An Investigation of Functional Brain Organization in Chronic Centralized Pain

By

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Dedication

To Tony, Doris, Desmond, Daisha, David and Khyra

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List of Abbreviations

ACC: anterior cingulate cortex
CNS: central nervous system
CPM: conditioned pain modulation
dACC: dorsal anterior cingulate
dlPFC: dorsolateral prefrontal cortex
CEN: central executive network
DMN: default mode network
DAN: dorsal attention network
FM: fibromyalgia
fMRI: functional magnetic resonance imaging
HADS: Hamilton Anxiety and Depression Scale
HC: healthy controls
IPL: inferior parietal lobule
M1: primary motor cortex
MD: mediodorsal thalamus
mPFC: medial prefrontal cortex
MFG: middle frontal gyrus
NMI: normalized mutual information
NRS: numerical rating scale
PCC: posterior cingulate cortex
Phi: Pearsons phi coefficent
QST: quantitative sensory testing
RSN: resting state network
S1: primary somatosensory cortex
SFG: superior frontal gyrus
SLN: salience network
SMA: supplemental motor area
SMN: somatosensory and motor network
STG: superior temporal gyrus
VAS: visual analog scale
V1: primary visual cortex
V3: visual association cortex
VIS: visual network

Abstract

Pain is a complex and subjective experience often associated with noxious stimulation. However, in the past several decades it has become clear that the central nervous system can augment or even cause pain in the absence of noxious input. For some individuals, the most common cause of pain is related to altered central nervous system function and collectively referred to as chronic overlapping pain conditions (COPCs). Together, these conditions are characterized by diffuse hyperalgesia (an elevated response to painful stimuli) and allodynia (perception of pain for non-noxious stimuli), in addition to sleep disturbances, fatigue, and mood disorders. Moreover, many chronic pain patients also have increased sensitivity to multiple sensory stimuli, that include light suggesting a global dysfunction in sensory processing. This dissertation aims to examine intrinsic network alterations in the central nervous system in fibromyalgia (FM), a prototypical chronic pain condition, and determine how aberrant visual processing may be associated with sensory hypersensitivity in chronic pain conditions.

Neuroimaging has enhanced our understanding of the neural correlates of pain. Pain processing in the CNS normally occurs via ascending and descending nociceptive pathways that integrate in several brain regions to include the insular cortex. Resting state functional MRI has shown aberrant intrinsic functional connectivity among chronic pain patients between the insula and the Default Mode Network (DMN) an intrinsic network associated with self-referential thinking. To elucidate the complex nature of pain and general sensory processing in chronic pain, we examine the relationship between multiple brain regions simultaneously using graph

theoretical analyses. Communities are a collection of nodes or brain regions that are more connected to each other than to other communities.

In Chapter 2, we investigate the network organization of the brain through intrinsic functional communities generated from resting state functional MRI data of individuals with FM and healthy controls. Composition of community structure between two networks were assessed using normalized mutual information (NMI). We found that the global network community structure in chronic pain patients is less stable (more variable, lower within-group NMI). Subsequent analyses of node community assignment revealed the composition of the communities differed between chronic pain patients and controls at rest.

Many individuals with chronic nociplastic pain conditions, such as fibromyalgia (FM), experience multimodal (i.e., tactile, visual, auditory, olfactory) sensory hypersensitivity. Moreover, the anterior insula plays a role in pain and conscious processing. In Chapter 3, we also measured brain network architecture with functional MRI during an aversive visual task (flashing checkerboard) in individuals with FM and healthy controls. We show that whole brain network community structure was more variable compared to that of HC during the visual stimulus (i.e., lower between-group NMI). As the anterior insula plays a role in pain, multisensory integration and gating conscious experience, we show the variability of insula community assignment during the visual stimulus was positively associated with clinical pain in FM.

Together this dissertation research demonstrates that intrinsic network communities differ substantially between individuals with FM and healthy controls. These differences may represent a novel aspect of the pathophysiology of chronic nociplastic pain.

Chapter 1 : Introduction

Pain is an unpleasant subjective experience that does not always completely match nociception, the afferent sensory transmission of painful stimuli, or stimulus intensity. The experience and expression of pain is expressed by self-report and based on the point of reference largely determined by prior experience [1]. An adaptive process to alert the body of threat or ongoing damage, pain can be modulated by internal and external factors. When pain is widespread and persistent, it may be augmented or caused by changes in central nervous system (CNS) processing, and considered nociplastic [2]. Pain perception, modulation, and experience that can contribute a negative impact on social and psychological well-being for many individuals, especially when of a chronic nature. Objective neural signatures of CNS processing associated with nociplastic pain and further hypersensitivity to pain and other sensory modalities among individuals remain the focus of many research programs and the focus of this dissertation.

Pain processing in the Central Nervous System

Normal brain function allows for the integration of sensory inputs to produce a physiologic response associated with its experience. Nociception occurs via the integration of ascending nociceptive pathways and descending modulatory pathways in the cortex and include the thalamus and brainstem, cingulate, somatosensory, prefrontal, and insular cortices [3-6]. Peripheral drivers, such as noxious stimuli or inflammation, activate nociceptors that send signals to the CNS via the spinothalamic tract. The thalamus then relays information to the somatosensory cortex and insula as well as the cingulate and prefrontal cortex. In aggregate these brain regions make up the ascending nociceptive pathway and are considered pain promoting, or pro-nociceptive brain regions meaning that when these areas are activated, pain is enhanced. From the cortex, neuronal projections from the anterior cingulate and insula cortex, as well as the prefrontal cortex project to the midbrain, terminating in the periaqueductal gray. The

periaqueductal gray plays a critical role in the endogenous modulation of pain indirectly, through interacting with the ventromedial medulla which has direct neuron projections back to the dorsal horn of the spinal cord. Together these brain regions make up the descending modulatory system and contribute to neural processes that modulate nociceptive processes in the central nervous system [3, 5-9].

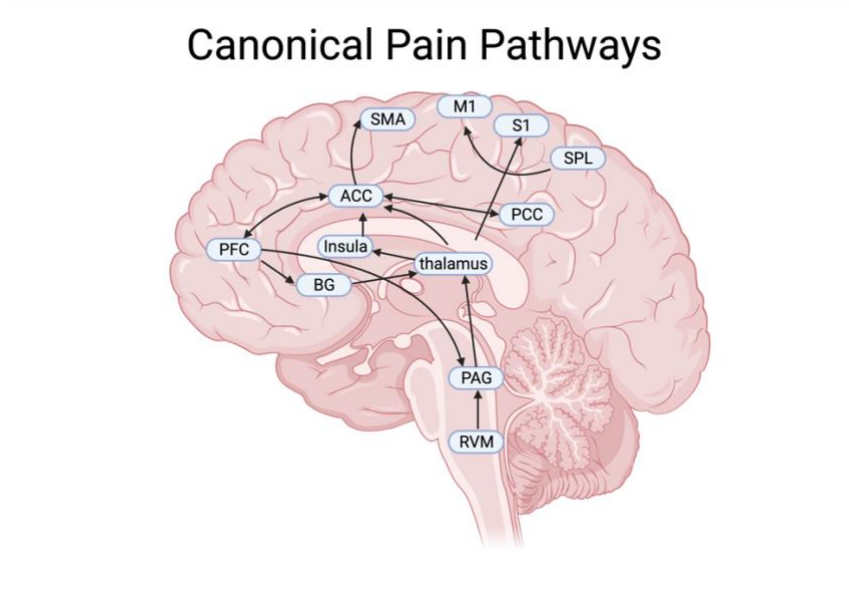


Figure 1.1 Canonical pain pathway: Sensory information from the peripheral enters through the ascending nociceptive pathway by way of the brainstem and thalamus, which send nociceptive signals to the primary sensory cortex, and insula. In turn, the descending pathway modulates the nociceptive information coming in through the anterior cingulate, insula cortex, periaquductal gray and medulla. and considered to be antinociceptive. Affective and cognitive components can amplify or modulate aspects of these pathways, as well as differences in neurochemistry and activity among many of these brain regions have been reported across chronic pain conditions.

The multidimensional aspects of pain processing can be divided into sensory, affective, and cognitive components and their corresponding neural correlates. It is clear the interaction of the neural processes that underlie these internal and external factors overlap and interact with the ascending nociceptive and descending modulatory circuits that influence or modulate the perception of pain. For example, the sensory component is initiated through noxious stimuli (e.g., thermal, or mechanical, or chemical) which is associated with increased activity in pro-nociceptive brain regions, associated with amplification of the pain percept measured through self-report [3, 7]. Mood or affect typically have negative effects and exacerbate the pain experience, while context contributes to whether stimuli are perceived painful or not. Anticipation of pain may also amplify the pain percept, whereas expectation of a

noxious stimulus modulates or reduces perceived pain. [6, 10-16]. Together these changes in percept are linked with activity in the brain in which reduced pain may be associated with reduced activity in ascending pathways or increased activity via descending modulatory pathways.

Yet, it is clear the central nervous system (CNS) can augment or cause pain in the absence of nociceptive input.

Nociplastic pain

Chronic pain affects approximately 20% of adults in the United States. Nearly 8% of adults in the United States experience chronic pain that impacts their everyday life [17]. There are three main mechanisms of pain identified by the International Association for the Study of Pain. These are: nociception, neuropathtic and nociplastic. As previously described, nociceptive pain arises from actual or the threat of damage to non-neural tissue and is activated by nociceptors. Neuropathic pain is a clinical descriptor of pain associated with lesions or disease that result in damage to neural tissue. Recently the International Association for the Study of Pain introduced a third category of pain defined as nociplastic pain, or clinical pain that occurs different from altered nociception and neuropathic pain, with sensitization as the major underlying mechanism [2] [18]. This type of pain is thought to originate from dysfunction in the CNS. However, the location of these central nervous system changes remains unknown. This abnormal state of responsiveness, may contribute to the development or maintenance of nociplastic pain [19, 20] Nociplastic pain can occur in the absence of peripheral insult and persist throughout the life for patients with chronic pain conditions such as fibromyalgia, chronic fatigue syndrome, migraine, temporomandibular disorder, endometriosis, and low back pain, collectively referred to as Chronic Overlapping Pain Conditions (COPCs) [21].

Fibromyalgia (FM) is a condition in which patients experience chronic widespread pain, fatigue, and sleep disturbances. Patients also report psychological or cognitive difficulties, mood disturbances, and heightened sensitivity, suggestive of a chronic multisymptom illness [22] [23] [24]. FM is often associated with a stressor whether it is known or unknown, that could be psychosocial, viral infections, physical trauma, or deployment to war. Left untreated, many patients may develop comorbidities

including depression and anxiety [25] [26] [27-29]. As a result, patients with chronic pain may also ruminate or catastrophize, in which they develop and/or express negative affect about their pain ultimately impacting the pain modulation [30, 31]. This highlights only one aspect of how cognitive and affective components of pain processing may interact with overlapping psychiatric conditions that impacts or influence pain perception and adds a layer of complexity to the overarching problem of identifying objective measures of pain and sensory processing.

