncture ritual, may inadvertently retain some of the specific component of real acupuncture [6]. We propose that neuroimaging in fibromyalgia (FM) patients, which display altered somatosensory neurocircuitry, will help elucidate *why* different forms of acupuncture can produce analgesia by teasing apart the different mechanisms of action underlying different components of this therapy: electrical (EA) versus sham acupuncture(ML) without somatosensation.

Our study will set the stage for future studies to explore specific mechanisms driving the linkage from brain response during treatment to eventual neuroplastic change over several months, in both animal and human models. Our study will also explore the role of heightened pain sensitivity and altered neurocircuitry in FM and how this predicts treatment response to acupuncture interventions with varying somatosensory afference. Such analyses will provide much needed information about which patients might benefit most from which types of acupuncture interventions.

6.2. Rationale

6.2.1. Need for a neurobiological model underlying acupuncture analgesia for FM

Our neurobiological model proposes that somatosensory afference imparted by different acupuncture procedures differentially modulates neuroimaging biomarkers that we have previously found to track with clinical pain. EA involves a unique ritual of deep somatosensory afference and, hence targeted somatic focus whereas ML does not. These different aspects (needle insertion/manipulation and electrical stimulation) may modulate pain

through differential modulation of (1) resting state brain networks such as the default mode network (DMN) and sensorimotor network (SMN), which are both modulated by pain and acupuncture [4, 17], and (2) Glx and GABA concentration in the insula.

If our hypotheses are proven true, *1.)* critical information will be provided to guide clinical decisions on whether and how to incorporate acupuncture into mainstream management of FM, 2.) individual differences in baseline neuroimaging markers can be used to predict analgesic response, 3.) our model would support combining acupuncture with other mechanistically well-understood interventions that may work synergistically with acupuncture to reduce pain, and 4) better understanding of acupuncture specific effects would aid development of more appropriate controls (e.g. ML).

6.2.2. Fibromyalgia (FM) is a common chronic pain syndrome and a major public health issue

Fibromyalgia is the second most common rheumatologic disorder, behind osteoarthritis, with 2 - 4% of the populations of industrialized countries affected [18-20]. Overall, it is estimated that FM costs the American taxpayers over $20 billion a year in lost wages and disability [21]. In part this burden on the US health care system is our lack of understanding of the specific pathophysiology of the disorder. While research suggests that FM is a central non-nociceptive pain syndrome [22-24], it is uncertain if the observed neurobiological outcomes are causally related to development of FM. Our study will provide much needed information into the pathology of this disorder. Moreover our data will synergize with emerging data indicating a generalized disturbance in central nervous system pain processing, which leads individuals to sense pain throughout the body in the absence of inflammatory or patho-anatomic damage [25, 26].

6.2.3. Acupuncture for FM and the role of somatosensation for both real and sham acupuncture

Complementary and alternative medicine (CAM) has been a popular option for treating FM, and many patients perceive a great deal of benefit of CAM for their pain [27]. However, for chronic pain in general [2], and FM specifically, multiple well powered clinical trials have found that real acupuncture demonstrates only minimal additional analgesia over sham acupuncture consisting of needling at non-acupoints, or even non-insertive tactile pressing on the skin [14, 15]. One explanation is that acupuncture does not have benefit beyond non-specific placebo effects. However, different acupuncture methods have shown differential efficacy for FM and a recent meta-analyses reports greater efficacy of EA than manual acupuncture [1], supported by two well designed and powered trials showing a significant effect on pain for EA [28, 29]. EA, which involves passing electric current across multiple pairs of acupuncture needles, evokes a different sensation from manual acupuncture [3], and imparts somatosensation continuously over the course of treatment. Conversely, manual acupuncture typically imparts somatosensation only at the beginning of the treatment, when needles are inserted and briefly manipulated, and only occasionally thereafter, over the course of the treatment session. These differences complicate combining results from EA and manual acupuncture trials in meta-analyses aimed at assessing acupuncture efficacy for FM.

As the specific effects of real acupuncture are not yet defined, current sham acupuncture designs meant to represent an inert placebo may also inadvertently incorporate acupuncture-specific effects [6]. For example, Wu et al. demonstrated robust brain response in pain-matrix brain regions (e.g. insula, ACC, posterior parietal) to a form of acupuncture needle stimulation dubbed “minimal acupuncture” [30]. This form of sham acupuncture has actually been used as a placebo control in previous randomized controlled trials (RCTs) [31, 32]. Somatosensation may play an important role in the efficacy of many commonly used placebo controls, as the acupuncturist palpates for the correct location and then simulates or actually inserts a needle. Both palpation and “insertion” stimulate cutaneous receptors. Interestingly, inordinately high “placebo” response for sham acupuncture is supported by a recent meta-analysis [33] and large RCT (n=270), which showed that sham acupuncture provided greater analgesia than a placebo pill for chronic pain [34], confirming earlier much smaller studies [35, 36]. Importantly, both real and sham acupuncture produce analgesia in FM comparable to usual care and FDA approved pharmacological agents [14, 15, 28, 29, 37-40]. ***Thus while we know that acupuncture relieves FM, we don’t know the specific effect.***

Our hypotheses suggests that this specific effect involves somatosensation. Somatosensory afference is part of most sham acupuncture designs, as they retain acupoint palpation and a tactile stimulus mimicking real acupuncture needle insertion. The induced somatosensation directs attention to specific body regions, and this somatic focus is reinforced with every (sham) acupuncture treatment. Such targeted somatic focus, when coupled with expectation, has been shown to produce target-specific endorphin-mediated placebo analgesia [41].

Furthermore, greater somatic focus has been shown to heighten placebo response [42]. The role of somatic focus in sham acupuncture has been hypothesized [43], but never formally tested. While the mechanisms behind how somatic focus might lead to beneficial clinical effects are unknown, one possibility is that repeated directed attention to the soma (body), will lead to a better subjective internalization of the patient's own body schema [44] and enhance their perception of bodily sensations. Thus we investigate the specific effect of somatosensation in sham acupuncture controls by incorporating mock laser acupuncture (ML), an intervention devoid of tactile sensation but retains acupuncture context and ritual.

6.2.4. Neuroimaging markers supporting a neurobiological model for acupuncture efficacy in FM

Aim 1 will evaluate not just the neurocircuitry underlying experimental pain hyperalgesia in FM (e.g. insula, cingulate, SMA, S1, S2, prefrontal cortex), supported by previous studies [22, 45], but also neuroimaging correlates for *clinical pain*. Specifically, we will evaluate resting (intrinsic) brain connectivity and neurotransmitter levels of patients' clinical FM pain at rest (see Approach). *Multiple neuroimaging approaches are necessary to evaluate the neurocircuitry underlying clinical and experimental pain, as they are sensitive to different physiological processes and will complement one another in evaluating the overall pain state*.

Brain imaging correlates of clinical pain have been notoriously difficult to measure [46, 47], and our recent publications provide preliminary evidence that our resting functional connectivity approach does indeed assay the neurocircuitry subserving clinical pain [48]. Resting functional connectivity MRI (fcMRI) is a recent adaptation of fMRI that examines intrinsic connectivity - defined as ongoing neural and metabolic activity that occurs in the resting basal state. Intrinsic brain connectivity may be important for maintenance of synaptic connectivity and as such modulates the efficiency and extent of neuronal transmission between brain regions. Intrinsic connectivity, as measured by neuroimaging methods, follows known structural monosynaptic and polysynaptic pathways [49, 50], likely reflecting meaningful neurophysiological activity [51] within known primary sensory, executive, and associative networks [52]. FcMRI investigations are conducted with subjects simply resting in the scanner. These data can then be analyzed with techniques such as independent component analysis (ICA) and seed-voxel connectivity.

While multiple resting state networks have been identified in healthy subjects [53], our Aims will focus on two cardinal networks known to be related to pain and somatosensory processing: the default mode network (DMN), and sensorimotor network (SMN) (**Figure 1**). The DMN [52, 54] is a constellation of brain regions thought to be engaged in self-referential cognition, which are "deactivated" during various externally focused task conditions. The DMN includes the inferior parietal lobule (IPL), the posterior cingulate cortex (PCC) and precuneus, areas of the medial frontal gyri, the hippocampal formation, and the lateral temporal cortex [55]. Pain is known to influence both DMN response and as well as cognitive capacity. While acute experimental pain

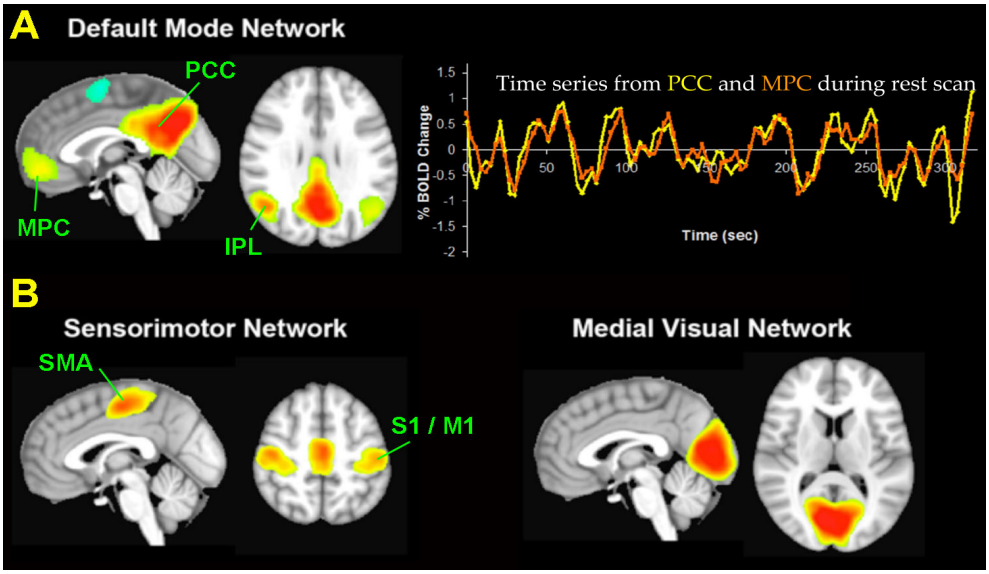


Figure 1 – (A) The default mode network can be identified through connectivity in resting fMRI data (e.g. PCC and MPC). (B) Other resting networks include the sensorimotor and medial visual networks. (adapted from Raichle and Snyder, 2007, and Beckmann et al. 2005)

induces DMN deactivation in healthy subjects [56], chronic back pain is associated with mitigated DMN deactivation to visual attention tasks [57]. Our own group's data in FM found increased correlation between the DMN and insula, a brain region thought to integrate the multiple dimensions of pain [58]. Furthermore, greater clinical pain correlated with greater correlation between the DMN and insula [48] and this connectivity was diminished following a longitudinal course of acupuncture (both MA and sham with somatosensation) [59]. The SMN includes bilateral primary and secondary somatosensory areas (S1 and S2), primary motor cortex (M1), and the SMA [53]. Our data shows that this network is modulated by both real and sham (with somatosensation) acupuncture [60], and that chronic pain patients have increased anti-correlation between the anterior insula and SMN (see preliminary results in Approach). The medial visual network (MVN), which includes bilateral VI in the calcarine sulcus and medial parastriate regions is mainly a primary sensory network for vision, has not been linked to chronic pain, and will be used as a negative control [53].

Proton magnetic resonance spectroscopy (^{1}H-MRS) provides another neuroimaging outcome and is complementary to fcMRI. In ^{1}H-MRS chemical spectra are obtained from volume-image elements or voxels within the human brain using radiofrequencies that excite protons [61]. Specific molecules are identified by their characteristic resonance frequency in the spectrum. Once acquired, spectra are analyzed to determine the relative concentrations of different molecules or CNS metabolites within the voxel or field of interest. Of most importance for this proposal, concentrations of specific neurotransmitters such as glutamate (Glu) and γ-hydroxybutyrate (GABA) can be quantified. Glu and GABA are respectively the major excitatory and inhibitory neurotransmitters of the mammalian CNS. These neurotransmitters both exert their effects via two types of receptors: ionotropic and metabotropic. Ionotropic receptors are ligand-gated ion channels generally involved in fast synaptic transmission, whereas metabotropic receptors are g-protein coupled receptors that are more responsible for neuromodulation.