Individuals with FM also report heightened sensory response to painful (hyperalgesia) or non-painful stimuli (allodynia). Of note, recent evidence suggest nociplastic pain may be associated with increased sensitivity that extends beyond the classic tender points to supraspinal regions as many individuals report increased sensitivity to bright lights and sounds as well. The prevalence in which nociplastic pain occurs varies across chronic overlapping pain conditions, as an individual may experience a combination of peripheral (nociceptive or inflammation) and central (sensitivity) drivers [19] [32] [33]. Moreover, central sensitization or amplification of sensory stimuli via the CNS is considered the primary cause of fibromyalgia (FM) [19, 32, 33].

In summary, acute pain is typically nociceptive in nature, but may also be chronic for some conditions, whereas chronic pain is often clinical wherein the perception of pain can be both spontaneous and persistent as well as exacerbated by physical activity. As nociceptive pain processing may begin in the periphery, faciliatory and modulatory processes occur in the CNS that may contribute to the pain experience. The nature of clinical pain can also be neuropathic, associated with damage to neural tissue, or nociplastic, due to changes central nervous system processing observed via increased sensitivity in the absence of sensory input [33, 34]. Of note, stimulus intensity does not always equal the amount of pain self-report, as individual differences in pain sensitivity in addition to cognitive and emotional factors (e.g., mood, expectation, anticipation, context) also influence the perception of pain and contribute to the pain experience [6]. Defining the central neural correlates of painful sensory processing provides an objective measure and deepens our understanding of the subjective experience of pain and increased sensitivity associated with nociplastic pain.

Measuring Pain through quantitative sensory testing and self-report

In the laboratory, well controlled experimental methods are used to investigate the relationship between sensory stimuli and the sensations they produce. These experiments (collectively termed quantitative sensory testing: QST) provide a psychophysical assessment of nociception that can be measured through the systematic administration of multiple sensory modalities (i.e., pressure, electric, thermal, auditory, and visual). The gain or loss of sensory function is measured through the detection of sensory thresholds, or ratings of self-report of the perceived stimulus [35-37].

In animal models, a behavioral response (e.g., reflex withdrawal) to remove the part of the body exposed to the noxious stimuli, potentially an attempt to reduce the experience associated with the noxious stimulus, is quantified to determine primarily nociceptive thresholds. In human studies, there are several questionnaires used to evaluate pain in healthy and clinical populations that vary in how they were developed, psychometric function and use, as well as their ability to detect change [38, 39]. The two more commonly used measures of pain ratings are visual analog scale of (VAS) and the numeric rating scale (NRS) as measures of painful intensity. Still, to date the most reliable and only true assessment of pain is self-report. However, higher self-report of pain sensitivity has been associated with anxiety and not correlated with actual pain thresholds emphasizing the importance both self-report and sensory testing to more adequately probe pain sensitivity [40, 41].

Quantitative assessment of neural processes associated with sensory stimuli identifies neural circuits and pathways of nociception and associated neural processes. These objective physiologic measures of sensations allow researchers to administer stimuli and quantify individual differences in pain thresholds, tolerance, and sensitivity in addition to modulation.

Sensory Sensitivity

Sensitivity to pain is widely distributed across the population and depicts a bell-shaped distribution. As such, individual differences in self-report of pain whether it be experimental or clinical pain largely overlap in both healthy and clinical populations [42-44]. To properly measure pain sensitivity, QST must assess both pain sensitivity and pain inhibiting (modulation) properties, separately.

Evoked pain and clinical pain may be due to separate phenomena and that using QST to probably probe pain sensitivity and inhibition is needed to further understand how sensory sensitivity interacts with nociplastic pain to understand the underlying mechanisms involved in sensory hypersensitivity in chronic pain populations [41, 44-46]. Recent analysis of children with widespread pain show that altered CNS connectivity (see below) may occur prior to the development of multisite chronic pain [47]. Understanding the transition from acute to chronic pain and how sensory sensitivity emerges, persists in nociplastic is a focus of ongoing research.

QST has been used to probe altered CNS function in FM. These patients report diffuse or widespread hyperalgesia and increased sensitivity to painful stimuli that extends beyond the tender point, lesioned area, or diseased structure [18, 21, 23, 35, 48, 49]. This increased sensitivity is in response to both noxious and non-noxious stimuli, and further suggests dysfunction of the nociceptive system and/or endogenous modulation (pain inhibition) activity or the interaction between them [46, 50, 51]. FM patients are also sensitive to supraspinal stimuli (e.g., sound, odor, and light) [19, 28, 52, 53], report significantly higher sensory sensitivity to both tactile and non-somatic (auditory and olfactory) stimuli [52]. FM patients have also been shown to be sensitive to visual stimuli [53, 54], wherein Harte and colleagues (2016) showed that FM patients report a visual stimulus to be more unpleasant, when compared to healthy controls [55]. Together this suggests that within nociplastic pain patients, altered CNS processing may be associated with sensory amplification or global sensory hypersensitivity [19, 55].

One way test CNS function is to assess conditioned pain modulation in which a test stimulus is presented and rated in isolation. A conditioning stimulus (which may engage the descending modulatory pathway) may as a result reduce the subsequent ratings of the test stimulus when presented concurrently. Similar to a pain inhibits pain phenomenon, individuals report lower pain ratings to the test stimulus [56-58]. This inhibitory modulation of noxious signals occurs at both the spinal and supraspinal levels and is engaged in response to not only painful stimuli but multiple psychological or cognitive factors as well [57, 59]. In humans, conditioned pain modulation (CPM) is such an indicator of altered CNS processing. Individuals with FM show deficient or failed CPM [60, 61] suggesting that this could be a mechanism of

chronic pain. Neuroimaging studies have revealed this mechanism engages the endogenous pain modulatory processes in which orbitofrontal cortex, rostral anterior cingulate, periaqueductal gray and medulla are associated [53, 62-64].

Lack of a CNS Locus for Pain

None of the brain regions involved in pain processing are uniquely associated with the perception of pain. They contribute to many other sensory, cognitive, and emotional processes outside of the pain processing [6, 65-68]. Therefore, coordinated activity within a network of brain regions is thought to facilitate and modulate the perception of pain. While pain is typically associated with sensation, sensitivity to pain (observe in nociplastic pain) may be associated with the balance of faciliatory (not always nociceptive) and modulatory (antinociceptive) processes. Furthermore, this coordinated activity may be disrupted in the context of chronic nociplastic pain.

Functional organization of the brain

To understand the complex nature of pain and sensory processing in chronic nociplastic pain it is imperative to understand the intrinsic organization of the brain and the integration of sensory, affective, and perceptual processes in the cortex. The brain consists of multiple levels of neural dynamics that largely depend on the connections between different brain regions or functional systems [69]. Resting state functional magnetic resonance imaging (rsfMRI) can be used to understand the intrinsic dynamics of brain (i.e., connectivity), which have elucidated the impact of pathological states in the central nervous system [70-72]. Functional connectivity between a particular brain region, or seed, and all other regions in the brain is determined by the covariance in BOLD signal between those regions over a fixed amount of time. This relationship provides useful information about how brain regions may be functionally linked and further their role in behavior or symptomology[73-75].

Multiple regions in the brain are intrinsically connected, forming structurally and functionally relevant sub-networks referred to here as resting state networks (RSN) [69, 76-78]. These networks include: the visual and somatosensory and motor network, which corresponding with primary visual cortex (V1) and primary sensory and motor cortex (S1/M1); the default mode network (DMN) [in which

the medial prefrontal cortex (mPFC) and posterior cingulate cortex (PCC) are major hubs] is typically associated with self-generated operations such as self-referential thinking; the central executive network (CEN) is involved in attention, goal-directed behavior and working memory [consisting of the dorsolateral prefrontal cortex (dlPFC) and posterior parietal cortex (PPC)]; and the salience network (SLN) [consisting of key hub structures such as the insula and dorsal anterior cingulate cortex (dACC)] which is involved broadly in salient driven emotion and sensory processing. Of note, the activity between the CEN and DMN are typically anticorrelated.

The perception of pain and other sensory processes are dynamic and require flexible integration of neural processes that can be observed among functional connections within and between many of these brain networks. This suggests a central mechanism of pain and sensory processing that coordinates the dynamic switching of neural processes and is involved in integrating both sensory and perceptual processes. The SLN is positioned as a central integrator of salient information for which internal (e.g., homeostatic) and external (cognitive) oriented behaviors follow [79-83]. Anatomically, the anterior insula receives input from the spinothalamocortical pathway via the posterior and mid insula [84]. The insula has structural connections to cingulate, supramarginal gyri and angular gyri (brain regions associated with inferior parietal lobule/DMN [85]. As part of the SLN, the insula and anterior cingulate serve specific roles with the insula being modality specific for sensory or salient processing and the anterior cingulate the modulation of signals to the cortex. The insula is also part of both the ascending and nociceptive pathway. As a part of the ascending nociceptive pathway, pain receives input from the thalamus. Moreover, the insula integrates sensory and affect processes contributing to the complex insula processing of pain perception [81, 86].

Central Nervous System changes in Chronic pain states

Neuroimaging techniques have identified structural, functional, and neurochemical changes in the brain associated with chronic pain states. Chronic pain patients have increased resting state functional connectivity or decreased anticorrelations between the default mode network and the anterior insula [87] [88]. Napadow and colleagues (2010) show that increased connectivity between the DMN and the

anterior and mid insula were correlated with increased clinical pain in individuals with FM [87]. Alterations in DMN and SLN activity and functional connectivity have been observed in several chronic overlapping pain conditions including chronic low back pain [89], knee osteoarthritis [90], rheumatoid arthritis [91], urologic chronic pelvic pain [92], and ankylosing spondylitis [93]. Patients with FM and urologic chronic pelvic pain also have higher insula connectivity to the SMN [92, 94].

The insula is also implicated in sensory and pain processing in chronic pain states. Insular increased regional blood flow and BOLD activity is increased when pressure is applied to the left thumb of individuals with FM during low and high pressure and only high pressure in healthy controls [95, 96]. Glutamate levels in the insula, measured by proton spectroscopy, are also higher in patients with FM compared to healthy controls [97]. In fact, improvement of pain and hyperalgesia (sensitivity to painful stimuli) following treatment with pregabalin was greater in FM individuals displaying higher baseline insular glutamate and DMN to insula connectivity. Also following pregabalin treatment, grey matter volume in the left and right posterior insula was reduced in FM [98]. Of note, evoked pain functional connectivity between the left posterior insula and the right inferior parietal lobule, and medial/middle frontal cingulate regions was affiliated with the DMN and further associated with reduced clinical pain. In aggregate, these findings suggest that the insula plays a key role in the relationship between brain connectivity, neurochemistry, pain sensitivity, and clinical pain in FM.

Recently, Harte and colleagues (2016) show that patients with FM have increased brain response in the insula to an aversive visual stimulation (flashing checkerboard), which also was associated with greater clinical pain [55]. Moreover, anterior insula activations during the presentation of the visual stimulus were reduced following pregabalin treatment. However, the relationship between brain network activity and neural mechanisms associated with visual hypersensitivity among chronic pain patients remain largely unknown.

Sensitivity in chronic nociplastic pain

Patients with FM report increased sensitivity to sensory stimuli that extend beyond touch to include light, suggestive of global sensory hypersensitivity [53, 55]. Additionally, women with FM find innocuous

tactile and auditory stimuli they encounter in their everyday environment significantly more unpleasant compared to HC [52]. Together, this suggests that in addition to hyperalgesia and allodynia, central sensitization may be associated with global sensory hypersensitivity.

Circuit model for salience in multisensory integration

The anterior insula and dorsal anterior cingulate cortex integrate external sensory information with internal affective and bodily signals and play a role in integrating multiple sensory and cognitive processes as a function of the SLN [8, 86, 99]. Menon and Uddin (2010) suggest the anterior insula plays a role in detection of salient stimuli, and the anterior cingulate cortex further modulates these responses to the rest of the brain, primarily sensory, motor and association cortices [80]. On a network level, these regions help integrate bottom-up attention with top-down control to further process incoming sensory stimuli and facilitate subsequent attention and cognitive processes via the central executive network while disengaging the DMN across multiple sensory modalities. This model suggests that the insula is integral to dynamically switch between stimuli to focus attention on external stimuli as a function of the SLN and perhaps multisensory integration.

Limits of traditional analytic methods of brain circuits

Combining noninvasive brain imaging with quantitative sensory testing, can enhance our understanding of CNS changes associated with central sensitization (hypersensitivity) in FM. Independent component analysis and seed based functional connectivity methods reliably identify resting state networks across several independent research studies, among healthy individuals, clinical populations, at rest, and even during a task [76, 100] [69, 101, 102]. However, investigations of the dynamic organization of the brain reveal the flexible interactions between brain networks largely supporting attention and cognition and their relationship with the SLN [79, 100, 103-105].

The influence of overlapping neural circuits in pain and sensory processing further suggests that functional connectivity may reflect overlapping neural processes associated with perceptual, sensory, or affective components of pain processing and overlap with the intrinsic dynamics of persistent nociplastic pain. While functional connectivity measures rely on an a priori regions of interests, application of graph

theoretical methods to functional neuroimaging provides the ability to examine interactions of the intrinsic fluctuations between multiple brain regions to examine the organization of functional brain at rest and during a task.

Identifying objective measures of pain becomes even harder to disambiguate in cases of chronic pain potentially caused by sensitization of the central nervous system, in which some patients experience persistent, widespread pain in the absence of tissue damage [19, 22]. For other chronic pain patients, the relationship between the pain percept and tissue damage are not correlated. Patients with FM often report increased sensitivity to noxious and non-noxious stimuli. Bivariate functional connectivity analyses alone provide limited insight into the degree in which two brain regions are involved in pain and general sensory processes. Multivariate analyses, such as graph theory, add a layer of complexity to examine network mechanisms by extending functional connectivity to multiple brain regions simultaneously to identify functional connections associated with increased sensitivity in nociplastic conditions like FM.

Assessment of brain structure and function through network theory

Across disciplines, graph theory provides a framework to understand the topographic structure of billions of nodes at multiple scales within a complex system [106-108]. In this the brain fits the graph theory framework in that the individual components or nodes of the graph are represented by neurons or brain regions, and the edges represent the relationship (e.g., functional connectivity) between them. The organization of a network is critical for its function and influences the efficiency and content of information flow among nodes [109-114].

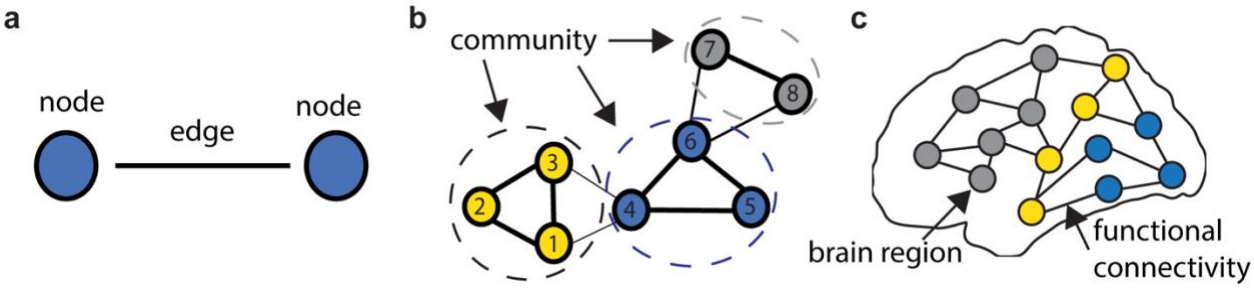


Figure 1.2 Graph theory overview. Graph theory is an interdisciplinary technique used to study complex systems (networks) by defining components of a network to understand complex properties of the system. (a) The individual components of the network are nodes, and the relationship between each node is represented by its edge. (b) Communities are modules within a network generated by community detection. the community structure of a network, may reveal novel interactions of the components

within your network to explain how the network or system functions. (c) Among brain networks, nodes are designated by individual brain regions are edges represent the functional correlation between them.

Within most networks, a collection of nodes that are intrinsically more connected to one another make up a community [115, 116]. The degree in which nodes are more connected within a community relative to between is quantified by modularity. Communities are specialized and integrated components of a network where information may be processed locally and then distributed across a network when necessary [100, 113, 117-119]. In this way, community structure may influence the flow of information throughout the network [120, 121]. Community structure in the brain is also hierarchical, with communities recognized at multiple levels within the brain that reveal neural pathways or circuits (e.g., dorsal and ventral stream of the visual system) [70, 122] or functional groups (e.g., RSN) [106, 107, 123].

Measuring the organization of the brain through a multivariate approach allows for unsupervised assessment of brain dynamics beyond a priori brain regions or resting state networks. Further changes to the functional architecture can shed light into how these changes in functional connectivity between brain regions or functional systems (resting state networks) may influence the larger mesoscale network architecture [100, 124]. Recently, our lab demonstrated that within the chronic pain network, the brain regions most crucial for information processing and integration, hubs, are in regions that are known to be pro-nociceptive [125]. The insula is one hub in the FM brain, and is more connected among FM networks of individuals with high clinical pain, compared to low pain. However, how these findings extend to the global network organization in individuals with FM remain largely unknown. It is unknown how this organization is altered during aversive sensory stimulation and how the segregation of brain regions into specialized functional systems (i.e., modules or communities) might be altered and contribute to pain and multisensory hypersensitivity.

Application of community detection in neuroscience

The construction of community detection algorithms is largely determined based on the following hypotheses here stated in the context of brain networks [119, 126]. First, the fundamental hypothesis is that the true community structure is encoded in the functional correlation matrix (i.e., adjacency, wiring

diagram). Second, nodes within a community are connected to each other and are more likely to be connected to each other, relative to other communities in the network. This suggests communities may overlap with predefined resting state networks but allows for a broad definition in how communities can be defined across the brain wherein the connections are stronger within the community than to other communities within the network. Third, functional correlation matrices based on random connections lack community structure. This provides the basis for measuring modularity and to determine if the community structure of the network occurs by chance or is "real". Lastly, for a given network the true community structure is based on the network with the highest modularity. For optimization purposes this is beneficial in constructing a network and its communities from the functional correlation matrix. However, there are a few limitations for modularity maximization [127].

Limitation of Modularity Maximization

In the context of brain networks, the true community organization of the brain is unknown. Furthermore, the modularity for networks over multiple iterations often generates many near maximum values that make it harder to identify the optimal network partition generated from the functional correlation matrix. As a result, consensus clustering across multiple iterations of community detection is used to determine the network organization of the brain [128].

Appropriate solutions of community detection may consist of grouping the nodes into communities that may provide an important way of uncovering the intrinsic structure of a-priori functional modules. Alternatively, communities may reveal new ways in which the nodes within the network may interact to provide knowledge about how the system or network may function [119, 129]. For large networks, there may be several natural organization levels of communities, in which communities divide further into sub-communities. Thus, for pain, it is desirable to use community detection methods that reveal this hierarchical structure to further assess the functional organization of the brain [129]. These findings will also highlight one way graph theory may be a useful tool to examine the

functional organization of the brain and how it may differ in chronic nociplastic pain at rest and during a visual stimulus.

Comparison of intrinsic functional communities

The community structure of the network reflects the functional connections of the brain. Differences in the composition of communities between two networks can be quantified using normalized mutual information (NMI) [130, 131]. Mutual information is a nonlinear statistical measure of interdependence that quantifies the degree to which the community label of a node between two network partitions. Mutual information equals one if the two partitions are identical and zero if they are completely independent. The normalized form of MI was considered as the differences in cluster number across individual networks is unpredictable. Normalized mutual information accounts for the the entropy of each network partition that quantifies how similar the community structure can be predicted between two or more networks. Specifically, NMI is calculated as a measure of between-subject similarity of subject-specific network communities.

Analyzing the brain as a complex network, we can empirically investigate intrinsic network architecture of the human brain and determine the differences between FM and healthy pain free controls (HCs). A group difference in community structure will result in higher average pairwise similarity within groups compared to between groups [ref Alexander-Bloch 2012]. Further, intrinsic functional connectivity shown to be altered in FM could further be observed through the heterogeneity of intrinsic community structure among individuals with varying degrees of clinical pain.

We therefore hypothesize that FM networks will be more variable and different in their community structure compared to HC. Differences in the intrinsic community structure, measured by the community assignment of the anterior insula, may then reflect the changes in intrinsic resting state connectivity observed in FM that are associated with clinical pain. This would suggest that global community structure is functionally reorganized in FM [132].

Goals of this Dissertation:

This dissertation seeks to examine neural signatures of visual and pain processing in the prototypical, chronic nociplastic pain condition, FM. Non-invasive neuroimaging techniques such as fMRI, are used to examine the functional dynamics of the entire brain at rest and during a visual stimulus. Combining QST, neuroimaging and computational data analysis methods, we examine changes in functional connectivity and extend these findings to whole brain network functional architecture at rest and during an aversive visual stimulus in individuals with FM as compared to HCs. The goal of this research is to identify neural signatures of hypersensitivity in chronic nociplastic pain.

Dissertation Aims:

Aim 1: Identify intrinsic differences in functional network architecture that are associated with clinical pain in chronic pain at rest.

Aim 2: Examine the relationship between insula functional connectivity and community structure during an aversive visual stimulation in patients with chronic pain patients.

Hypothesis:

Across both studies, we compare the community structure of individual networks of patients with FM and healthy controls at rest and during a presentation of an aversive visual stimulus (flashing checkerboard). We hypothesize that altered intrinsic community structure would be observed among FM patients, compared to HC. Consistent with previous findings, functional connectivity between the default mode network and anterior insula will be associated with clinical pain self-report. Increased correlation between the insula and visual cortex during presentation of a sensory stimulus may also be associated with the subjective experience of the sensory stimulus among patients with FM.