Our group was the first to use ^{1}H-MRS to study Glu and combined Glu+glutamine (Glx) levels in FM and their response to acupuncture. We showed that FM patients with greater reductions in Glx following acupuncture displayed greater improvements in both clinical and experimental pain [62]. We subsequently went on to show that Glx and Glu levels within the posterior insula are also significantly elevated in FM, as compared to pain-free controls, and that this elevation was associated with evoked pain sensitivity [63]. FM patients may have more Glu within their synaptic vesicles, higher numbers or densities of glutamatergic synapses, or even less reuptake of Glu from the synaptic cleft. All of these could result in enhanced excitatory neurotransmission. This "phenotype" of elevated Glx within the FM brain has been subsequently reported in the amygdala [64], the posterior cingulate [65], and the ventral lateral prefrontal cortex [66], by other groups. Thus it appears that there are multiple loci within the FM brain where elevations in Glx are observed and that this is related to heightened pain expression

The importance of GABA, the brain's major inhibitory neurotransmitter, in pain has also been recognized for some time [67]. GABA receptors are widely distributed in the spinal cord, thalamus, and cortex, both pre- and post-synaptically. Many of the early studies showing proof of concept that GABA plays a critical role in pain transmission involved demonstrating that baclofen, a GABA-B receptor agonist, was effective in both preclinical models of acute and chronic pain [68, 69]. Of relevance to this proposal, the action of GABA specifically within the insula has been shown to modulate pain. Decreases in insular GABA levels exacerbate pain whereas blocking GABA degradation, specifically within the insula, relieves pain [70]. In relationship to chronic pain in humans, we were the first to directly assess the level of GABA within the FM brain [71]. Decreased GABA levels were detected within the insula suggesting a disinhibition of cortical activity (i.e. enhanced activity) within this structure. Patients with lower GABA were also more sensitive to pressure pain. Overall FM patients display dysregulation of excitatory to inhibitory neurotransmitter balance within the insula.

While altered CNS neurotransmitter levels and resting brain connectivity are likely contributing factors for centralized chronic pain, it is unknown how these factors respond specifically to somatosensory afference in FM. We hypothesize that individuals with greater centralized pain may respond preferentially to acupuncture interventions with greater somatosensory afference, a major aim of this proposal. Since Glx and GABA are likely to be involved, we speculate that a neurotransmitter imbalance of Glx/GABA in pain and somatosensory processing regions may be critical in setting the "gain" on FM pain and its subsequent response to acupuncture.

6.3. Innovation and Importance of Research

6.3.1. Novel neuroimaging approach for evaluating the neurocircuitry subserving clinical pain and analgesia following acupuncture: resting (intrinsic) brain connectivity

Innovation in our proposal comes from coupling neuroimaging with clinical pain outcome measures incorporated into a longitudinal treatment trial framework. In addition to evaluating brain response to experimental pain and hyperalgesia, which is a more conventional approach, we will also investigate the neurocircuitry subserving clinical pain using resting functional connectivity. Neuroimaging markers reflecting FM patients' clinical pain are crucial to this longitudinal design, as (a) they conceptually link more closely to important clinical outcome measures, compared to neuroimaging markers for experimental pain hyperalgesia and allodynia, and (b) their differential susceptibility to different forms of therapy may underlie the ultimate mechanisms of action. Recent efforts have been increasingly aimed at developing a neurobiological model for chronic pain, and our proposal adds to this burgeoning field of research [46, 72].

6.3.2. Novel neuroimaging approach for evaluating neurochemical imbalance in the chronic pain brain treated by acupuncture: excitatory (Glx) and inhibitory (GABA) neurotransmitters

Emerging data from our group [62, 63] and others [64-66] suggests an elevation in the excitatory neurotransmitter glutamate within the FM brain. These data add to the growing neurobiology field demonstrating altered brain function and structure within chronic pain [46]. The innovation of ^{1}H-MRS is the addition of key neurochemical information, which is arguably a fundamental factor in the activity of the brain. Our recent work extends our glutamate investigations to also explore the role of the inhibitory neurotransmitter GABA, using a novel spectral editing technique [71]. While a rich literature of basic science studies implicate GABA as a key mediator in chronic pain, the innovation of our data extends basic research findings of GABA to a human chronic pain state. Moreover in this study we also demonstrate for the first time the importance of Glu and GABA in acupuncture for FM by associating changes in these neurotransmitters with successful treatment. This information is impactful as therapies that target specific neurotransmitters could be used in conjunction with imaging to predict which patients could benefit from a specific treatment. For example FM patients with low baseline levels of GABA and high glutamate within the insula may respond variably to interventions with varying somatosensory afference. Information of this type could greatly improve clinical care.

6.3.3. Incorporating an innovative non-tactile sham acupuncture control within a neuroimaging trial

In order to explore the influence of somatosensation in sham acupuncture methods, we will employ mock laser acupuncture (ML) – an intriguing, recently developed sham acupuncture control [73-75], which lacks somatosensation. Verum laser acupuncture has been used as a therapeutic option to stimulate acupoints with low-intensity, non-thermal laser irradiation [76], and is a credible form of acupuncture. ML acupuncture involves the use of a laser device whose irradiation source has been deactivated. Past studies have found ML to be as equally credible as needle acupuncture [75, 77]. In our study, the ML control will be applied while the patient's vision is masked and will retain the ritual of acupuncture, but will not induce specific somatic focus and therefore will not have somatosensory afference. Several groups have successfully used mock laser as a placebo control for needle acupuncture [73, 74], and have demonstrated significantly greater pain reduction following acupuncture (compared to mock laser) in an RCT of chronic neck pain [74].

7. STUDY DESIGN

7.1. Overall Design

Our overall goal is to understand the role somatosensation plays in modulating analgesia and neuroimaging markers related to altered neural processing of pain in FM (Table 1; Figure 2). Eighty (80) FM women patients will be evaluated with MRI at baseline and after acupuncture treatment (electro-acupuncture, EA; mock laser acupuncture, ML; n=40 per group). Treatments will be delivered at 2x/week for 4 weeks, for a total of 8 treatments. An additional 40 healthy age-matched adult women will serve as a control for neuroimaging markers collected from baseline FM patients, and will allow us to assess central pain pathology in our population (Aim 1). The HCs will not be treated with acupuncture since the goal of this investigation is to investigate potential mechanisms for acupuncture in chronic pain patients. In addition to our neuroimaging outcomes, we will also assess clinical and experimental pain outcomes during the study period from all subjects. These data in combination with other outcomes will be used to perform secondary analyses.

Table 1 – Neuroimaging Outcomes

Outcome	*fMRI Protocol*	*Rationale*
Clinical Pain	- resting fcMRI and ^{1}H-MRS	Neuroimaging markers for clinical pain directly reflect our primary clinical outcome. Markers are needed for localized brain neurochemistry and associated network connectivity and their relation to clinical pain.
Experimental Pain	- fMRI with pressure pain and conditioned pain modulation.	Protocols for brain response to experimental pain are more mature than for clinical pain (facilitating comparison to prior research) and allow the assessment of hyperalgesia and allodynia.

7.2. Schema

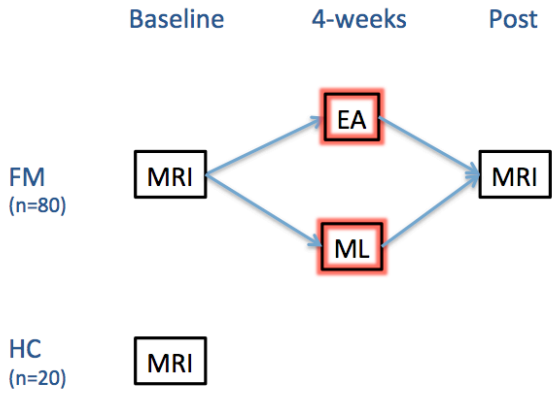


Figure 2– Experiment overview. EA = electro-acupuncture, ML = mock laser "sham" acupuncture (without somatosensation).

8. PATIENT SELECTION AND ENROLLMENT

8.1. Patient Eligibility

8.1.1. Inclusion Criteria

Inclusion Criteria for Fibromyalgia Participants

- Must have self-reported FM symptoms for at least one year and also meet the newly proposed Wolfe et al 2011 criteria for FM. Continued presence of pain more than 50% of days.
- Pain greater than or equal to 4 on a 10cm Visual Analog Scale (VAS) for pain; 7-day recall; [Note: The VAS is widely used in clinical pain research and as such we chose to use it for inclusion criteria and for clinical pain assessment below. Within our group numerical ratings scales 0-100 are more commonly used in quantitative sensory assessment, and as such we chose to use NRS scales for evoked pain assessments below.]
- Willing to limit the introduction of any new medications or treatment modalities for control of FM symptoms during the study.
- Able to travel to the study site to receive acupuncture treatments up to two times weekly.
- Over 18 and under 65 years of age.
- Right-handed.
- Female.
- Capable of giving written informed consent.

Inclusion Criteria for Healthy Control Participants

- Over 18 and under 65 years of age.
- Female.
- Right-handed.
- Pain equals 0 on a 10cm Visual Analog Scale (VAS) for pain; 7-day recall
- Willing to complete all study procedures.
- Capable of giving written informed consent.

8.1.2. Exclusion Criteria

Exclusion Criteria for Fibromyalgia Participants:

- Acupuncture within last 6-months.
- Presence of a known coagulation abnormality, thrombocytopenia, or bleeding diathesis that may preclude the safe use of acupuncture.
- Presence of a concurrent autoimmune or inflammatory disease such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, etc. that causes pain or any other chronic pain condition with pain greater than FM pain.
- History of head injury with substantial loss of consciousness
- Peripheral neuropathy of known cause that interferes with activities of daily living.
- Routine daily use of narcotic analgesics, marijuana, or history of substance abuse.
- Stimulant medications, such as those used to treat ADD/ADHD (e.g., amphetamine/ dextroamphetamine [Adderall®], methylphenidate, dextroamphetamine), or the fatigue associated with sleep apnea or shift work (e.g., modafinil), are excluded.
- Concurrent participation in other therapeutic trials.
- Pregnant or nursing.
- Severe psychiatric illnesses (current schizophrenia, major depression with suicidal ideation).

- Active substance abuse disorder in the past 24 months as determined by subject self-report
- Use of PRN over the counter (OTC) pain medications (NSAIDs, etc.) on day of MRI scan.
- Use of PRN narcotic pain medication 48 hours prior to MRI scan.
- Contraindications to fcMRI, fMRI, or ^{1}H-MRS methods. These may include but are not limited to: surgical clips, surgical staples, metal implants, and certain metallic dental material. [Note: a more formal description of contraindications for MRI is present in our DSM Plan].
- Any impairment, activity or situation that in the judgment of the Study Coordinator or Principal Investigator that would prevent satisfactory completion of the study protocol. This includes unreliable, or inconsistent pain scores as deemed by the principal investigator.
- Current, habitual, or previous use within the last 12 months of artificial nails, nail enhancements, or nail extensions that cover any portion of either thumbnail. Exceptions, including brief and/or occasional use, may be permissible at the discretion of the principal investigator.
- Contraindications to the Electrostimulator device: Participants with electrical implants (i.e. pacemakers, defibrillators), cardiac rhythmic disorders, seizure disorders, any skin disorders in the vicinity of the electrode or any malignant diseases in the region of application.
- History vascular surgery in lower limbs or current lower limb vascular dysfunction.
- Subjects with Worker's Compensation, Workman's Compensation, civil litigation or disability claims pertinent to the subject's fibromyalgia; current involvement in out-of-court settlements for claims pertinent to the subject's fibromyalgia; or currently receiving monetary compensation as a result of any of the above.

<u>Exclusion Criteria for Healthy Control Participants:</u>

- Have a diagnosis of FM or meet the newly proposed Wolfe et al 2011 criteria for FM."
- Presence of a concurrent autoimmune or inflammatory disease such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, etc. that causes pain.
- History of head injury with substantial loss of consciousness
- Peripheral neuropathy of known cause that interferes with activities of daily living
- Routine daily use of narcotic analgesics, marijuana or history of substance abuse.
- Stimulant medications, such as those used to treat ADD/ADHD (e.g., amphetamine/ dextroamphetamine [Adderall®], methylphenidate, dextroamphetamine), or the fatigue associated with sleep apnea or shift work (e.g., modafinil), are excluded.
- Concurrent participation in other therapeutic trials.
- Pregnant or nursing.
- Severe psychiatric illnesses (current schizophrenia, major depression with suicidal ideation).
- Use of PRN over the counter (OTC) pain medications (NSAIDs, etc.) on day of MRI scan.
- Use of PRN narcotic pain medication 48 hours prior to MRI scan.
- Active substance disorder in the past 24 months, as determined by subject self-report.
- Current, habitual, or previous use within the last 12 months of artificial nails, nail enhancements, or nail extensions that cover any portion of either thumbnail. Exceptions, including brief and/or occasional use, may be permissible at the discretion of the principal investigator.
- Contraindications to fcMRI, fMRI, or ^{1}H-MRS methods. (see above section)
- History vascular surgery in lower limbs or current lower limb vascular dysfunction.
- Any impairment, activity or situation that in the judgment of the Study Coordinator or Principal Investigator that would prevent satisfactory completion of the study protocol.