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Chapter 2 : Altered Network Architecture of Functional Brain Networks in Chronic Nociplastic Pain[1]

Introduction

An elusive goal of pain research is the identification of an objective marker for the chronic pain state. Ideally this marker would be more salient among chronic pain patients and would quantitatively track with the severity of clinical pain sensation [1]. In nociplastic pain conditions, such as fibromyalgia (FM), altered central nervous system function contributes to pain perception in the absence of peripheral tissue damage or inflammation [2-4]. Resting state fMRI can be used to understand the intrinsic dynamics of brain (i.e. connectivity), and previous studies have identified disrupted intrinsic functional connectivity in patients with chronic pain [5-13]. Yet, these analyses examine functional connections between isolated brain regions based on *a priori* hypotheses (e.g., seed connectivity) or isolated resting state networks (RSNs) (e.g., Independent Component Analysis) [14]. The brain can also be studied as a *network* of nodes (i.e. brain regions) and edges (i.e. connections between each node) to measure the contribution of *all* functional connections present in the brain [15]. Adopting a network-based approach provides insight to the global and local topology of large-scale functional networks.

Within most networks, a collection of nodes that are intrinsically more connected to one another make up a community. Community structure within a network can be quantified using modularity, where networks with communities that are more segregated would have higher modularity values [16, 17]. Differences in the composition of communities between two networks can be quantified using normalized

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mutual information (NMI) [18]. Specifically, NMI quantifies how similar the community structure is between two networks. Yet, it remains unknown how the functional brain intrinsically organizes into modules or communities in FM.

We hypothesized that the organization of functional communities within the brain provides a brain-based marker of FM. We also hypothesized that altered community assignment of specific nodes, or brain regions, contributes to differences in network structure in FM and is associated with the level of pain self-report.

Material and Methods

Participants. The novel analysis for this study included female fibromyalgia (FM) patients and healthy controls (HC) from previously published studies that investigated univariate resting state functional connectivity, hub structure among chronic pain patients. Participants were divided into distinct discovery [19-22] and replication [8, 23, 24] datasets of nonoverlapping patients and HC. Moreover, participants in both FM and HC groups were age matched within 2 years across discovery and replication datasets. The discovery dataset consisted of 43 female FM patients and 20 (HC). 8 participants (5 FM and 3 HC) were removed from the analysis due to excessive motion or artifacts in fMRI data, resulting in a total of 38 FM patients and 17 HC in the discovery dataset. A separate cohort of 34 female FM patients and 21 HCs were combined to create our replication dataset. 3 participants (2 FM and 1 HC) were removed from the analysis due to excessive motion in fMRI data, resulting in a total of 32 FM patients and 20 HC in the replication dataset (Appendix Table A.1). 1 FM patient was not included in the analysis among low, medium and high pain due to missing clinical variables. The Institutional Review Board at the University of Michigan approved the studies and analyses. Written consent was obtained from all participants in accordance with the Declaration of Helsinki. Inclusion and exclusion criteria varied across studies and were previously outlined in [21]. General inclusion criteria for FM patients in the discovery dataset were: meeting the American College of Rheumatology (ACR) 1990 criteria for FM [25] and at least 6 months of self-reported chronic widespread pain, a score of ≥ 40 mm on a 100 mm pain Visual Analog Scale (VAS) at the time of consenting. All participants were between 18 and 75 years of age, female, right-handed, and capable of

giving written informed consent. HCs were excluded if they met the ACR criteria for FM. Additional exclusion for all participants were: contraindications of fMRI, positive urine drug screen or history of drug or alcohol abuse within the past two years, pregnant or nursing mothers, body mass index greater than 36, sever psychiatric illness, concurrent autoimmune or inflammatory disease that causes pain or systemic malignancy or infection such as HIV or hepatitis. In the replication dataset, inclusion criteria comprised: meeting the 1990 ACR FM criteria with at least one year of disease duration, self-reported persistent pain for more than 50% of each day, willingness to forgo new treatments and medication use for FM during the study, between 18-75 years of age, right-handed and capable of giving written informed consent. Participants were excluded based on current or past opioid or narcotic analgesics, history of substance abuse or severe psychiatric illness, concurrent autoimmune or inflammatory disease that may contribute to pain, or concurrent participation in other therapeutic trials.

Clinical Assessment of pain, depression and anxiety. Clinical variables (pain, depression and anxiety) ratings for each participant were acquired prior to the scan session (Table A.1). Clinical pain was assessed on a visual analog scale (0 – 100), with 0 being "no pain" and 100 being "worst pain imaginable" prior to the scan. In our discovery data, Hospital Anxiety and Depression Scale (HADS) were used to assess anxiety and depression [26]. A subset of patients in the replication dataset completed the Center for Epidemiologic Studies Depression Scale (CES-D) to assess depression and State-Trait Anxiety Inventory (STAI) to assess anxiety [27, 28]. In order to compare clinical variables across all subjects, cutoff values for depression and anxiety were used to split patients into two groups: possibly depressed or anxious, or not. [29-31]. Cut off values were as follow: HADS – Depression: greater than or equal to 8; HADS – Anxiety: greater than or equal to 9; STAI: greater than 40; CESD: greater than or equal to 18.

fMRI data acquisition and preprocessing. Imaging data were acquired on a 3T GE Signa 9.0, VH3 scanner (Milwaukee, WI) using an 8-channel head coil. Six minutes of resting state fMRI data was acquired using a T2* weighted spiral-in sequence (TR/TE:2000/30ms) with 180 volumes, as previously described [21] (Table A.2). A T1-weighted high-resolution anatomical scan was also acquired. During the resting state scan subjects were instructed to stay awake with their eyes open on a fixation cross. Since cardiac and

respiratory fluctuations are known to influence brain connectivity within several networks, physiological data were collected simultaneously using a chest plethysmograph and infrared pulse oximeter [32, 33].

fMRI data were preprocessed and analyzed using Statistical Parametric Mapping (SPM) software package version 12 (Wellcome Department of Cognitive Neurology, London, United Kingdom), and the Conn functional connectivity toolbox (Cognitive and affective neuroscience laboratory, MIT, Cambridge, USA) both running on MATLAB 2014a. Upon collection of fMRI data, physiological artifacts are first removed using RETROICOR algorithm in MATLAB and slice time corrected using FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) software. Preprocessing steps include motion correction, realignment, co-registration to anatomical image, normalization to standard MNI template, and smoothing (FWHM Gaussian kernel of 8mm). Preprocessed fMRI data were entered into the Conn Toolbox in which CompCor including six subject-specific motion parameters, fMRI signal from white matter and CSF, and their first order derivatives as confounds [34]. A temporal filter of 0.01 – 0.1 Hz was applied to focus on low frequency fluctuations in the fMRI data.

Motion. Subjects were excluded at multiple steps in the preprocessing pipeline due to excess motion, consistent with previous methods from our group [21]. Motion parameters were calculated from the six head realignment parameters (x, y, z, pitch, yaw, roll) during the resting state scan session for each participant (Figure A.1). First, participants were removed from analyses if the framewise displacement exceed ± 2mm translation or ± 1° rotation in any directions prior to preprocessing. The mean and standard deviation for each of the six motion parameters were also calculated for each subject to determine group differences in motion parameters for each dataset. The six motion parameters were combined into a scalar quantity, instantaneous framewise displacement [35]. Mean and maximum framewise displacement (meanFD and maxFD) were calculated as the average and maximum instantaneous framewise displacement across the entire fMRI scan session for each participant. Lastly, outliers were identified as participants that had meanFD more than three standard deviations (i.e. z-score > 3) above the mean and were also excluded from analyses. To confirm motion does not contribute to group differences in NMI or phi , meanFD and

maxFD were included in the final model to test the group difference NMI (FM-FM vs HC-HC) as nuisance covariates, and correlations between meanFD and maxFD were made with NMI (Figure A.3).

Graph theoretical analyses. The steps for the creation of individual subject graphs are outlined in Figure 1. Preprocessed fMRI data were entered into the Conn toolbox to create functional correlation matrices from regions of interest were based on the 264 brain regions previously shown to produce functional network topologies at rest and during task [36, 37]. Using the Brain Connectivity Toolbox and custom Matlab codes, functional networks were generated for individual subjects using community detection methods that maximize modularity and consider the fully connected and weighted correlation matrix with 1000 repetitions [15, 17, 38, 39]. Consensus analyses were used to generate the subject-specific network. Specifically, agreement matrices were generated which calculate the number of times, within the 1000 repetitions, two nodes are in the same community. A threshold, tau, was applied to each matrix from 0.1 to 0.9 at an interval of 0.1 which indicates the proportion of time in which two nodes appear within the same community to generate each subject-level partition [40, 41].

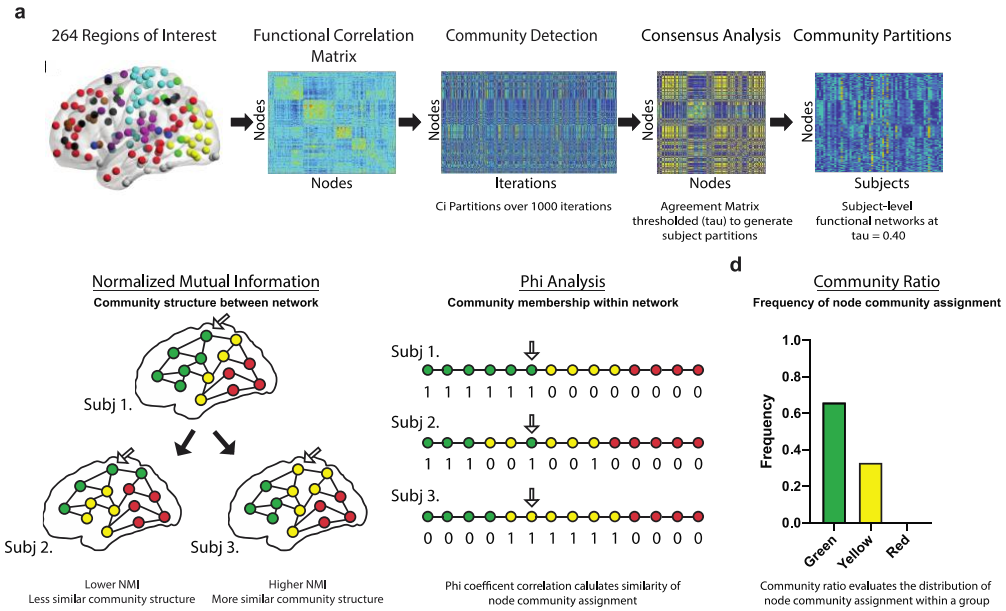


Figure 2.1 Functional brain networks were constructed from resting state fMRI data.(a). fMRI time series were extracted from 264 predefined brain regions to construct subject level functional correlation matrices for each participant. Using the fully connected and weighted correlation matrices, networks were generated using the modularity maximization community detection algorithm with 1000 repetitions. Next, consensus analyses were performed to generate a subject-specific network for each subject. (b) Normalized mutual information (NMI), a measure of network similarity was used to determine whether network organization was conserved within and between groups of individuals. Higher NMI values indicate greater similarity in community structure between two networks. (c) Pearson's phi coefficient (phi), quantified the similarity of node community

assignments between two networks from a binary vector denoting whether two nodes are assigned to the same community (1) or a different community (0). (d) The frequency distribution of node community assignment further assessed how nodes may differ in their community assignment between groups. The community ratio quantifies how nodes may differ in their community assignment within a group. Communities were labeled in representative networks as Green, Yellow, Red. White arrow identifies node used to determine frequency distribution

Modularity. A partition of networks into non-overlapping communities or modules can be achieved using modularity maximization [39, 42]. Measures of modularity quantify the goodness of modular partitions such that networks with stronger connections within a module compared to between modules have higher values. Modularity values for each individual network were calculated based on the fully weighted matrix and the the consensus generated subject level partition [17, 39].