8.2. Study Enrollment Procedures

8.2.1. Recruitment

The primary recruitment method will be through phone solicitation of participants currently enrolled in the Chronic Pain and Fatigue Research Registry (University of Michigan Institutional Review Board # HUM00063489, PI: Clauw). These individuals reside mainly in Southeast Michigan and have previously agreed to being solicited regarding participation in clinical trials.

We will also utilize the University of Michigan research recruitment portal www.UMClinicalStudies.org This online portal has been created by the CTSA sponsored Michigan Institute of Clinical Research (MiCHR) to facilitate study team-volunteer communications. This website posts all available, ongoing research projects for interested volunteers and is available to the general community as well as the University of Michigan internal workforce and engages social networking to promote U-M research activities.

Other avenues that will be used to recruit patients include posting publically accessible flyers throughout University of Michigan clinics, public advertisements in the *AnnArbor.com*, the *Metro Times* and the *Detroit Free Press*, participation in the *Fibromyalgia Workshop* held at the UM CPFRC, and other local media outlets.

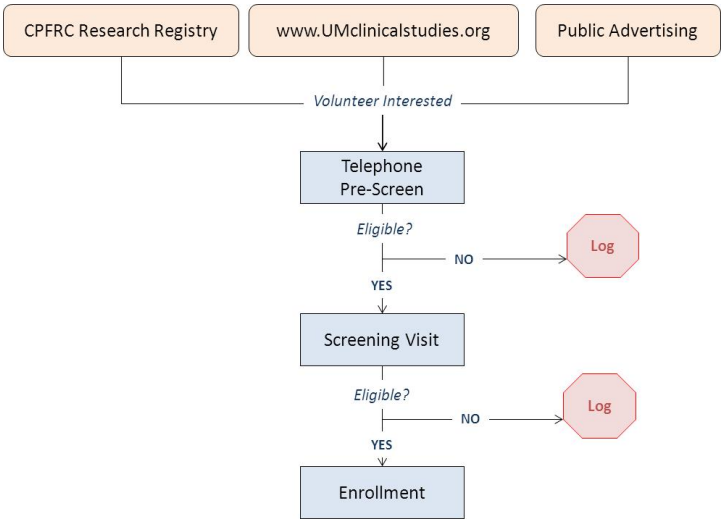
Participants will be recruited into the study by a dedicated member of the study team. All materials used for recruitment, and ongoing participant engagement, will be approved by the University of Michigan IRB. The initial step will be a telephone screen in which the inclusion and exclusion criteria will be described along with the general procedures for the study. Potential participants will have an opportunity to ask questions about the study involvement and if still interested will be invited to attend a screening visit.

At the screening visit, potential participants will go over the study requirements in more thoroughly with the study coordinator. They will sign the informed consent document at this time if they wish to proceed with the screening exam. Once screened, and deemed eligible for the study they will be considered "enrolled".

All potential volunteers will be assigned a unique screening ID. Those volunteers who do not meet the eligibility criteria or who choose not to participate in the study at either the telephone Pre-Screen or the Screening Visit will be logged. Personal identifiers will be kept until the study has been closed to recruitment, at which time they will be destroyed. This will prevent repeat screenings of potential volunteers.

8.2.2. Informed Consent

The informed consent document will be approved by the University of Michigan IRBMED. The informed consent interview will be conducted by trained study staff and will include a verbal and written explanation of the study, including the purpose, testing procedures, time commitment, inclusion/ exclusion criteria, risks and benefits, alternative treatments, confidentiality, compensation, study personnel contacts, and required regulatory information. All individuals will be given the opportunity to ask questions. Once all questions and concerns are addressed to the participant's satisfaction, the participant will sign the consent form. Only the subject herself will sign the consent form. Following informed consent, the study participant will be assigned a unique study identification number.



From this point forward, only the study ID number will be used to identify the individual, and the signed informed consent document will be stored securely and separately from all other research materials.

8.3 Screening and Enrollment

Potential subjects who are interested in participating in the study will be contacted by a member of the study team responsible for enrollment. Interested candidates will have the study and the eligibility criteria explained to them. If at this point they wish to continue with enrollment the candidate will be invited to participate in a study screening visit

The screening visit begins with the informed consent interview. Once the subject has formally consented to participate they will undergo a screening examination by a member of the study team. This includes:

- Completing demographic, sociodemographic, and medical history questionnaires
- Reviewing patient's self-reported 7-day recall VAS pain scale and Brief Pain Inventory (BPI)
- Assessing fibromyalgia status by tenderpoint examination and completing Fibromyalgia Symptom Questionnaire (FSQ) [*FM and HC cohorts*] and Fibromyalgia Impact Questionnaire (FIQ-R) [*FM cohort only*]
- Conducting a physical assessment (height and weight)
- Completing Psychological Screening questionnaires (HADS, PHQ-9)
- Administering a urine pregnancy test to women of childbearing potential
- Tenderpoint examination
- Reviewing current medications and therapies with subject.

The subject will be deemed enrolled upon meeting the study eligibility criteria. At the completion of screening visit, the subject will be provided with a copy of the informed consent document and a study schedule of appointments.

9. INVESTIGATIONAL PLAN

9.1. Acupuncture Treatments

9.1.1. Randomization and Blinding

After each FM participant has given written informed consent, they will be randomized to one of two arms: EA or ML. Computer generated random numbers will be used to develop the randomization scheme which will utilize a permuted block design, with variable size (4, 6, 8). This will help reduce the chance of participants breaking the blind and determining the next patient's assignment. Sealed envelopes will be generated by an individual in the Chronic Pain and Fatigue Research Center that will have no access to the study data nor influence on outcome assessment. Participant treatment allocation within these envelopes will be given to the acupuncturist on each participant's first treatment day. The acupuncturist will open the envelope to determine to which group the subject has been randomized. Following randomization, FM participants will receive 8 acupuncture treatments over the course of a month (two times per week for four weeks). During all treatment sessions, participants will be blind folded to ensure masking of the treatments. All acupuncture and sham treatments will be performed by a trained acupuncturist with board certification from the National Certification Commission for Acupuncture and Oriental Medicine.

The statistician and the study PIs will remain blinded until the study database is locked. The only study personnel that will not be blinded are the acupuncturists and the project manager, in order to facilitate randomization codes and treatment delivery. Due to this study utilizing dual database data entry, un-blinded non-essential personnel may need to enter data. However, this entry will not occur until the participant has exited trial *(see 12.2 Data Management*).

9.1.2. Electro-Acupuncture (EA)

This group will receive EA at 3 pairs of real acupoints: right side LI-11 to LI-4, left side GB34 to SP6, and bilateral ST36. Needles will also be inserted in Du-20, right ear shenmen, and left LV3. EA needles will be stimulated with low intensity, low frequency (2 Hz) electro-acupuncture using a constant-current electro-acupuncture device which allows for flexible setting of pulse width (1ms), frequency (2Hz), and shape (biphasic rectangular) parameters. The current intensity will be set at the midpoint between sensory and pain thresholds. Stimulation will last 25 minutes per session. ***Selection of active needle sites****:* Different types of acupuncture have been reviewed in detail [78]. These reviews emphasize the heterogeneity of traditions and styles even within a specific school of acupuncture. The optimal needle sites for FM have yet to be defined; consequently, the selection of active acupuncture sites is based on treating the predominant FM symptoms including: 1) diffuse, chronic myofascial and joint pain; 2) headache; 3) gastrointestinal pain and dysfunction; 4) disrupted or non-restorative sleep; and 5) chronic fatigue and weakness.

EA device: http://www.harmonymedical.co.uk/product/3153-as-super-4-digital-needle-stimulator

9.1.3. Mock Laser (ML)

For mock laser treatment, a laser acupuncture device (VitaLaser 650, Lhasa OMS) will be positioned 1-2cm over all of the same acupoints used in EA. There will be no palpation prior to positioning these devices, and there will be no physical contact between device and skin. The laser will not be turned on during the treatment. Switching the Timer toggle to 10 minutes for “on” and to 5 minutes for “off will produce an audible click, as detailed by Irnich et al. [74, 75]. ML treatments will last 25 minutes per session.

ML device: http://www.lhasaoms.com/Vita_Laser-47-1300-page.html

9.1.4. Deception

One of the aims of our research is to study whether acupuncture alters the way the brain processes pain signals. To do this we needed to compare an active acupuncture treatment to an inactive, or “sham”, acupuncture treatment, however to maximize patient participation and retention throughout the course of the study, it is important that those receiving the sham treatment believe that they are in fact receiving active treatment. For this reason, the sham/inactive acupuncture treatment, ie."mock laser", is to be described as non-traditional "laser acupuncture" in the informed consent document and throughout the IC process and study materials. It is described as a Placebo Comparator: Non-tradition Acupuncture" in Clinicaltrials.gov.

The sham treatment poses no additional risk to the subject. All participants regardless of assigned group will be debriefed by the Project Manager at the conclusion of their final visit (visit 12).

9.2. Pain Outcome Measures

Pain outcomes will be assessed at the University of Michigan by trained clinical research coordinators.

9.2.1. Clinical Pain

Pain severity and functional interference due to pain will be assessed using the Brief Pain Inventory (BPI). The BPI has been recommended by IMMPACT as a measure of choice for the assessment of pain in clinical research [79]. Clinical pain immediately before and after resting MRI scans will also be assessed using a 10cm visual analog sale, anchored by “no pain” and “worst pain imaginable.” Visual analog scales are used commonly by our group and others in the assessment of clinical pain.

9.2.2. Quantitative Sensory Testing

We will use the MR-compatible Multidimensional Automates Sensory Testing (MAST) System (US Patent No. 9,307,906)[80], which features a control computer running custom-design software, a touch screen display for patient feedback, and a wireless pressure actuator. The pressure actuator applies blunt force, delivered by a 1-cm^2 hard-rubber probe, to the thumbnail bed. The probe is attached to a cylindrical transducer housed inside a plastic “joystick” designed to be held comfortably in either hand. The transducer is driven by a miniature servo-motor

and a digital load-cell measures the exact pressure applied to the thumb to ensure accurate and repeatable testing.

Our group has extensive experience using thumbnail pressure as an evoked pain stimulus and its validity in the measurement of pain sensitivity has been discussed extensively [45, 81-88]. The MAST system will deliver an ascending series of 5-s duration stimuli at 25-s intervals, beginning at 0.25 kg/cm^2 and increasing in 0.25 – 0.50 kg/cm^2 intervals up to tolerance or to a maximum of 10 kg/cm^2 to the patient's dominant thumbnail. Pain intensity will be rated after each stimulus on a 0-100 numerical rating scale (NRS), with "0" representing "no pain" and "100" representing "extreme pain". These ratings will be used to compute pain threshold and a psychophysical function of each subject's suprathreshold pain sensitivity with pressure intensity and pain magnitude represented on the x- and y-axes, respectively. These curves will be used to compare single subject and group changes in pain sensitivity longitudinally throughout each project. These methods have been used extensively in our group to assess sensitivity to experimental stimuli.

Any data elements that are not obtained during the QST sessions due to unplanned yet common circumstances for this type of behavioral research (e.g., patient or facility time constraints, technical issues with equipment, or patient refusal to complete a particular test) will be considered "missing data" and will not be reported as a protocol deviation.