Normalized Mutual Information. To quantify group differences in network organization among participants, we used normalized mutual information (NMI) to measure pairwise similarity between two network partitions, across all participants [18, 43] (Figure 2.1b). Stemming from information theory, mutual information (MI) quantifies the degree to which two clusters are similar. MI is 0 when two networks are completely random and 1 when networks are identical. Normalized mutual information (NMI) improves the sensitivity of network comparison of two networks with varying number of communities. Networks that have similar community structure have higher NMI values, as lower NMI suggests that networks are different in their organization. Details of the use of NMI measures on brain networks have been described previously [14, 43, 44]. For each subject, within-group NMI values were calculated from the mean NMI between all subjects within the same group. We also calculated between-group NMI as the mean pairwise NMI between all subjects in a different group. Higher NMI values suggest greater similarity in community structure between two networks.

Phi Analysis. A node's community assignment is a local measure that identifies its contribution to the organization of a network. We examined which brain regions contributed to group differences in NMI using a Phi test to quantify pairwise similarity of node community assignments as previously described [43, 45]. (Figure 2.1c) For each subject, a binary matrix was generated based on whether a given node was in the same community as all other nodes (1) or in a different community (0). The binary matrices were then used

to generate a node similarity measure (phi) using Pearson's phi coefficient. The within-group mean phi was calculated for all subject-by-subject node pairs. Higher phi values suggest the node is frequently a member of the same community across participants (i.e. strong allegiance). Low phi indicates that the node community assignment is variable and frequently shifts allegiance between different communities across participant networks.

Group consensus functional networks. Group consensus networks were constructed as previously described [43]. Briefly, subject network partitions at tau threshold 0.40 were used to generate an agreement matrix for the FM and HC group independently. In this framework, an agreement matrix measures how consistent two nodes appear within the same community across subjects within the FM and HC group separately. Group consensus partition or networks were based on tau thresholds between 0.1 to 0.9, at intervals of 0.1. We found that the number of communities increase with tau thresholds. For the group consensus analyses we applied a tau threshold of 0.50 (50% agreement within the group) to each agreement matrix because the number of communities were consistent between FM patients and HC at this threshold, (Figure A.2).

Community Ratio. Communities are a collection of nodes identified by stronger connections between each other relative to other nodes or group of nodes within a network. We examined the variation of community assignment for each node within each group of low and high pain participants to assess how node community assignment (See examples in Figure 2.7). Variation of community structure may reflect individual differences across individuals due to a variety of external or extrinsic factors. However, we are specifically interested in assessing whether node community assignment is related to varying degrees of pain among individuals with FM. Among both groups, we discovered that nodes were either assigned to one community, while others were split between two or more in our dataset. We grouped these nodes based on their patterns: Pattern A, Pattern B, Pattern C, and Pattern D. Nodes that follow Pattern A were split between communities 1 and 3. Pattern B consisted of nodes that are predominately in community 2. The nodes grouped in Pattern C and D are primarily in community 3 and community 1, respectively.

The community ratio examines the variation in node community assignment further through quantifying how the distribution of node community assignment differs between low and high pain FM networks. The alpha ratio quantifies the average frequency nodes were assigned to community 1 and 3 relative to community 2. A lower Alpha ratio suggests that a node may be primarily found in community 2. The Beta ratio examines whether nodes are assigned to community 1 or 3, quantified by the frequency nodes were assigned to community 1 relative to community 3. A lower Beta ratio suggests a node were primarily in community 3 within a group of networks. An alternative interpretation of the community ratio is that the Alpha ratio determines the scale in which a node exhibits Pattern A relative to Pattern B within a group. The Beta ratio explains the proportion in which a node exhibits Pattern D relative to Pattern C.

ROC Analyses. Discrimination indices were determined based on receiver operator curve analyses in R statistical software. At each threshold, within-group NMI values were used to determine whether network topology accurately identified FM or HC group membership. Threshold values were determined based on the value that maximizes the distance to the identity line [46]. Sensitivity, specificity, and accuracy values were also calculated from this threshold value.

Whole brain regression analyses. To explore the relationship between our Graph Theory measures and traditional intrinsic resting state networks (RSN), we conducted whole brain regression analyses correlating NMI with changes in functional connectivity of resting state networks shown to be altered in FM patients. RSN seeds were generated from independent component analysis using the GIFT toolbar [47]. From the estimated components previously reported to be altered in chronic pain, salience (SLN) and default mode networks (DMN) were identified by spatial correlation with RSN templates (Beckmann et al 2005). Binary masks were generated for each RSN and used for seed-to-whole brain functional connectivity analyses in the Conn toolbox. To determine changes in RSN connectivity that were associated with NMI in FM, individual beta-maps were entered into a second-level whole brain regression analysis with NMI (within-group NMI:FM-FM) in SPM12. Age and motion (meanFD and maxFD) were included as nuisance regressors.

Statistical Analysis. Independent t-tests were used to test differences in motion, clinical variables and modularity values between groups in R statistical software. We tested for group differences in within-group NMI values using permutation tests with age and motion (e.g. meanFD and maxFD) as regressors of no interests. Permutation test significance was determined by the number of occurrences the 10,000 random permuted group differences in NMI were more extreme than the observed difference. Age and motion (both meanFD and maxFD) were included in the model as covariates of no interest. To correct for multiple comparisons across thresholds, permutated p-values were corrected based on false discovery rate. Between-group NMI values were calculated based on the mean of the NMI values associated with the comparison of each FM patient to each HC. Paired-t-tests were conducted to determine whether the within-group NMI were significantly different from the between group NMI. Results were deemed significant at $p < 0.05$. For the phi analyses, we ran permutation tests to determine whether the observed within-group differences were different from 10,000 random permutations. Age and motion were included as regressors of no interest. Permutation test significance were determined by the number of times the observed value were more extreme than the permutated difference. To correct for multiple comparisons, across 264 nodes in the phi analysis, results were deemed significant at a false discovery rate (FDR) corrected p-value < 0.05. Results of whole brain regression analyses were deemed significant based on a FDR cluster level corrected P value < 0.05 derived from a voxel-wise uncorrected p-value < 0.001. Post-hoc correlations were performed between functional connectivity values and NMI (within:FM-FM) using R statistical software ($p < 0.05$).

Results:

Intrinsic network architecture is altered in chronic pain patients

We first determined whether there were differences in network architecture between FM patients and HC. We compared the organization of brain networks both within a group (FM to FM and HC to HC) and between groups (FM to HC). We found that within the FM group, the mean NMI between each pair of FM patients was lower than between pairs of HCs, suggesting that the organization of functional networks is more variable in FM. HCs, on the other hand, had relatively stable network configurations (high pairwise NMI). The within group NMI was significantly different between FM and HCs and this

remained true across thresholds (for tau = 0.4, permutation test *P* value < 0.0001; Figure 2.2a, Figure A2). Next, we compared network similarity across groups. We found that FM patients have significantly lower between-group NMI relative to the within-group FM NMI measure (*paired t-test,* $t(37)=19.916$, $p < 2.2 \times 10^{-16}$), suggesting that FM network organization is even less similar to HC. There were no differences in the average number of communities and modularity between FM and HC (Figure 2.2b-c). Motion was assessed through mean framewise displacement for the entire length of the scan for each subject. While meanFD was significantly different between FM and HC, there were no significant correlations between NMI and motion (i.e. meanFD and maxFD) suggesting that motion does not contribute to the variability of subject networks (NMI). Further, group differences in NMI remained when meanFD and maxFD were included as nuisance covariates (Figure A.2).

Next, we tested if these results replicated in a separate population of FM patients and HCs. In our replication cohort, we again found that FM patients had lower within- and between-group NMI compared to HCs (within-group NMI - permutation test P value < 0.0001; between group NMI - paired t-test $t(31)=12.796$, $p < 6.567 \times 10^{-14}$; (Figure 2.2c-e, Figure A.2). In short, chronic pain patients have lower within- and between-group NMI compared to HCs, suggesting that their network architecture is more variable and different. The lack of group difference in both modularity and number of communities further implies that while the strength of connections within relative to between communities are similar, the nodes that make up the communities differ between groups.

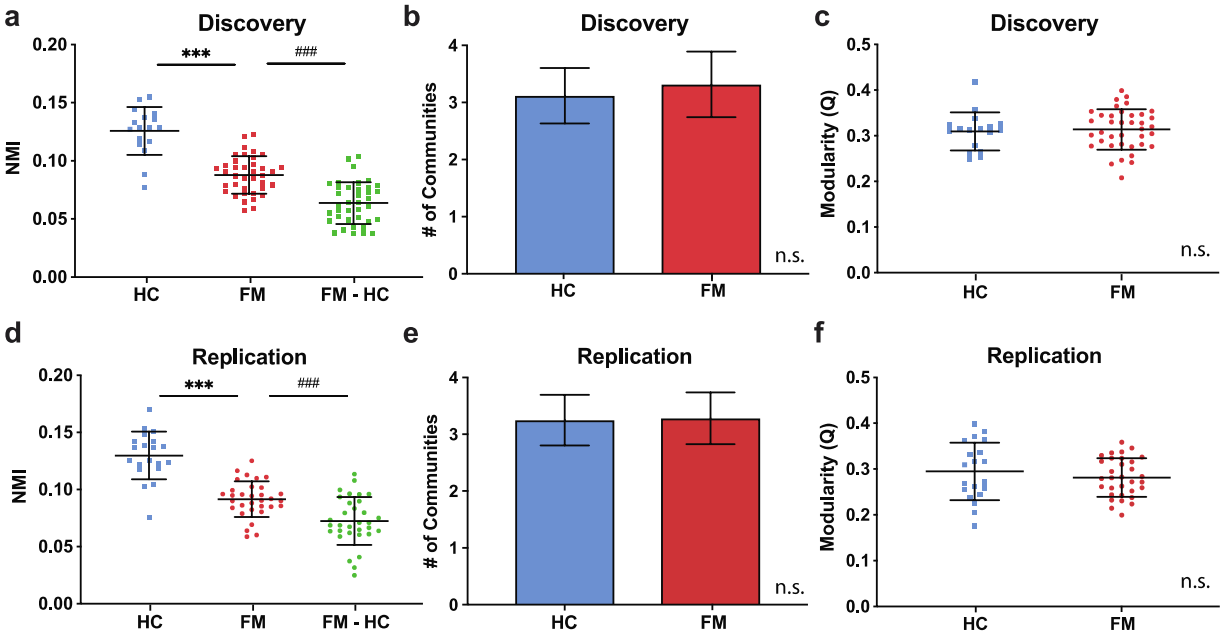


*Figure 2.2 Comparison of functional networks between FM and HC. Lower normalized mutual information (NMI) indicated that network organization was more variable among FM patients (red) relative to within HCs (blue) Further, FM and HC networks were even less similar to each other (green) (a). There were no differences in the number of communities (b) or modularity (c). These results were consistent in the replication group (d-f). (*** designates significance based on permutation test – $p < 0.001$; ### designates significance based on paired t-test - $p < 0.001$; n.s. not significant)*

Sensitivity and specificity of NMI identifies FM networks

To investigate whether global network structure can be used to identify FM networks, we measured whether NMI can accurately discriminate networks of FM patients and HCs. Receiver operating curve (ROC) analyses showed that within-group NMI successfully identified patients and controls across multiple thresholds, with an average (±SD) accuracy of 85 ± 13.9 and 86 ± 10, and area under the curve (AUC) of 87 ± 10.8 and 86 ± 12.8, for the discovery and replication datasets, respectively (Figure 3a). Across thresholds up to 70% agreement (tau = 0.70), within-group NMI accurately identified FM patients from controls with an average 88% sensitivity and 94% specificity in the discovery dataset, and average 79% sensitivity and 91% specificity in the replication dataset (Figure 2.3b). In both datasets the accuracy, sensitivity, specificity and AUC decreased as tau increased over 0.7.

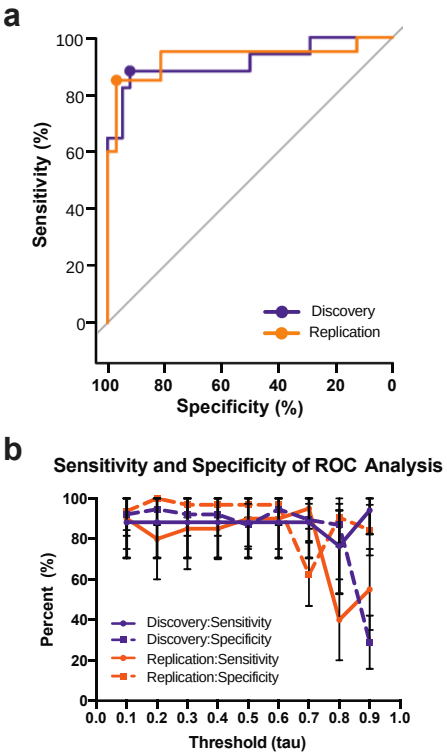


Figure 2.3 Within-group NMI reliably discriminates FM patients from HC. (a) ROC curves for within group NMI at tau = 0.40 for the discovery (AUC = 91%) and replication, (AUC = 93%) datasets. (b) NMI could discriminate networks FM patients from HC between tau thresholds of 0.1 and 0.7.

Changes in resting state network connectivity are associated with normalized mutual information

To explore the relationship between intrinsic network connectivity and NMI in FM, we conducted whole brain regression analyses correlating resting state network connectivity with NMI. Functional connectivity between the salience (SLN) and default mode (DMN) networks were associated with within group NMI (FM-FM). Specifically, we found salience connectivity between the posterior and anterior cingulate/subcallosal gyrus, cuneus, middle temporal gyrus and angular gyrus were negatively associated with NMI (Figure 2.4a). Functional connectivity between the salience network and the anterior cingulate cortex (ACC) suggests stronger within salience network connectivity is associated with lower NMI. As the posterior cingulate cortex (PCC) and angular gyrus are part of the default mode network, these results suggest that functional connectivity between the salience network and default mode network is also associated with FM networks that were less similar among the group. Of note, we also found that DMN

functional connectivity between the insula cortex, lingual, inferior frontal, supramarginal, and precentral gyri is negatively associated with NMI in FM (Figure 2.4).

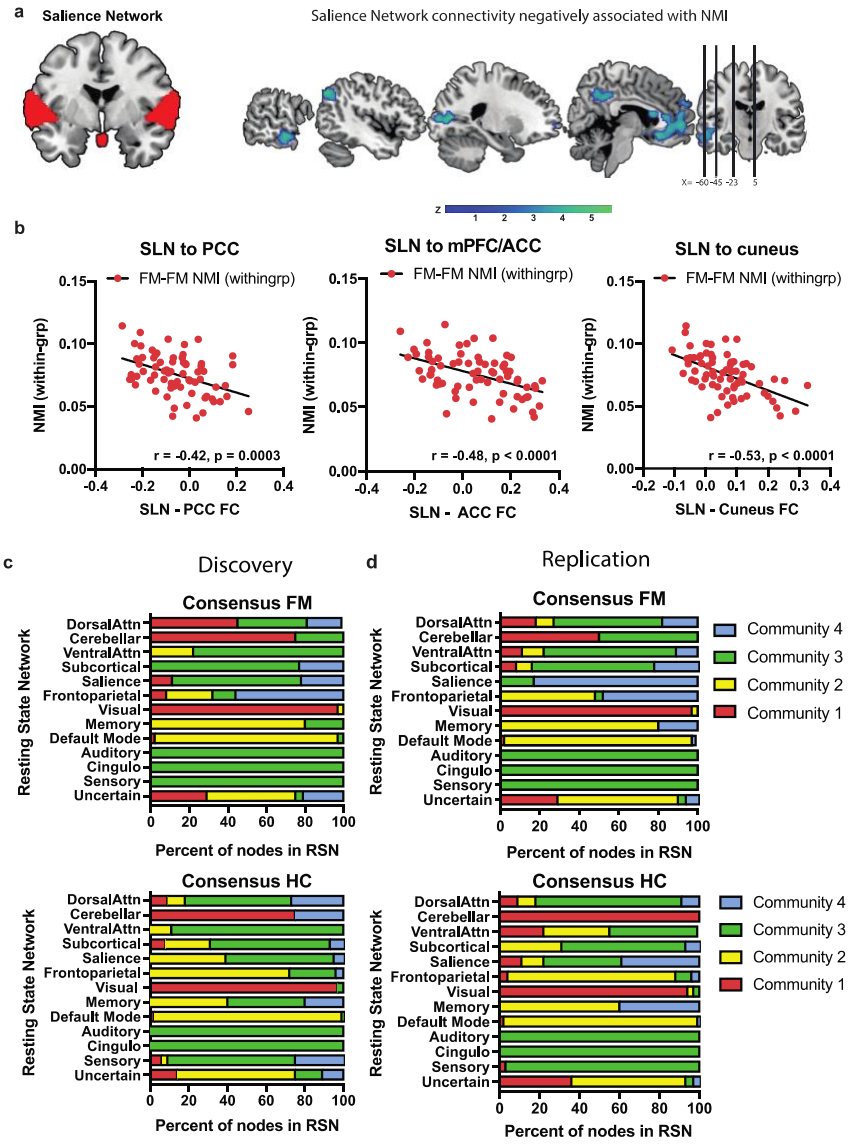


Figure 2.4 Intrinsic resting state network functional connectivity is associated with NMI. (a) Salience network connectivity between the posterior cingulate cortex (PCC), mPFC/ACC, cuneus, middle temporal gyrus and angular gyrus were negatively associated with NMI. (b)Within the FM group, FM networks that were less similar have stronger functional connectivity. (c and d) Group consensus networks were constructed from subject networks generated at tau of 0.40 in the discovery and replication dataset. Resting state networks spanned multiple communities. Moreover, the community assignment of nodes within an RSN were different across FM and HC groups. The composition of communities 1 (visual), 2 (default mode and frontoparietal), and 3 (sensorimotor and attention) were consistent among FM and HC networks across both datasets. Community 4 was the smallest community with respect to the number of nodes and included nodes from different RSN between datasets and groups suggesting community organization may be different in FM. (Community 1 – red; Community 2 – yellow; Community 3 – green; Community 4 – blue)

Group consensus analyses reveals community structure

To visualize the community structure of FM patients and HC we generated consensus networks based on 50% agreement (tau=0.50) within each group separately. We chose 50% as the agreement threshold because the number of communities between patient and control consensus networks were consistent (Appendix A. Figure 2.5). To examine how nodes within resting state networks (RSNs) spread across communities, we quantified the percent of nodes in each community for each resting state network. (Figure 2.4c and d) Nodes in the visual network (VIS) and default mode network (DMN) were found predominately (i.e. at least 90% of the nodes) in one community for both FM and HC networks respectively. In contrast, sensorimotor (SMN) nodes were split between communities 3 (66%) and 4 (26%) in the consensus HC network, yet only in community 3 in the FM network from the discovery dataset. Frontoparietal, salience and subcortical nodes also differed in how they were distributed across multiple communities between FM and HC.

To determine the composition of each community, we quantified the percent of nodes in each resting state network, within each community (Figure A.5). The composition of communities 1, 2, and 3 were consistent among patients and controls across both datasets. Community 1 consisted of nodes from the visual and cerebellar networks, community 2 consisted of nodes from the DMN, and community 3 was composed of sensorimotor, cingulo-opercular, subcortical, dorsal and ventral attention network nodes in FM and HC in both datasets. Community 4 was the smallest community with respect to the number of nodes and included different RSN between datasets and groups. In the discovery dataset, 48% of the nodes in community 4 were comprised of nodes from the frontoparietal network in the FM consensus network, whereas 45% of the nodes in community 4 of the HC network corresponded with the sensorimotor network. In the replication dataset, 32% of the nodes in community 4 in the FM consensus network consisted of frontoparietal network nodes, consistent with the discovery dataset. However, salience network nodes in both FM and HC consensus networks made up 41% and 50% of the nodes in community 4, respectively. Overall, these data support the notion that nodes within each resting state network differ in community assignment across FM and HC groups.

Chronic pain patients have nodes that are more variable in their community assignment

Differences in consensus networks suggest that communities consist of nodes from multiple resting state networks which may reveal differences in intrinsic resting state network connectivity. Moreover, the organization of nodes into different communities within the networks of FM patients compared to HCs may reflect differences in network architecture in chronic pain and also be associated with patients' clinical pain intensity. We next examined whether there were differences in the composition of communities between FM and HC. Pearson's phi coefficient (phi), quantifies the pairwise similarity of node community assignments between two networks from a binary vector that defines whether two nodes are within the same community (1) or not (0) [43, 45] (Figure 2.1c). Higher phi values indicate a node is consistently a member of the same community across individuals within the FM or HC group. Many brain regions significantly differed in their community assignment between groups, having lower Phi values among FM patients compared to HC. Specifically, we found that 118 (44.7%) and 121 (45.8%) nodes had a significantly lower phi value among FM patients in the discovery and replication dataset respectively (permutation test; FDR-corrected $p < 0.05$), (Figure 2.5a). The remaining nodes were not statistically different in their phi values between FM patients and HCs. Together, 47 (17.8%) of the 264 nodes had consistently lower phi in FM individuals across the both discovery and replication datasets, suggesting that they were significantly more variable among FM patients (Figure 2.5b).