9.2.3. Cuff pain and Conditioned Pain Modulation.

Calibrated cuff pain stimuli will be delivered to the gastrocnemius area of the dominant leg using a validated cuff pain device that has been recently adapted to the MRI scanning environment. A computer controlled air compressor (Hokanson Rapid Cuff Inflator) will inflate the cuff to a pre-specified pressure, and maintain the pressure at that level. Subjects will participate in two separate test sessions of cuff pain on different days: one training session and one brain imaging session. In both sessions, pressure will be maintained for blocks of 14 seconds during the evoked pain fMRI scan to evaluate brain response to experimental pain. Pressure pain will also be applied for a continuous 6-minute period in a separate scan to evaluate functional brain connectivity response to deep pain (see below). One advantage to using cuff algometry pain is that unlike more superficial methods (e.g. heat pain), cuff pain responses are unaffected by sensitization or desensitization of the skin, indicating that this procedure primarily assesses sensitivity in muscle and other deep tissues. We have considerable experience applying these techniques in both healthy adults and chronic functional pain patients, and have found that all subjects are able to tolerate these procedures without any lasting discomfort. We will also repeat the thumbnail pain procedures above during and after the application of cuff pain to the gastrocnemius in order to assess conditioned pain modulation, which is used to evaluate the descending inhibitory pain modulation systems in the brain. During this test, alternating (on) and (off/touch) pressures will be applied to the thumbnail bed of the patient's dominant hand using a computer-controlled pneumatic pressure device compatible within the fMRI scanner environment. This system uses the QST Suite (Arbor Medical Innovations, Ypsilanti, MI, USA) Windows software to design the pneumatic pressure profile generated by the IPC-1000 pressure controller (Arbor Medical Innovations, Ypsilanti, MI, USA) via a USB link.

Training Session. A training session with the cuff will be used to familiarize subjects with the stimuli and rating procedures and determine appropriate stimulus intensities to be used subsequently in the imaging session (see below). This will take place during the in-clinic evaluation visit. During training, subjects will be maintained in a seated position in a chair with the left foot resting on a support at a slightly elevated position (but at a lower level than that of the hips, in order to facilitate blood circulation in the leg).

Testing will began with an ascending series of pressure stimuli starting at 60 mmHg and increasing 20 mmHg increments. Ten seconds after the end of each stimulus, subjects will complete two 0-100 numerical rating scales: pain intensity (0 = "no pain", 100 = "worst pain imaginable/extreme pain") and pain unpleasantness (0 = "neutral", 100 = "extremely unpleasant"). The ascending series will end when a pain intensity rating of >70/100 is obtained. A descending series will then be administered starting with the last stimulus delivered during the ascending series and decreasing in 20 mmHg decrements until a pain intensity rating of 0/100 is obtained. For each pressure delivered, the mean pain rating obtained from the ascending and descending series will be calculated and then plotted against the corresponding pressure level to obtain a first "approximate'" stimulus-response (S-R) curve. From this curve, the pressure values corresponding to pain intensity ratings of 10, 20, 30, 40, 50, 60 or 70/100 (i.e., "Pain10" to "Pain70") will be obtained by interpolation. In addition, the highest pressure value that was consistently rated as non-painful (i.e., associated with a pain intensity rating of 0 in both

ascending and descending series) will be defined as Pain0. As the awareness that the next stimulus will be higher (or lower) is likely to bias the ratings, a new S-R curve will then calculated based on the ratings recorded during the presentation of these Pain0-Pain70 stimuli in pseudorandom order. Adjusted Pain0-Pain70 stimulus pressures will be determined by interpolation from this new curve. At the end of this calibration phase, subjects will receive each of the adjusted Pain0-Pain70 stimuli three times, for a total of 24 stimuli. The stimuli will be delivered in a pseudorandom order in three separate runs (8 stimuli per run).

Based on this testing, subjects will be eligible for participation in the imaging session if they are able to reliably differentiate stimuli of different intensities (i.e., if they reported increasing pain intensity ratings in response to stimuli of increasing intensity). This training session will have the effect of rendering subjects non-naïve to the experimental conditions in the imaging session, an aspect that might be argued to have some impact on the imaging results (particularly with regard to brain activity underlying cognitive and emotional functions). However, the training session has several advantages that outweigh these concerns: (1) identification and exclusion of individuals with unstable ratings, (2) a thorough training in the use of the rating scales, and (3) the reduced potential for developing experiment-related anxiety and head motion in the imaging session.

9.2.4. Visual Stimulation Assessment.

In addition to hypersensitivity to painful stimuli applied to the body, many chronic pain patients report hypersensitivity to nonpainful sensory stimuli, including to auditory, olfactory, and visual stimulation [81, 89, 90], suggesting a global state of multisensory amplification in centralized pain. To measure nonsomatic sensitivity, we will present to each participant a series of nonpainful yet aversive visual stimuli [91]. This 10 min task, conducted both inside and outside of the MRI scanner, consists of different visual stimuli presented in an alternating block design. The control stimulus is a fixed crosshair centered in the middle of a solid color background, whereas experimental stimulus is a flashing annulus checkerboard. These stimuli will be interleaved into independent 10-30 second blocks throughout the duration of the task. Participants will view the stimuli on either a standard LCD computer screen or through video goggles. Stimuli will be adjusted to varying degrees of frequency (checkerboard flashing only), color, shape, motion, and/or brightness level, and rated on a NRS and/or Box Scales[87] of sensory intensity and unpleasantness. No adverse events were associated with this task in a previous study of chronic pain patients (HUM00021096). However, to avoid possible adverse events in vulnerable patients, this visual stimulation task <u>will not</u> be performed if the participant has a history of migraines. These participants will still be allowed to complete all other QST procedures.

9.2.5 Assessment of Diet.

To assess typical dietary intake over the last 12 months we will use the 80out 2007 Harvard green FFQ questionnaire, which is a 61-item semi quantitative food frequency questionnaire that takes on average 20 minutes to complete. The semi-quantitative food frequency questionnaire have been developed at Harvard University is the result of over thirty years of continued development, evaluation, refinement and re-evaluation. It was originally created to be used as a self-administered, mailed questionnaire. The reproducibility and validity of the questionnaire has been assessed by comparing its estimates with those of diet records or multiple 24-hour recalls and with relevant biochemical indicators of nutrient intakes. Such studies have been conducted among adults of all ages and both sexes, and among a variety of socio-economic groups; many of these validation studies have been published [92-94]. The results of the validation studies have indicated that the method is remarkably robust; similarly valid results have been obtained from virtually all the groups that we have studied. We will give participants the FFQ once at baseline to take home and complete in the following week. FFQ will be returned via mail and all participants will receive $10 for completion of the FFQ.

Imaging session. On the day of the imaging session, the Pain0-Pain70 pressures will be briefly recalibrated prior to scanning using procedures similar to those used during the training session. The first "approximate" S-R curve, however, will be calculated based on the ratings of Pain0, Pain10, Pain40 and Pain70 from the training session (instead of the full ascending and descending series). The Pain0-Pain70 stimuli pressures interpolated from this curve will then be presented in a pseudorandom order for calculation of an adjusted S-R curve. The definitive Pain0-Pain70 pressures used during fMRI will be determined by interpolation from this latter curve.

During the fMRI scan runs, subjects will receive three pressures: touch (30mmHg), equal pressure (130mmHg) and an equal pain condition (P40; 40/100). Each stimulus will be preceded by a 4-second visual cue (a cross changing color from black to green) that signals the upcoming stimulus to limit the stimulus-onset startle reflex. Ten seconds after stimulus offset, subjects will be presented with the intensity and unpleasantness scales, each for 10 seconds. Cuff pressure calibrated to each subject's Pain30-50 will also be applied for a continuous 6-min period in a separate fMRI scan to evaluate functional brain connectivity response to tonic deep pain. (see below)

9.3. Neuroimaging

Our team has successfully developed several different neuroimaging outcome measures that appear to differentiate FM patients from HCs, vary between patients based on their spontaneous clinical pain state, and longitudinally track with clinical pain levels. Our MRI scan sessions will include (1) structural MRI, (2) resting fMRI, (3) ^{1}H-MRS in the right anterior (aIns) and posterior insula (pIns), and (4) fMRI with evoked (experimental) pain (**Figure 3**).

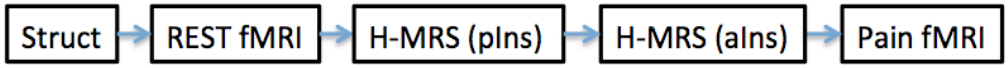


Figure 3 - Schematic of the neuroimaging scan session

Functional neuroimaging will include BOLD fMRI at 3T using a T2*-weighted gradient echo sequence. The pain fMRI scans will include 1) a phasic cuff pain block design scan, 2) a 6-minute sustained cuff pain scan, and 3) a conditioned pain modulation scan, where phasic thumbnail pain stimuli are delivered with or without concurrent sustained cuff pain. The entire is imaging session will take no more than two hours.

9.3.1. Functional connectivity MRI

Resting 6min scans, performed with subjects resting comfortably in the scanner with eyes open, will be completed at the beginning of the scan session. In order to quantify intrinsic brain connectivity, functional MRI data will be analyzed with both a dual-regression ICA approach and seed-voxel approach. These approaches are complementary, as they quantify intrinsic brain connectivity on a network level (dual regression pICA) and a more specific region-focused level (seed-voxel). Physiological data will also be collected simultaneously to the fcMRI data, as cardio-respiratory fluctuations are known to influence fMRI intrinsic connectivity estimation within several brain networks [95, 96]. A similar approach will be used to evaluate the sustained cuff pain 6min scan.

Dual Regression ICA Approach: The within- and between-subject resting fMRI data analysis will be performed using ICA through Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC, an FSL tool) and a previously validated dual regression approach [97]. This technique allows for voxel-wise comparisons of resting state functional connectivity by first temporally concatenating resting fMRI data from all subjects, followed by back-reconstructing the group networks for individual subjects, which are then used for within- and between-subject group and difference maps. This technique does not regress out global signal (enhancing interpretation of anti-correlations), and has been found to have moderate to high test-retest reliability in previous studies [98]. This process will be completed for networks of interest (DMN, SMN). The MVN will also be evaluated as a control network. Group analyses will be performed to evaluate intrinsic brain connectivity differences between FM and HC groups, how this intrinsic connectivity covaries with spontaneous pain intensity in FM patients, and how networks differentially change following EA and ML. The results will be threshold at $p < 0.05$, cluster-corrected for multiple comparisons.

Seed-voxel Functional Connectivity Approach: For exploratory analyses, we will also use a complementary approach to evaluate intrinsic connectivity involving seed-voxels from distinct brain regions (see below). The average timeseries from these regions is used as a regressor in a whole brain GLM to find which other regions contain correlated timeseries. While this approach can yield more focused inferences, there is some bias in choice of exact seed location and contour [99]. Thus seeds will be carefully chosen based on the results of the evoked pain fMRI scan, with particular focus on insular clusters.

Feasibility of BOLD Approach: Importantly, a similar approach has been successfully implemented in our previous studies [10, 59, 60]. One such study contrast resting connectivity in 18 FM patients and 18 HCs, and evaluated the relationship of intrinsic connectivity to spontaneous clinical pain [48]. We found that FM patients had greater connectivity between the DMN and insula as well as S2 (**Figure 4**) – brain regions known to process evoked experimental pain. Furthermore, greater spontaneous pain at the time of the scan correlated with greater intrinsic connectivity between the insula and DMN. Our most recent publication showed that a longitudinal course of manual or sham acupuncture (with somatosensation) was able to decrease DMN-insula connectivity [59].

We also have pilot fcMRI data from N=18 chronic low back pain (cLBP) patients. Resting fMRI data were collected before and after provocation of patients own clinical pain with specific maneuvers, similar to our recent published study [99]. In addition to replicating again our finding that DMN-insula connectivity is correlated with increasing spontaneous clinical pain, we also noted that higher clinical pain, both before and after maneuvers, was correlated with more pronounced anti-correlation between anterior insula and SMN (**Figure 5**). Moreover, the after-before maneuver increase in pain was negatively correlated with change in SMN connectivity to the left anterior insula. These results support our hypotheses for increased anti-correlation between SMN and insula in chronic pain (here FM) patients, and its relationship to clinical pain.

Other studies recently published by our group noted increased DMN connectivity to anti-nociceptive areas such as the PAG immediately following real, but not sham, acupuncture in HC subjects [60]. SMN connectivity following real acupuncture was enhanced to the dorsal ACC, pre-SMA, and cerebellum, while sham acupuncture reduced connectivity between SMN and dlPFC, a brain area heavily implicated in placebo analgesia [100-102]. These results demonstrated proof-of-concept that acupuncture stimulation does indeed modulate DMN and SMN connectivity. In this proposal, we will extend these short-term, acute acupuncture findings, by investigating how resting brain connectivity changes following a longitudinal trial of therapy.