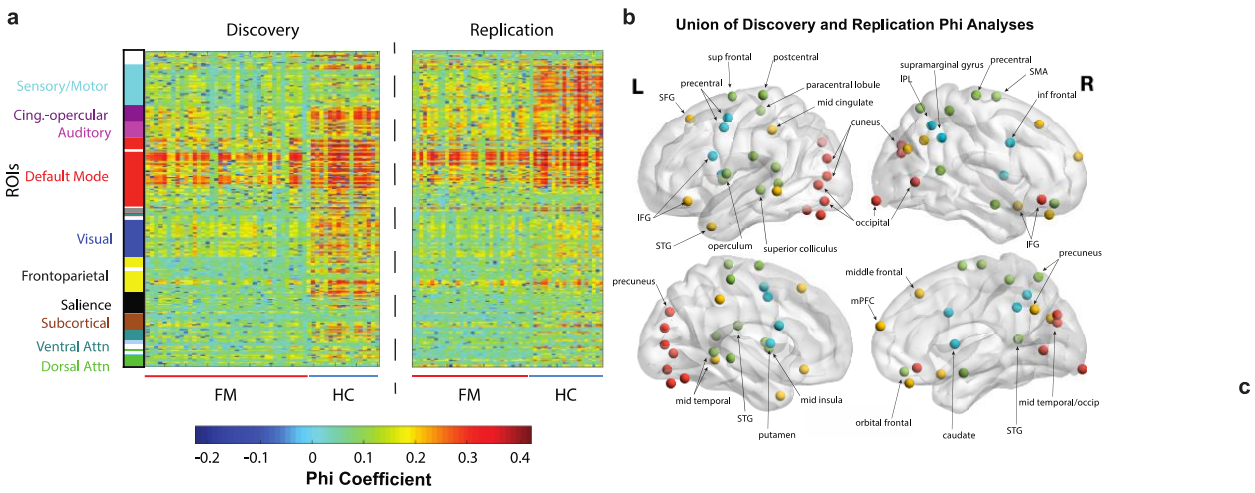


Figure 2.5 Phi analysis identifies variable nodes in brain networks of FM patients. (a) Lower Phi values were observed for nodes across functional networks in FM patients compared to HC. 118 (44.7%) and 121 (45.8%) out of 264 nodes had significantly lower phi value among FM patients in the discovery and replication dataset, respectively. (b) Together, 47 (17.8%) nodes have significantly lower phi values in chronic pain patients across both datasets. Node color based on community assignment from consensus network of FM patients. (Community 1 - red; Community 2 - yellow; Community 3 - green; Community 4 – blue)

Intrinsic functional network structure differs between patients with varying degrees of clinical pain

While nodes appear more variable among FM networks, we were particularly interested in identifying whether the increased variability of community structure in FM is associated with clinical pain, and whether these differences are attributed to changes in community organization. To examine this possibility, FM networks from both datasets were combined and then split into tertiles (n = 23 per group) based on clinical pain ratings (Visual analog scale from 0 = "no pain" to 100 = " worst pain imaginable"), creating a low (31.04 ± 12.92), medium (58.48 ± 5.99) and high pain (76.87 ± 7.32) group. High pain patients had significantly lower within-group NMI compared to low pain patients (tau = 0.40, permutation test, p = 0.0142), suggesting differences in community structure between patients with low and high pain (Figure 2.6a). There were no differences in within-group NMI between low and medium pain, or medium and high pain patients (low versus medium: permutation test *P* value = 0.3765; medium versus high: permutation test *P* value = 0.0977). Cluster analysis showed that these nodes form a two-cluster solution that differ between low and high pain cohorts further suggesting the variability of nodal community assignment is associated with pain intensity among FM patients. (Figure A.6).

To explore the relationship between clinical pain and the variability of community structure of chronic pain patients, we identified which nodes may be contributing to the differences in network structure between low and high pain patients (Figure 2.6b). Phi test revealed forty-three nodes that were significantly different in their community assignments between low and high pain patients (permutation test; FDR-corrected p <0.05; Figure 2.6b). Twelve of the forty-three nodes were stable in their community assignment among high pain patients (i.e. higher within-group phi values among high pain patients). These nodes were associated with the default mode, frontoparietal and salience networks (Figure 2.6c). The remaining thirty-one nodes were stable in their community assignment among low pain patients and associated with the sensorimotor, cingulo-opercular, visual, attention, frontoparietal and salience resting state networks (Figure 2.6d).

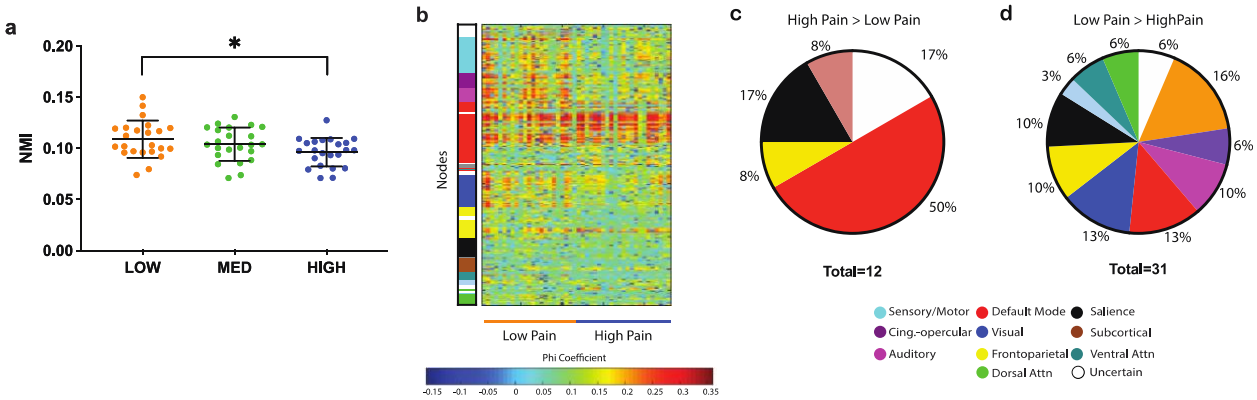


Figure 2.6 Network structure tracks with level of clinical pain. FM patients from both the discovery and replication dataset were combined and then split into tertiles based on the degree of spontaneous pain: low pain (31.04 ± 12.92), medium pain (58.48 ± 5.99) and high pain (76.87 ± 7.32). (a) Differences in network structure were observed between patients, such that high pain patients had lower within-group NMI compared to patients with low clinical pain. (b) Forty-three nodes significantly differed in their community assignment between patients with low and high pain. (c) Twelve out of forty-three nodes were more stable among patients with high pain compared to low. These nodes consisted of primarily DMN (red), SLN (black), FPN (yellow) and Uncertain (white) nodes. (d) Thirty-one of the forty-three nodes were more stable among low pain patients and consisted of nodes across all RSNs. (designates significance based on permutation test – p < 0.05; DMN-default mode network; SLN-salience network; FPN-frontoparietal network)*

Community ratio reveals differences of node community assignment across chronic pain intensity

Lastly, we used the community ratio to identify how the community membership of the nodes may differ across varying degrees of clinical pain. For the nodes that significantly differed in their community assignment between low and high pain networks, we quantified how the variation in

community assignment differed between low and high pain networks by calculating the frequency distribution of node community assignment for each group.

We measured the alpha ratio for nodes with greater phi in high pain networks. These nodes were differentially assigned to community 1 and 3 (Pattern A) versus community 2 (Pattern B). For example, the right angular gyrus was commonly found between communities 1 and 3 (as opposed to community 2) and had a higher alpha ratio among high pain networks relative to low pain networks. The right anterior insula in contrast was primarily found in community 2 in networks of high pain patients and had a lower alpha ratio (Figure 2.7a and c). We also calculated the beta which. assessed how nodes stable in low pain differed in community assignment between community 3 (Pattern C) or community 1 (Pattern D). For instance, the left postcentral gyrus was assigned to community 3 across low pain networks. As a result, the beta ratio is lower in low pain compared to the high pain group. The medial orbital frontal is found in community 1 among low pain networks compared to high pain networks where they are found more commonly in community 3. As a result, the beta ratio for this node is lower in the high pain group compared to the low pain group (Figure 2.7b and d).

Using the community labels based on the group consensus analysis, differences in the alpha ratio revealed that nodes in the middle temporal gyrus, superior frontal gyrus, mid frontal gyrus, and angular gyrus, affiliated with the default mode network, are stable in high pain and are found in communities associated with visual and sensorimotor networks. The two nodes affiliated with the salience network (i.e. the anterior insula and frontal precentral) were found in the default mode network communities among high pain patients (Figure 2.8a and 2.8c). Of note, the medial thalamus differences between low and high pain networks reflect nodes assigned to community 2 or 3 between low and high pain networks. The beta ratio highlights differences in visual and sensorimotor network community assignment associated with varying pain intensity (Figure 2.8b and 2.8d). Across high pain networks, the medial orbital frontal, superior temporal gyrus, supplemental motor area/paracentral lobule, dorsal anterior cingulate nodes have a lower beta ratio indicating these nodes were assigned to the sensorimotor network in high pain. In low pain networks, these nodes varied between visual and sensorimotor communities and

as a result the beta ratio was closer to 1. Interesting, a third group of nodes that include the precentral gyrus, middle insula, superior temporal gyrus frontal middle orbital gyrus and visual regions: lingual, inferior temporal and inferior occipital gyrus, also have a higher beta ratio in high pain compared to low pain. However, these nodes appeared to be in the visual community in high pain networks more than community 3 in low pain networks. Together these results demonstrate that differences in community composition is associated with clinical pain intensity in FM.