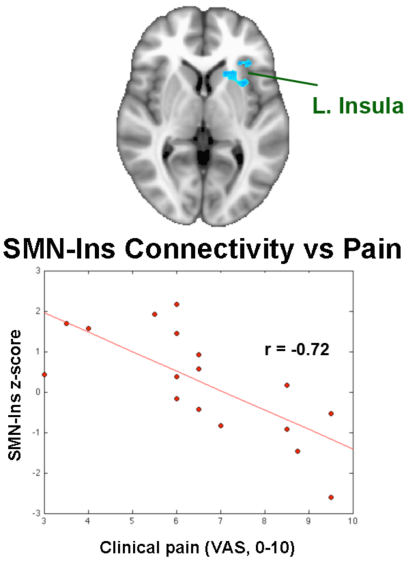


Figure 5: SMN-insula connectivity is more anti-correlated with increasing pain.

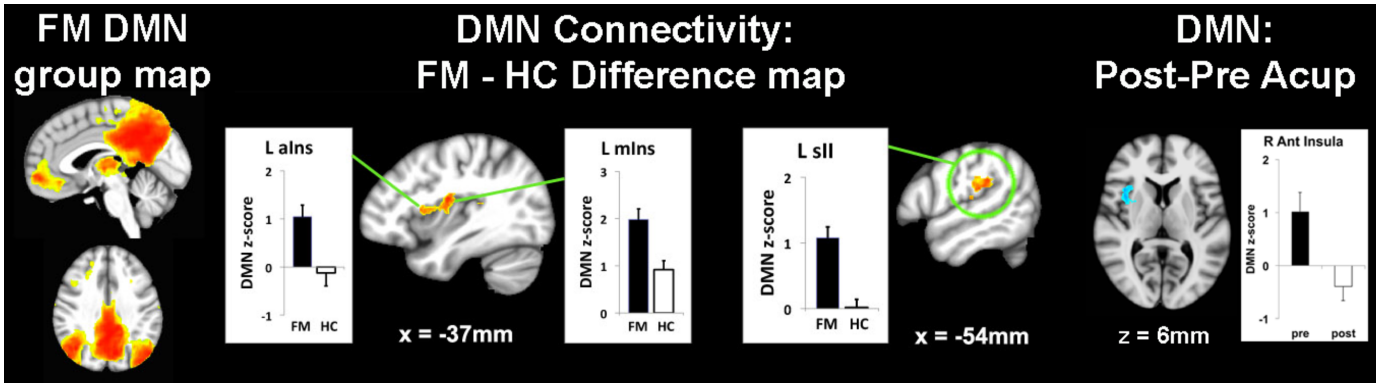


Figure 4: Intrinsic DMN connectivity to insula and S2 is greater in FM [10] and is reduced following acupuncture [60].

9.3.2. ^{1}H-MRS acquisition and processing.

^{1}H-MRS provides metrics amenable to longitudinal studies such as those proposed here. High-resolution anatomical scans isolate identical brain structures within individuals over time thus minimizing error that otherwise would occur because of slight differences in voxel location from one evaluation to the next. Previous ^{1}H-MRS studies, performed by our group and others, have utilized this approach to examine changes in the levels of CNS metabolites in test-retest studies [100].

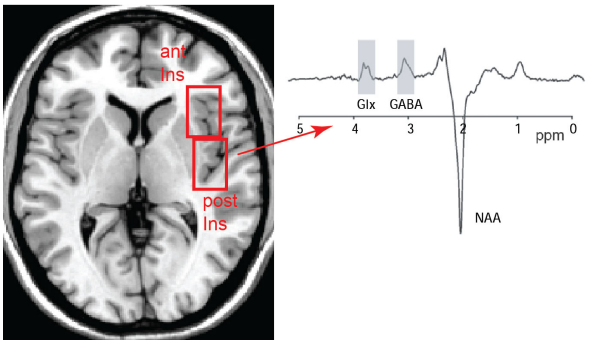
^{1}H-MRS will be focused on two specific regions of the brain: the anterior insula cortex and the posterior insula cortex (see Figure 6 below). The voxels will be placed on the right side of the brain. The right side of the brain was chosen, as this is contralateral to the pressure pain testing stimulus used in our evoked pain fMRI studies. The posterior insula is chosen as our main outcome given we have shown it to be dysregulated in FM [62, 63].

^{1}H-MRS studies will be performed on a Philips Ingenia 3 Tesla system using a 15 channel receive head coil. Details of our ^{1}H-MRS methods are presented in our previously published work [62, 63]. In brief, single voxel MRS will be performed on each region of interest with voxel sizes of 2x3x3 cm. The order of acquisition will be randomized across subjects. Standardized voxel placements will be guided by the Philips Smart Brain function

and visual inspection of anatomical T1-weighted images as reported previously [63]. The ^{1}H-MRS scanning protocol will consist of: 1) Survey images (1 minute scan time), 2) SENSE Reference images (2 minute scan time), 3) B0 field map sequence for shim adjustment (2 minute scan time), 4) Axial 3D-MP-RAGE sequence isotropic 0.9 mm resolution, 150 slices, TR/TE 8.2/3.7 msec, matrix 192x256, FoV 184 x 230, flip angle 8˚, SENSE factor (R) 2.0 (4:37 minutes), and 5) PRESS single voxel spectra (TR/TE=2000/35 ms) will be acquired from each of the regions of interest with and without VAPOR water suppression with 64 averages and a total scan time of approximately 2 minutes for each voxel. The water signal will be recorded using 8 averages (scan time 16 sec). Participants will be at rest during the ^{1}H-MRS session.

The raw data from each single-voxel MR spectroscopy sequence will undergo manual post-processing using ^{1}H-MRS software (LCModel; Stephen Provencher, Oakville, Ontario, Canada). LCModel uses a linear combination of individual spectra obtained from pure molecular species to fit the experimental spectra [101]. Values for Glx and Glu will be calculated as absolute concentrations using the water signal for normalization. Resulting metabolite absolute concentrations will be reported in arbitrary institutional units. Since our voxels incorporate CSF, and the volume of CSF dilutes ^{1}H-MRS-derived metabolite values, we will also correct our metabolite levels for CSF volume for each participant as reported previously [63]. For this we will use Voxel Based Morphometry, a toolbox that operates within the image analysis program Statistical Parametric Mapping (SPM; (Functional Imaging Laboratories, London, UK). Drs. Harris and Foerster will conduct the analysis of the MRI data at the CPFRC at UM.

Figure 6 ^{1}H-MRS Regions of Interest (ROIs) and Resulting Spectra from Posterior Insula Resolving Glx and GABA Signals



In addition to assessing Glx, we will also perform more sophisticated ^{1}H-MRS to measure GABA levels in FM and HC, as in our pilot study [71]. In brief, using the same 3 Tesla MRI magnet as described above, two single-voxel point resolved spectroscopy (PRESS) spectra (TR/TE=2000/35 ms) will be acquired for the anterior and posterior insula cortex. MEGA-PRESS using editing for GABA will be performed with the following parameters: TE=68 ms (TE_1=15 ms, TE_2=53 ms); TR=1.8 s; 256 transients of 2k data points; spectral width=2 kHz; frequency selective editing pulses (14 ms) applied at 1.9 ppm (ON) and 7.46 ppm (OFF). Refocusing will be performed using amplitude-modulated pulse 'GTST1203' (length=7 ms, bandwidth=1.2 kHz). Our MEGA-PRESS spectroscopy will be analyzed using in-house post-processing software in Matlab with Gaussian curve fitting to the GABA and inverted N-acetylaspartate (NAA) peaks. GABA will be measured relative to the NAA signal in the edited spectra to calculate a ratio based on the concentration of NAA as generated by the MEGA-PRESS technique [102]. After calculating this NAA:GABA ratio, GABA levels will be estimated by multiplying the ratio by the NAA concentration determined from LCModel analysis of a short-TE PRESS spectrum of the same voxel. Cerebral spinal fluid correction will be performed as described previously [63]. Metabolite concentrations will be used only if the Cramér-Rao bounds are less than 20%.

Feasibility of ^{1}H-MRS Approach: Importantly, all of the ^{1}H-MRS procedures outlined above have been successfully implemented in our published preliminary studies [71, 103, 104]. These studies suggest that (1) FM patients have higher levels of Glu and Glx within the posterior insula and (2) that changes in Glu and Glx within the posterior insula are strongly correlated with improvements in pain, with reductions in clinical pain being associated with lower Glu levels. We hypothesize that our acupuncture and/or sham interventions may be directly modulating Glu and Glx within the posterior insula. However since our previous analyses combined both acupuncture and sham treated groups, we have recently tested whether acupuncture and sham acupuncture *differentially* modulate Glu levels within the insula. 19 FM patients were randomized to receive either nine manual acupuncture (MA; n=9) or nine sham (with somatosensation; n=10) treatments over the course of a month. All subjects underwent two ^{1}H-MRS sessions once prior to and once following treatment. Changes in Glu within the posterior insula, post-pre treatment, were significantly greater in the MA group as compared to the sham group, (mean difference in concentration post-pre treatment (s.e.m) in AIU: MA -0.82(0.32); SHAM 0.20(0.32); p=0.04). Participants receiving MA displayed slightly greater reductions in Glu within the posterior insula, suggesting that there may be subtle differences between MA and sham acupuncture with somatosensation.

No differences were detected between groups for NAA, Cho, or myoInositol (all p>0.05). No differences were detected between groups for any metabolites in the anterior insula (all p>0.10).

9.3.3. Functional magnetic resonance imaging (fMRI) of evoked pain.

fMRI Data acquisition. We were the first to use fMRI of evoked pain in FM and have published extensively in this field [22, 105-110]. Whole-brain BOLD functional images will be acquired on the same 3 Tesla scanner used for ^{1}H-MRS and fcMRI, using a T2* weighted echo-planar sequence. Parameters will be: TR/TE 2500/30msec, flip angle = 90°, FoV = 220 mm, 48 AC-PC aligned slices, thickness 3mm. Each participant will undergo 5 minute block design scans, during which three cuff pressures (touch, 30mmHg; equal pressure, 130mmHg; and equal pain, 40/100) will be applied to the left calf using the pressure cuff device in pseudo-random order. Each pressure stimulus will be applied for 14 seconds. Participants' head motion will be minimized using foam pads placed around the head along with a forehead strap. A separate scan will assess brain connectivity during sustained 40/100 cuff pain on the same leg. A final 5 minute scan will assess conditioned pain modulation by applying 40/100 thumb nail pain with or without concurrent 40/100 cuff pain.

Pre-processing and First-level Analysis. Data will be quality checked, pre-processed and analyzed using SPM5 running under Matlab 7.5b (Mathworks, Sherborn, MA, USA). The first 5 images will be discarded from the data set and not used for further analysis in order to correct for equilibration effects. Pre-processing steps will include: physio-correction, motion correction, co-registration, normalization, and smoothing (FWHM 6mm). First level analyses will be performed using the general linear model implemented in SPM. Motion parameters from re-alignment will be modelled as regressors of no interest. Pain blocks will be convolved with a canonical hemodynamic response function. For group level analyses, we will compare HC and FM at baseline (Aim 1), and the effects of EA and ML on cortical activations either pre-post treatment (Aims 2), or predicting treatment response (Aim 3), all using SPM software version 5. Briefly single subject contrast maps of equal pressure and equal pain versus touch will be passed up to a general linear model (GLM) group level analysis. For Aim 1 we will compare activations between HC and FM participants at baseline. For Aim 2, effects of acupuncture treatment, post-pre treatment will be estimated and we predict that EA will demonstrate greater reductions in activation of somatosensory and pain areas as compared to ML which will have no effect. For Aim 3, the degree of activation of cortical structures in response to evoked pain at baseline will be correlated with change in clinical pain response. All statistical maps will be corrected for multiple comparisons on the cluster level at $p < 0.05$. To ensure that there are no differences in baseline fMRI outcomes we will also perform ANOVA across groups at all pre-treatment scans.

9.4. Schedule of Assessments

The schedule of study procedures and assessments is tabulated in Section 3. The descriptions of the procedures to be performed at each visit are provided below.

9.4.1. Screening

- Informed Consent review and documentation
- Review study procedures and requirements with the patient
- Collect demographic and sociodemographic information
- Record tender point count
- Complete 7-day recall VAS pain scales
- Conduct psychological screening interviews (HADS and PHQ-9). Confirm that the patient is not experiencing active suicidal ideation or intent. Any participant experiencing active suicidal ideation will be directed to seek medical help.
- Obtain medical history and fibromyalgia treatment history *(FM cohort only)*
- Conduct a physical assessment (height and weight) and confirm and document results of the Wolfe et al 2011 criteria for FM .
- Urine pregnancy test for women of childbearing potential
- Document current concomitant medications

- Collect:
    - Brief Pain Inventory - Severity and Interference scales [BPI]
    - FM Survey Questionnaire [FSQ]
    - Fibromyalgia Impact Questionnaire [FIQ-R] *(FM cohort only)*
- The Screening visit and Baseline Behavioural visit may occur on the same day.

9.4.2. Baseline Behavioural Session – V1

- Review inclusion/exclusion criteria to assess patient's continued eligibility for enrolment
- Urine pregnancy test for women of childbearing potential
- Administer Neuroimaging Safety Screen
- Evaluate and record any changes in concomitant medications
- Collect:
    - BPI
    - FSQ
    - PROMIS-29
    - Perceptions of Bodily Sensations [PBS]
    - Pain Catastrophizing Scale [PCS]
    - PainDETECT
    - Fibromyalgia Impact Questionnaire [FIQ-R] *(FM cohort only)*
    - Desire of Relief Scale [DRS] *(FM cohort only)*
    - Expected Relief Scale [ERS]*(FM cohort only)*
    - Multiple Ability Self Report Questionnaire [MASQ]
    - Pittsburgh Sleep Quality Index [PSQI]
    - VAS -Present Pain
    - Menstrual Questionnaire
    - After-Cuff Questionnaire *(post QST assessments)*
    - *Harvard Food Diary (FFQ)*
- Quantitative Sensory Testing (QST) – Assessment of ascending pressure pain thresholds and conditioned pain modulation (CPM) using MAST and cuff, and visual stimulation assessment.
- Collect and record pre-treatment adverse events
- Patients will be reminded to refrain from taking any OTC pain medication on the day of the scan (NSAIDs, etc.)
- Patients will be reminded to refrain from taking any PRN narcotic pain medication 48 hours prior to the MRI scan.

9.4.3. Baseline MRI – V2

- The baseline MRI is to occur within 3 days of the baseline behavioural session, but not be completed on the same day as the behavioural session.
- Review inclusion/exclusion criteria to assess patient's continued eligibility for enrolment
- Evaluate and record any changes in concomitant medications
- Urine pregnancy test *(if not performed at the behavioural session)* for women of childbearing potential
- Collect:
    - BPI
    - VAS-Present pain (pre and post MRI)
    - After-Cuff Questionnaire *(post MRI)*

- Baseline Neuroimaging (fcMIR, fMRI, and ^{1}H-MRS) with conditioned pain modulation and visual stimulation assessement
- Collect and record any MRI-related adverse events

9.4.4. Treatment – V3-10

After baseline, patients will return to the clinic twice a week for acupuncture treatments. At the first treatment session, the acupuncturist will open a sealed envelope containing the treatment to administer. Every effort will be made to have two treatments per week (only one acupuncture treatment per day may be conducted). Visits not completed within the acupuncture visit window will be considered "missed", and not reported as a protocol deviation.

- Randomization to Acupuncture group (EA or ML)
- The study coordinator will evaluate and record any changes in concomitant medications
- Patient complete the FIQ-R *(once a week at visits 4, 6, 8 and 10)*
- Administer acupuncture procedure
- Evaluate and record patient recall of needling sensations (using MASS questionnaire) **every treatment* Credibility Scale *(FM cohort only visit 3 and 10 only)*
- Adverse Events

9.4.5. Follow Up Behavioral Session – V11

- Review inclusion/exclusion criteria to assess patient's continued eligibility for enrolment
- Conduct psychological screening interviews
- Urine pregnancy test for women of childbearing potential
- Administer Neuroimaging Safety Screen
- Evaluate and record any changes in concomitant medications
- Administer:
    - BPI
    - FSQ
    - FIQ-R
    - PROMIS -29
    - PBS
    - PCS
    - Credibility Scale
    - PainDETECT
    - DRS
    - MASQ
    - PSQI
    - VAS-Present Pain
    - Menstrual Questionnaire
    - After-Cuff Questionnaire *(post QST assessments)*
- Quantitative Sensory Testing (QST) – Assessment of ascending pressure pain thresholds and conditioned pain modulation (CPM) using MAST and cuff, and visual stimulation assessement.
- Collect and record post-treatment adverse events
- Patients will be reminded to refrain from taking any OTC pain medication on the day of the scan (NSAIDs, etc.)
- Patients will be reminded to refrain from taking any PRN narcotic pain medication 48 hours prior to the MRI scan.

9.4.6. Follow Up MRI – V12

- Review inclusion/exclusion criteria to assess patient's continued eligibility for enrolment
- Evaluate and record any changes in concomitant medications
- Urine pregnancy test *(if not performed at the behavioural session)* for women of childbearing potential
- Administer:
    - BPI (Severity and Interference)

- VAS-Present pain (pre and post MRI)
- After-Cuff Questionnaire *(post MRI)*

- Baseline Neuroimaging (fMRI and ^{1}H-MRS) with conditioned pain modulation and visual stimulation assessement
- Collect and record any MRI-related adverse events
- Complete the study close-out documentation.
- Debrief participant to their treatment assignment

9.5. Concomitant Interventions

9.5.1 Allowed

Allowable medications/interventions include:

- FDA Approved medications for Fibromyalgia - Milnacipran (Savella®), duloxetine (Cymbalta®) and pregabalin (Lyrica®).
- Acetaminophen, aspirin, and other nonsteroidal anti-inflammatory agents (NSAIDs) will be allowed however not the day of MRI scanning
- Enrolled patients will be allowed to take as needed (PRN) narcotic pain medications during the study however not within 48 hours of scanning.

All remedies, whether prescription or OTC, will be tracked and recorded on the concomitant medication CRF. Participants will be asked to refrain from changing their existing ongoing treatments during the course of the trial. Participants will be allowed to be on stable doses of antidepressants.

9.5.2. Prohibited

Prohibited medications/interventions include:

- Daily use of narcotic analgesics are not permitted while enrolled in the study.
- As needed (PRN) narcotic pain medication are prohibited 48 hours prior of scanning
- Stimulant medications, such as those used to treat ADD/ADHD (e.g., amphetamine/ dextroamphetamine [Adderall®], methylphenidate, dextroamphetamine), or the fatigue associated with sleep apnea or shift work (e.g., modafinil), are excluded.
- Acupuncture treatment is not allowed during the trial however, meditation, Tai Chi, and yoga are permitted.

10. SAFETY ASSESSMENTS

Participant safety will be monitored while individual is enrolled in the study. Table 10.1. lists expected adverse experiences by study intervention/procedure, as well as criteria for management and modification of the study intervention regimen or participant assessments if an adverse event occurs.

Expected Adverse Event	Criteria for Management	Intervention Modification, if any
Acupuncture		
Nausea/Vomiting	Participant complains of significant nausea or "stomach upset". Participant vomits.	Needles (if any) will be removed and intervention will be stopped. Intervention modification will include assessment of nausea at next session.
Fainting	Participant loses consciousness during treatment.	Needles (if any) will be removed and participant feet will be elevated.

		Intervention modification will include assessment of lightheadedness at next session.
Localized skin infection or bleeding	Skin redness and/or pain following 48 hours after last treatment.	Participants will be asked to take NSAIDs for pain. Acupuncture treatments will be given on the opposite side of body.
Quantitative Sensory Testing		
Residual Soreness at testing site	Participants displaying sustained pain over 48 hours post testing.	Participants will be allowed to take NSAIDs. Stimuli duration and timing may be reduced.

10.1 Safety Parameters

We will assess changes in fibromyalgia symptoms throughout the trial using the Fibromyalgia Impact Questionnaire-Revised (FIQ-R). FIQ-R symptom scores will be logged and reviewed in aggregate by the IMC annually. Significant changes in patient FIQ-R symptom scores will be logged and reported in aggregate to the IMC, IRB, and NCCAM annually. Other safety measures will include verbal contact with the patient throughout the trial. This will be in the form of patients' response to the questions:

"Since your last acupuncture session, have you experienced a significant worsening change in any of the following?"

(a) Nausea/Vomiting

(b) Fainting

(c) Localized skin infection or bleeding at the site of the treatment

(d) Increased Pain other than the normal symptoms of fibromyalgia pain

There are no specific laboratory blood tests within this study. As such there will be no safety measures in place to monitor blood outcomes.

Suicidal ideation will be assessed at screening using the Patient Health Questionnaire (PHQ-9). Depression and Anxiety will be assessed at screening, baseline and follow-up. Subjects scoring between 10-14 on the PHQ-9 ("Moderately Depressed") will be eligible to enroll but will require additional monitoring and will be asked to retake the PHQ-9 at the baseline behavioral visit (visit 1) and the third treatment visit (visit 5), in addition to the scheduled survey administration of visit 0 and visit 11. Subjects scoring between 15-19 ("Moderately Severe" depression) will be referred to the PI for a decision. Should they be enrolled in the study, they will also complete the PHQ-9 at visit 1 and visit 5. Any score above 20 will be considered "Severe" depression, and the subject will not be eligible. Any subject that reports a score of > 0 on question 9 ("Thoughts that you would be better off dead, or of hurting yourself") of the PHQ-9 will be referred to support services offered by the University of Michigan Hospital System. Any subject deemed ineligible based on their PHQ-9 will be referred to a support services offered by the University of Michigan Hospital System.

10.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Acupuncture and electro acupuncture are generally considered safe interventions and as such we do not expect to have many adverse or serious adverse events in our trial. However to assess if participant fibromyalgia pain worsens significantly over the course of the trial, we will administer the FIQ-R once per week during the treatment phase of the study and at each evaluation. This measure will be used for safety purposes only and will not be used as a research data gathering tool. As stated above, if the FIQ-R score displays a clinically significant increase during the course of the trial, we will advise the research participant to seek care from their primary care physician.

The acupuncturist or unblinded team member will complete the post-acupuncture safety assessment at the completion of each treatment administration. This case report form will be reviewed with the unblinded project manager prior to the completion of the treatment visit.

10.3 Adverse Events and Serious Adverse Events

An **adverse event (AE)** is defined as: "Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporarily associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research." An adverse finding can include a significant change in baseline symptoms, abnormal assessments (such as bruising or minor bleeding with needles), or any combination of these.

Examples of AEs are as follows:

- Changes in the general condition of the patient
- Subjective symptoms offered by or elicited from the patient
- Objective signs observed by the Investigator or other study personnel
- All concurrent diseases that occur after the start of the study, including any change in severity or frequency of preexisting disease such as a clinically significant increase in the FIQ-R score.
- Clinically relevant physical findings that occur during the study, this includes the presentation of known side effects from acupuncture treatment (bleeding, fainting, localized skin infection, increased pain, nausea, or vomiting)

Changes in the pain or occurrence of fibromyalgia symptoms noted in the patient's baseline self-report questionnaire will be logged as part of the patient's research record and reported annually. Clinically significant changes, as assessed by the Investigator, will be recorded and assessed for seriousness. This includes the signs and appearance of symptoms of fibromyalgia.

A **serious adverse event (SAE)** is defined as any adverse event that results in one or more of the following outcomes:

- Death
- A life-threatening event
- Inpatient hospitalization or prolongation of existing hospitalization relating to study treatment
- A persistent or significant disability/incapacity hospitalization relating to study treatment
- An important medical event based upon appropriate medical judgment

10.4 Reporting Procedures

All adverse events, regardless of attribution, will be recorded by the study coordinator in the patients research file using the AE CRF. In addition, AEs will be tracked in aggregate using the AE tracking log.

This log will also document corrective actions required and attribute severity, relatedness and expectedness.

The PI will review and acknowledge (by signing the CRF) all recorded adverse events. The AE Log will be reviewed by the Independent Monitoring Committee annually, or more frequently if requested. The U-M IRB will receive at least an annual summary of all AEs in aggregate. This log will also be included in the annual report to NCCAM.

Serious AEs will be reported within 7-days of the occurrence notification. Unanticipated problems or privacy violations/breach of confidentiality will be treated as serious and also reported within 7-days of occurrence to the appropriate oversight body.

10.5 Follow Up for Adverse Events

Any participant that has an adverse event will be followed weekly until either the event has resolved or the event is considered stable by the participant and the research team. Adverse events that persist for an extended duration (such as over the duration of the study) will also be followed weekly until either the event is resolved or considered stable by the PI and the research participant.

10.6 Safety Monitoring

The PIs have designated an Independent Monitoring Committee (IMC) to perform an independent review of ongoing study progress and safety. The Monitoring Committee for this study is comprised of Sawsan As-Sanie, MD, MPH, Scott Peltier, PhD, and Kelley Kidwell PhD. Drs. As-Sanie, Peltier, and Kidwell are not associated with this research project and thus work independently of the PIs, Drs. Richard Harris and Vitaly Napadow. They are not part of the key personnel involved in this grant and each are qualified to review the patient safety data generated by this study because of their unique areas of expertise.

Dr. As-Sanie is an Assistant Professor (Obstetrics and Gynecology) at the University of Michigan specializing in gynecology, the medical specialty that focuses on chronic pelvic pain. Dr. Peltier is an Associate Research Scientist and Laboratory Manager, at the University of Michigan Functional MRI Laboratory and an Associate Research Scientist, Biomedical Engineering. Dr. Peltier's research deals with functional MRI data acquisition and analysis. Current areas of interest include: 1) resting-state functional connectivity; 2) real-time fMRI; 3) multivariate and data-driven analysis techniques; and 4) multimodal imaging. Dr. Kidwell is a Research Assistant Professor, Department of Biostatistics, University of Michigan specializing in clinical trials, adaptive treatment strategies, and survival analysis

The sponsor contact for medical emergencies and SAE reporting:

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The Independent Monitoring Committee will meet annually to review safety assessments and study progress. Refer to the DSMP in Appendix A, for a detailed description of study monitoring activities.

11. STATISTICAL CONSIDERATIONS

11.1. Aim 1: At baseline, characterize altered somatosensory neurocircuitry underlying chronic pain in FM.

Hypothesis 1: Using resting fcMRI, the insula in FM patients will show more correlation with DMN, and more anti-correlation with SMN, compared to HC.

We will use dual-regression independent component analysis (ICA) to evaluate resting connectivity between DMN/SMN and insula. Two-sample t-tests will compare FM vs. HC, cluster-corrected at p=0.05.

Based on out previous studies, assuming DMN connectivity z score (middle insula region) of 1 for a fibromyalgia patient vs. 0.5 for a healthy control with a common standard deviation of 0.5, we will expect a typical relative effect of 1 (mean ICA-derived z score over its standard deviation). Similar effects (negative connectivity values) are expected for SMN-derived biomarkers. With the planned numbers of subjects we will have the power exceeding 97% to detect the effects by a two-sided t-test.

Hypothesis 2: Using ^{1}H-MRS, FM patients will show pain related increased Glx and reduced levels of GABA within the insula and S2 as compared to HC.

Metabolite concentration from regions of interest will be assessed using spectra data input into LCModel. A two-sample T-test will compare FM vs. HC, significant at p=0.05.

Our sample size calculation for difference between FM and HC assumes (from pilot data) that Glx, a proxy for glutamate concentration, has mean(SD) = 10.5(1.4) in arbitrary institutional units (AIU) and a FM-HC relative difference in excess of 10%. Thus we conservatively hypothesize an average Glx around 11.5 AIU and GABA around 1.3 mmol/kg in the FM group. We conclude that we will have the power of at least 84% for the GABA and 81% for the Glx based on a two-sided t-test with unequal N (80 in FM vs. 20 in HC) at the significance level of 0.05.

Hypothesis 3: Using evoked pain fMRI, FM patients will be more sensitive to evoked pressure pain and demonstrate greater insula activation to pain stimuli, compared to HC.

A general linear model will assess brain response to experimental pain from a block design BOLD fMRI run. A group level FM - HC difference map will use a two sample t-test, cluster corrected at p=0.05.

Changes in connectivity z-score for a typical connectivity biomarker are expected to follow a different slope in FM vs. HC patients in response to pressure pain. Based on our previous studies we expect the slope of 1.3 vs. 0.8 (FM vs. HC, respectively) with the standard deviation of the pain score of approximately 1 and standard deviation of the connectivity score at a given level of pressure pain of around 0.5. This ensures we will have over 86% power to detect a difference in connectivity responses to evoked pain.

ROC Analysis

In order to characterize the potential of biomarkers to predict the disease, a decision rule predicting FM status, a binary outcome, based on connectivity, Glx/GABA and evoked pain biomarkers. Our primary method for rule development will be logistic regression. Stepwise variable selection procedures using Likelihood Ratio tests and Akaike Information Criterion (AIC) will be performed to identify the set of significant predictors. A cutpoint on the linear predictor in the logistic model will define the decision rule with values above the cutpoint for a particular subject interpreted as prediction of treatment success for the subject. A set of rules obtained by varying the cutpoint will be summarized by the Receiver-Operating-Characteristic (ROC) curve plotting sensitivity by 1-specificity of the rule. The area under the ROC for the model will characterize the quality of prediction. The area under the ROC will serve as the overall measure of discriminative ability of the model. In order to obtain a realistic estimate of ROC, cross-validation will be used when estimating sensitivity and specificity corresponding to various cutoff values.

The assessment of power is based on a test for area under the ROC. We hypothesize that an area of 0.7 can be achieved. A test for area <0.6 vs. >0.6 shows that we will have 83% power to discriminate area of 0.7 from 0.6 or less with 80 FM patients and 20 controls, using a two-sided test at the significance level of 0.05. This indicates that we will have the precision of at least 0.1 on the area under ROC scale.

11.2. Aim 2: Evaluate longitudinal desensitization effects of electro- and mock laser acupuncture interventions on the neurocircuitry underlying chronic pain in FM, correlate the change in pain with changes in neurocircuitry physiology, study neuroimaging biomarkers as mediators for the treatment effect.

This aim evaluates a ***mechanistic*** value of the neuroimaging biomarkers by studying their response to treatment and correlation between the change in biomarker measurements and changes in pain induced by treatments, and biomarker mediation effects.

Hypothesis 1: EA, which involves greater somatosensory afference, will more readily reduce resting insula connectivity to SMN and DMN compared to ML. The change in pain will correlate with change in insula connectivity for EA, but not ML. Insula connectivity will represent a mediator for the treatment effect.

Treatment effects on connectivity

We will perform dual-regression ICA with group level estimates for baseline and post-treatment fcMRI data (DMN and SMN) entered into a linear mixed model with factors STIM (levels EA, ML) and TIME (levels baseline and post-therapy). Time by treatment interaction effect will be of main interest in the analysis. If a significant main effect or interaction is found, we will perform post-hoc difference map analyses, to evaluate which group produced the greatest change in resting connectivity.

Power analysis for fcMRI is based on data from the preliminary study on intrinsic connectivity and its short-term response to acupuncture. From preliminary data on 15 subjects, we estimate the SD of the number of voxels activated before and after acupuncture in the default mode independent component to be 2860 and 4559, respectively. The covariance between these before and after counts is 3388878. This implies the standard deviation of the within-subject change in connectivity of $\text{sqrt}(2860^2+4559^2-2*3388878)=4710$. Based on the same data, we find the before and after means to be 7078 and 9844, respectively. Then, a two-sided paired t-test for the presence of change will have the effect size of 0.6 and the power of 95% with 40 patients in the EA group.

Based the similar mean connectivity difference of around 3000 between ML and EA, and the same SD of 4710 we will have 80% power to detect the difference between treatments with respect to the mean within-patient change in connectivity.

Correlation with pain

A linear mixed model will be used to regress BPI pain measurements on the neuro-imaging biomarkers. Correlation between clinical pain and biomarkers is the primary target.

We hypothesize a correlation coefficient of at least 0.5 between pain and connectivity z score in the EA arm. This will give us 94% power to detect the correlation.

Interim analysis. After 50% of the subjects have been enrolled and evaluated, we will determine if fcMRI outcome metrics are sensitive enough to continue using for the biomarker for the duration of the study. Screening of biomarkers (brain regions) will be done by computing a post-hoc power based on 20 patients per arm. Biomarkers showing post-hoc power lower than 20% will not be pursued further. This decision corresponds to dropping biomarkers showing correlation coefficients less than 0.18 in the interim analysis.

Mediation Analysis

In the linear mixed model regressing BPI pain score on treatment and time, the effect of treatment on pain is expressed as a regression coefficient for time specific to treatment group (or a treatment by time interaction). Mediation effect will be measured as the proportion of treatment effect explained by the biomarker (PTE) representing a relative change in the regression coefficient when biomarker is included in the model (relative to the coefficient before biomarker inclusion into the model). Standard error for the PTE will be estimated by bootstrap, and a two-sided 95% interval for the effect will be constructed. Wald test with the statistic of the form (change in regression coefficient)/(bootstrap SE) will be used to test for the mediation effect. Similar analyses will be done using a logistic regression model with clinically relevant reduction in pain (defined as 50% and 30%) as a response.

Power. We use Sobel's test based on linear regression to compute power. Power analysis targets biomarkers with the mediation effect of PTE=0.5. We assume that the regression coefficient in the model for the treatment effect on connectivity is 3000; treatment effect beta in the model of change in pain excluding the biomarker is 2; standard deviation of the randomized treatment variable 0.5; standard deviation of the change in connectivity over time 4500 voxels; squared the correlation between the treatment variable and the change in connectivity 3.7e-05; standard deviation of errors in the full model regressing change in pain on both treatment and the biomarker 1.5. These assumptions are consistent with the ones made previously in the power calculations related to different lags of the mediation analysis, with our preliminary data, and with the acupuncture effects on pain reported in the literature. Under these assumptions we will have the power of 81% to detect the mediation effect by Sobel's test.[114]

Hypothesis 2 : EA will more readily reduce Glx and increase GABA in the insula as compared to ML, which will not reduce Glx and GABA. The change in pain will correlate with change in insula GABA and Glx for EA but not ML. Glx and GABA will represent mediators for the treatment effect on change in pain.

Similar to above, linear mixed models and paired t-test analyses will evaluate, separately, Glx and GABA concentration levels taken from baseline and post-therapy H-^{1}MRS scans.

A reduction in the Glx of about 1.5 arbitrary institutional units (AIU) with a standard deviation not exceeding 0.6 is expected in the EA group. At the same time in the ML group a much smaller effect is expected (around 1 AIU, same standard deviation). This ensures 95% power when using a two –sided test. A similar analysis for differences in the change in GABA of 0.3 vs. 0.1 mmol/kg with a standard deviation of 0.25 gives 94% power to detect a difference.

Correlation between Glx/GABA and clinical pain (BPI) will be analyzed similar to the fcMRI biomarkers. The study has 94% power to detect correlations of 0.5 and higher.

Similar to fcMRI biomarkers, if Glx or GABA show less than 20% post-hoc power after half of the patients have been measured (correlation less than 0.18), their longitudinal measurements will be discontinued for the remaining patients.

Mediation Analysis is similar to the one proposed for connectivity.

Hypothesis 3: EA will more readily reduce pain-evoked fMRI activation in the insula as compared to ML, which will not reduce pain-evoked fMRI activation. The change in pain will correlate with change in insula response to evoked pain for EA but not ML. Insula response to evoked pain will represent a mediator for the treatment effect on change in pain.

Parameter estimates from evoked pain block-design fMRI scans will be used in a linear mixed model and post-hoc paired t-tests, similar to the approach described above.

Changes in connectivity z-score for a typical connectivity biomarker are expected to follow a different slope in EA vs. ML patients in response to pressure pain. Assuming the slope of 1.3 vs. 0.5 with the standard deviation of the pain score of approximately 1 and standard deviation of the connectivity z score at a given level of pressure pain of around 1. This ensures we will have over 94% power to detect a difference in connectivity by pressure pain slope responses with 40 patients per treatment group.

Change in pain-evoked fMRI activation will be correlated with the change in clinical pain. To do this evoked pain responses will be added as covariates into the linear mixed model regressing BPI pain on other covariates.

Mediation Analysis is similar to the one proposed for connectivity.

11.3. Aim 3: Evaluate the ability of altered neurocircuitry at baseline to predict clinical response.

This aim evaluates a ***predictive*** value of the neuroimaging biomarkers by studying whether their measurements at baseline correlate with pain responses to treatments.

Hypothesis 1: Greater baseline DMN-insula/S2 connectivity and SMN-insula/S2 anti-correlation, will display greater clinical response to EA vs. ML

We will use baseline estimates of DMN and SMN connectivity, taken from the dual regression ICA analysis, as independent variable in a multiple regression model with dependent variable set as the change score (post-therapy – baseline) for clinical pain (Brief Pain Inventory; BPI). Regression slopes will be contrast between different pairs (e.g. EA vs. ML) set in the same regression model. We will explore within-group correlations for individual therapies if the difference map for any two therapies is significant, at cluster corrected p=0.05.

Hypothesis 2: FM patients with greater baseline insular and S2 Glx/GABA ratios will display greater clinical response to EA as compared to ML.

An identical approach will be used as for hypothesis 1 above, but with Glx/GABA ratio as dependent variable.

Hypothesis 3: FM patients with greater baseline pain-evoked insular activation will display greater clinical response to EA as compared to ML.

An ROI analysis will determine baseline insula response to evoked pain. This parameter estimate will then be used as a dependent variable in an identical multiple linear regression approach, as detailed above.

Power. The expectations for correlations between the baseline measurements of biomarkers and clinical pain responses are set similar to the mechanistic setting. We expect to uncover correlations of 0.5 between measurements at baseline and the change in pain in response to treatment (mainly in the EA arm). We will have 94% power to detect such correlations.

Interim analysis. Similar to the mechanistic study we will conduct an interim analysis after 20 patients in each arm have been measured. Given the focus of this study on mechanistic value of the biomarkers, mechanistic analysis will take priority to the predictive setting, and the results of the predictive interim analysis will be treated informally. A study team decision will be issued on biomarkers that do show mechanistic value yet do not make the cut in the predictive analysis.

11.3.2 Exploratory analysis of diet and brain neurochemistry and functional connectivity

Data from the Harvard food diary obtained at baseline for both FM and healthy control participants will be correlated with molecular (glutamate and GABA) and functional connectivity MR data. A general linear model

will be constructed within SPM having the neuroimaging data be the dependent variable and diet outcomes and group status be the independent variables. As this is an exploratory analysis, no *a priori* hypotheses are made.

11.4. Handling of missing data: Reasons for missingness will be analyzed using multinomial logistic regression with treatment as one of the covariates. The results of formal missing data imputation by predictive-matching algorithms will be compared to the results of missing data exclusion in a sensitivity analysis.

12. DATA COLLECTION AND QUALITY ASSURANCE

12.1 Data Collection Forms

All study related information will be collected either via interview with assessments recorded directly onto a case report form, or via self-report questionnaire as per the table below.

FORM NAME	Data Collection Method
Screening & Demographics	
Informed Consent	Study Coordinator/Team Member
Tender Point (1990 ACR FM Criteria)	Study Coordinator/Team Member
FM Survey Questionnaire (FSQ - Wolfe et al 2011 criteria for FM)	Patient
FM Treatment History	Patient
VAS Recall (inclusion) Pain	Patient
Medical History	Patient
Physical Assessment (Height/Weight)	Study Coordinator/Team Member
Urine Pregnancy Test	Study Coordinator/Team Member
HADS	Patient
PHQ-9 (suicidal ideation)	Patient
Socio-Demographics	Patient
Demographics	Patient
Concomitant Medications	Patient
Self-Report	
BPI – Pain Severity and Interference	Patient
FM Survey Questionnaire (FSQ)	Patient
PROMIS - 29	Patient
Perception of Bodily Sensations	Patient
Pain Catastrophizing	Patient
Credibility Scale	Patient
PainDETECT	Patient
Pittsburgh Sleep Quality Index (PSQI)	Patient
MASQ (cognition)	Patient
VAS-Present pain	Patient
Expected Relief Scale (ERS)	Patient
Desire for Relief Scale (DRS)	Patient
Menstrual Questionnaire	Patient
Quantitative Sensory Testing	
Pain Assessment	Study Coordinator/Team Member

After Cuff Questionnaire	Patient
CPM	Study Coordinator/Team Member
Visual Stimulation Assessment	Study Coordinator/Team Member
Imaging	
Neuroimaging	Imaging Technician/Patient/Study Coordinator/Team Member
Treatment	
Randomization	Acupuncturist
Assessment of Needling Sensation (MASS)	Patient
Acupuncture Procedure	Acupuncturist
Safety	
Adverse Event form	Acupuncturist/Project Manager/Study Coordinator/Team Member
Fibromyalgia Impact Questionnaire – Revised (FIQ-R)	Patient
Debriefing / Study Exit Form	Project Manager/Study Team Member

All subjects will be assigned a unique, study-specific identification code. Only the subject ID will be used to associate the subject with source data listed above. Any personal identifiers required to manage the subject while they are participating in the study will be stored separately from the research data files. Those volunteers who do not meet the eligibility criteria or who choose not to participate in the study at either the telephone Pre-Screen or the Screening Visit will be logged. Personal identifiers will be kept until the study has been closed to recruitment, at which time they will be destroyed. This will prevent repeat screenings of potential volunteers.

12.2 Data Management

The University of Michigan Chronic Pain and Fatigue Research Center will be responsible for the collection of all source information. All Case Report Forms will be approved by the University of Michigan IRB.

Participants will complete paper-based questionnaires; no electronic survey completion will occur. Data will be verified and checked for completeness and initialed by the study coordinator or other team member prior to the completion of the visit. All data will be entered into a research database (REDCap) by the study team using double data entry. A blinded team member will enter data into database one, while another study team member will enter the data into the second database. In extenuating circumstances an unblinded study team member may enter data into database two if needed. Each participant's data will be entered only after the participant has exited the study. Cross-referencing the two databases for disparities will occur annually. Discrepancies will be corrected in REDCap via source document verification.

Study data will be collected and managed using REDCap electronic data capture tools hosted at University of Michigan. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

12.3 Imaging Repository

12.3.1 Pain and Interoception Imaging Network (PAIN)

This trial has a data-sharing agreement with the Pain and Interoception Imaging Network (PAIN), an NCCAM-funded imaging repository at University of California Los Angeles (UCLA). The PAIN initiative is aimed at the characterization of brain signatures associated with chronic pain disorders. The PAIN repository is specifically targeted towards using neuroimaging for discovery of mechanisms and biomarkers related to chronic pain and chronic pain treatment. (http://pain.med.ucla.edu/)

12.3.2 Shared Data

High quality structural and resting state functional images taken from the baseline and follow-up imaging sessions (visit 2 and 12) for fibromyalgia participants and from healthy control's sole baseline imaging session (visit 2). In addition to these image files, we will also submit relevant metadata (sex, age, diagnosis). A list of subject IDs and acquisition dates will be provided to UCLA each time data is transmitted.

12.3.3 Confidentiality

All transferred data will be stripped of identifiable information and will be transferred to the PAIN Repository using the SFTP Interface for Archived and Standardized Data. Data are separated from personal identifiers through the use of a code. The code key will be held by the contributor only, and the PAIN Repository will never retain this linking file or have access to the code. The code will be kept in a secure location, which can only be accessed by the research team at the University of Michigan.

12.3.4 Transmission and Storage of Repository Data

The PAIN Repository transfers all data via secure network protocols (https and sftp). Member permissions and provenance are maintained via user and group accounts and a relational database management system. Images are parsed and scanned to both extract scan specifications and ensure long-term file integrity. Algorithmic fingerprint "checksums" protect against digital deterioration (bitrot) by mathematically matching the stored image to the original. Data are stored on a dedicated system separate from user application servers. Only administrators and automated system processes (eg. backup) can access data outside of collaboratively-defined repository datasets. Servers run on NIST Common Criteria compliant Red Hat Enterprise Linux with NSA-developed role-based security (SELinux) enabled. Network security includes firewalls and private local area networks (LANs) with perimeter network (DMZ) access to web servers.

PAIN data are stored in a scalable, secure, fault-tolerant, high availability location at the Oppenheimer Center for Neurobiology of Stress at the University of California, Los Angeles. Data submitted to PAIN will be stored indefinitely.

12.3.5 Accessibility

PAIN data will be used for the purposes of research and analysis, and is accessible by PAIN network authorized investigators only, who have an accepted data sharing agreement and pre-approval from the PAIN Executive Committee (EC).

12.3.6 Participant Authorization

Participants will have the option to decline participation in the PAIN repository portion of the study. A separate section of the informed consent form (ICF) will be reserved for participants to choose whether they agree to participate in the repository by checking "Yes" or "No" and initial. Participants may withdraw their consent to future donation of data to the repository, however any data already submitted to PAIN will not be retractable but will remain coded.

12.4 Quality Assurance

12.4.1 Training

All study team members are required to be certified by the University of Michigan Program for Education and Evaluation in Responsible Research and Scholarship (PEERRS). In addition, research staff are required to follow study specific operating procedures as documented in the Manual of Operations and approved by the study PI.

Dr. Harte will be responsible for training the study team in testing procedures, and together with Ms. Scott, will ensure study is conducted in accordance with GCP.

Acupuncturists will be trained in the treatment techniques by Dr. Richard Harris, study PI. Acupuncturists will be instructed to follow a script throughout their interactions and will be required to attend re-training sessions.

12.4.2 Quality Control Committee

There is no quality control committee for this trial.

12.4.3 Metrics

All outcome measures will be assessed for quality. If an outcomes value is above or below the possible range for that outcome, the data will be flagged for review.

12.4.4 Protocol Deviations

Protocol deviations will be documented on the visit checklist at each visit by the study team member responsible for that visit. The study coordinator will review the deviation and report to the study team via the Deviations Tracking Log. Corrective Actions, if required, will be noted in the subject file and study documentation.

12.4.5 Monitoring

NCCAM Clinical Monitoring Service will conduct periodic on-site reviews of the study, including study initiation visit, periodic site visit and a study closeout visit.

The study team will meet at least quarterly to review and calibrate testing procedures.

Internal quality assurance reviews performed by the study team will occur annually. This process will entail the review of 10% of randomly selected cases.

The acupuncturist(s) performing the treatment will meet with Dr. Harris at least annually to review and calibrate treatment procedures.

13. PARTICIPANT RIGHTS AND CONFIDENTIALITY

13.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the U-M IRB.

13.2 Informed Consent Forms

Written Informed Consent will be obtained from all participants at the screening visit and prior to undergoing any research related activity. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A signed consent form will be obtained from each participant. A copy will be given to each participant or legal guardian and this fact will be documented in the participant's record.

13.3 Participant Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NCCAM, and the OHRP.

13.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NCCAM, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

14. COMMITTEES

There are no additional committees beyond the DSM committee associated with this study.

15. PUBLICATION OF RESEARCH FINDINGS

The study governance does not include a Steering Committee.

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17. LIST OF APPENDICES

A. AcuAfference Data Safety Monitoring Plan

B. Outcome Measures
- Fibromyalgia Survey Questionnaire (FSQ)
- VAS Pain Scale (Present Pain)
- VAS Pain Scale (7 day recall)
- Hospital Anxiety and Depression Scale (HADS)
- Patient Health Questionnaire (PHQ-9)
- Brief Pain Inventory (Severity and Interference scales)
- PROMIS – 29
- Pain Catastrophizing Scale (PCS)
- Credibility Questionnaire
- PainDETECT
- Menstrual Questionnaire
- After Cuff Questionnaire
- Desire of Relief (DRF)
- Expected Relief Scale (ERS)
- MGH Assessment of Needling Sensations (MASS)
- MASQ
- Perceptions of Bodily Sensation (PBS)
- Pittsburg Sleep Quality Inventory (PSQI)

C. Case Report Forms / Safety Review Forms
- Pre-Screen Interview
- Enrollment / Registration Form
- Demographics Form
- Sociodemographics Form
- Tenderpoint Count
- Medical History
- FM and FM treatment history (including acupuncture/alternative therapies)
- Health Status (including results of pregnancy test)
- Medications
- Eligibility Checklist

- Neuroimaging Safety Screen
- fMRI form/checklist
- Visit checklist / Protocol deviation form
- Post-Acupuncture AE assessment
- Fibromyalgia Impact Questionnaire - Revised
- AE reporting form
- Early termination / study exit
- Adverse Event Tracking Log
- Minor Deviation Tacking Log

D. Spreadsheet of Study Activities