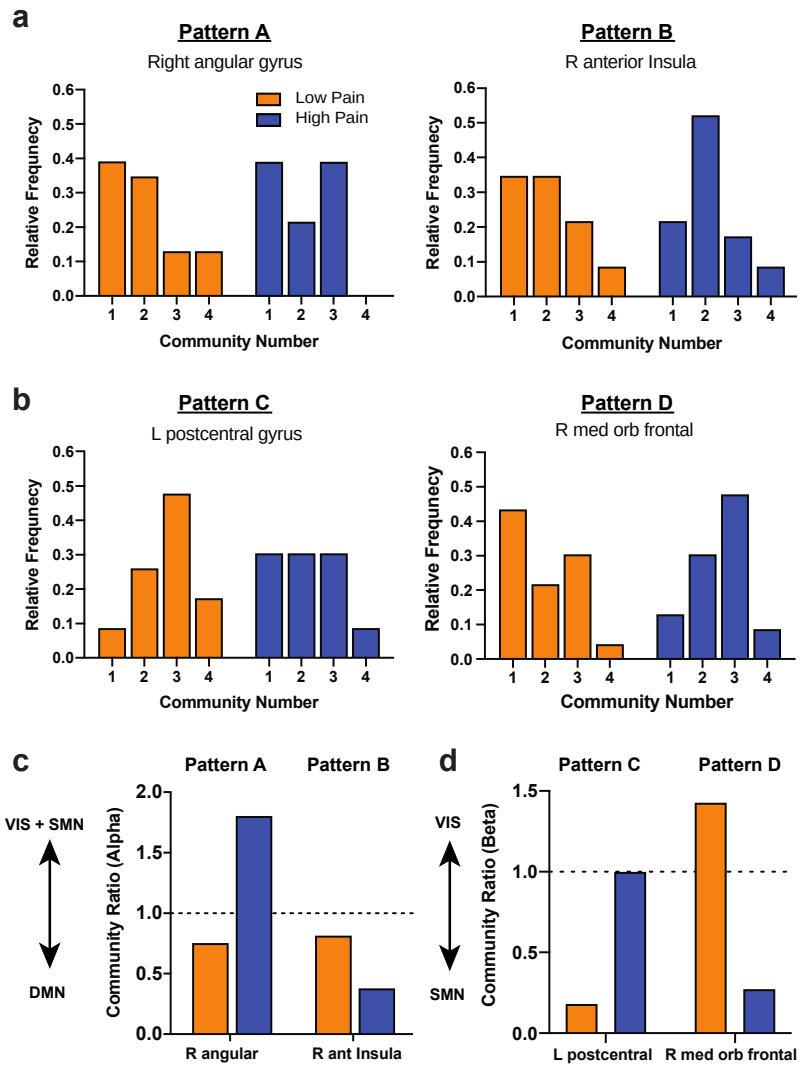


Figure 2.7 Community Ratio reveals the distribution of node community assignments across low and high pain. Of the nodes that were different in their community assignment between FM patients with high and low pain, the relative frequency in which nodes were assigned to different communities displayed distinct patterns of community distribution across the networks. (a) Pattern A consisted of nodes typically assigned to communities 1 or 3 among high pain patients. Pattern B among high pain patients were nodes found in community 2. (b) Pattern C consisted of nodes assigned to community 3 and Pattern D consisted of nodes assigned to community 1 among low patients. The community ratio of these nodes quantifies the distribution of nodes across communities within a network. (c) Pattern A nodes have a higher alpha ratio, indicating these nodes were assigned to communities 1 and 3. Pattern B nodes have a lower alpha ratio. (d) The beta ratio measures whether nodes are assigned to

community 1 or community 3. The beta ratio was lower for Pattern C nodes and higher for Pattern D nodes among low pain patients. The relationship of the community ratio reveals how nodes are assigned to different communities between low and high pain networks. (Blue – low pain patients; orange – high pain patients. DMN-default mode network; SLN-salience network; SMN-sensorimotor network.)

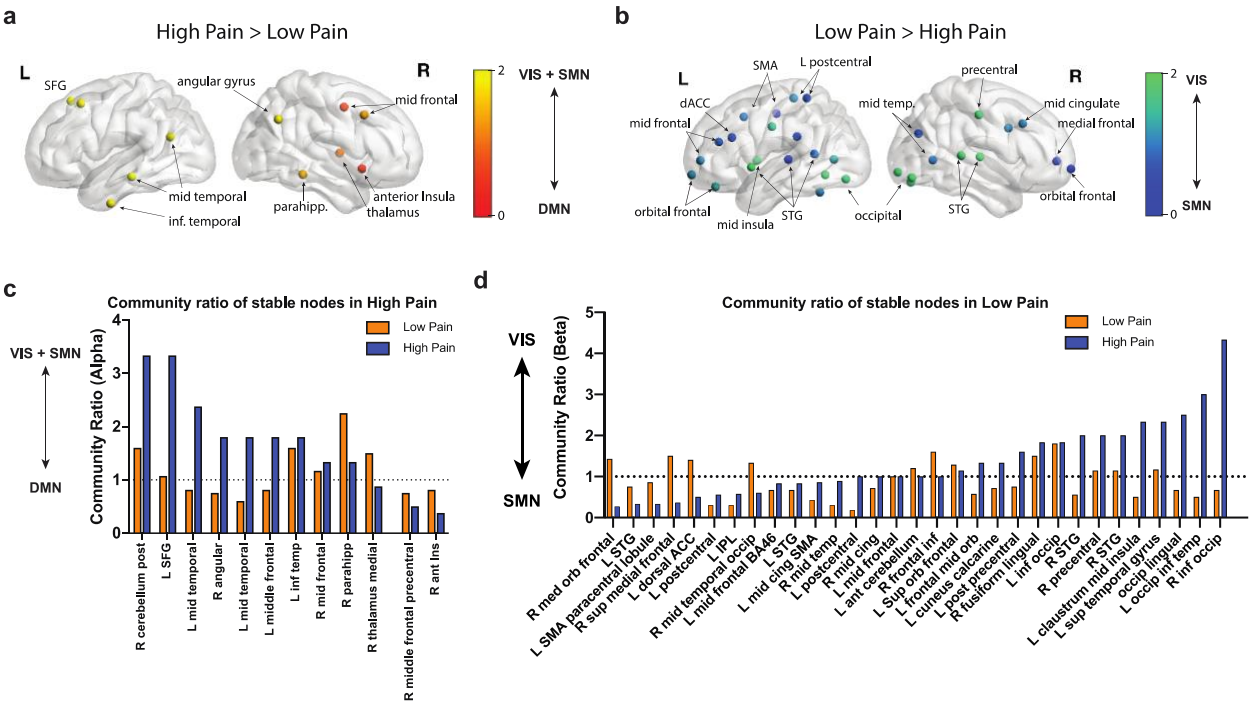


Figure 2.8 Network reorganization among FM patients. FM patients have more variable community structure compared to HC. This variability in network structure is attributed to differences in node community assignment across FM networks with varying degrees of clinical pain, as changes in the composition of communities are associated with clinical pain. The community ratio reveals how nodes are assigned to different communities between low pain and high pain groups for the nodes stable across (a and c) high pain networks and (b and d) low pain networks. (a) The alpha ratio measures whether nodes are assigned to the DMN (i.e. lower alpha ratio) or sensory communities (i.e. higher alpha ratio) for nodes stable among high pain networks. (b) The beta ratio measures whether nodes are assigned to visual (i.e. higher beta ratio) or sensorimotor (i.e. lower beta ratio) for nodes stable across low pain networks. (c) Differences in the alpha ratio show that DMN nodes are found in visual and sensorimotor communities while SLN nodes were found in the DMN communities among high pain networks. (d) SMN nodes have a higher beta ratio in high pain relative to low pain indicating they are assigned to the visual community more in high pain networks relative to low pain networks. (Orange-yellow color bar for nodes stable among high pain networks is based on alpha ratio within the group. Blue-green color bar for nodes stable among low pain networks is based on beta ratio within the group. DMN-default mode network; VIS-visual network; SLN-salience network; SMN-sensorimotor network.)

Discussion:

The main finding of this investigation was that NMI, a measure of pairwise similarity between community membership, is different between chronic pain patients compared to HC. FM patients had significantly lower within- and between-group NMI, suggesting that the composition of communities is more variable amongst FM patients and significantly different from HCs. Furthermore, NMI accurately

predicted whether a brain network came from an FM patient or HC with 85% accuracy. We found that the differences in community structure were attributed to changes in node allegiance across networks in chronic pain patients. In short, certain brain regions were more variable or less predictable in their community assignment among chronic pain patients. Next, we determined if these changes in community structure were related to clinical pain levels. Patients with high clinical pain had lower NMI compared to low pain chronic pain patients. These differences in network topology were attributed to intrinsic composition of functional communities across networks and associated with varying levels of clinical pain (Figure 2.8). A significant strength of this study is that we replicated the differences in community structure in a novel cohort of FM and HC participants.

Through expanding the scope of functional connectivity analyses to whole brain networks, this work makes three crucial contributions to the field of pain and neuroimaging: First, connectome-based measures of the brain may improve diagnosis of conditions with underlying brain pathology or prediction of subgroups or states within a clinical population [1]. We show that NMI reliably identified networks within FM patients from healthy controls in both our discovery and replication datasets. Previous work in schizophrenia, major depression disorder and chronic low back pain have also observed altered network structure among patients compared to healthy controls, supporting the notion that global network structure may be a neural marker of brain pathology [14, 43, 48, 49]. It is important to note that prior to application of network measures in a clinical setting, we understand how these measures relate to the underlying pathophysiology of these conditions, separately. In FM, salience nodes more stable in their community assignment in high pain networks had a lower alpha ratio, indicating these nodes are more commonly found in community 2 with other DMN nodes. Moreover, these nodes overlap with changes in DMN connectivity negatively associated with NMI, linking the relationship between differences in functional connectivity and network structure to clinical pain intensity in FM [5, 6, 9, 10] (Figure A.4). The community ratio also revealed that DMN nodes stable in high pain have a higher alpha ratio and are found in community 1 and 3. These regions may have increased connectivity to sensory or attention communities and decreased connectivity to the DMN in networks of high pain FM patients [49].

Interestingly, the nodes with higher phi values in low pain differ largely between sensory communities (e.g. visual and sensorimotor) when comparing networks of low and high pain. The insula, superior temporal gyrus and occipital brain regions, have a higher beta ratio in high pain compared to low pain, suggesting these nodes were typically found in community 1 and may be more connected to visual regions in high pain networks. The beta ratio for the precentral and postcentral nodes were also higher in high pain compared to low pain, but largely reflect in the increase variability in community assignment among high pain network. The dorsal ACC, medial orbitofrontal cortex, superior medial frontal gyrus and SMA had a lower beta ratio among high pain patients compared to low pain patients. Of note, these nodes were found in community 3 in high pain networks compared to being more variable or community 1 in low pain patients (Figure 2.8). These brain regions are involved in modulation of pain via placebo analgesia and the descending inhibitory pathways. Clinical pain may arise from disrupted descending pain modulatory activity in chronic pain patients [50-54]. Whether the differences in the assignment of these nodes into their respective communities reflect altered pro- or antinociceptive mechanisms among chronic pain conditions is a point worth exploration in future studies.

Second, analyses of the intrinsic mesoscale organization of the brain through communities explores changes in resting state network connectivity that are associated with clinical pain intensity. Functional connectivity between the salience and default mode network is negatively associated with NMI such that stronger connectivity was associated with networks that are more variable in network structure in FM. These findings were consistent whether we used the salience or default mode network as regions of interest indicating changes in the intrinsic resting state functional connectivity and may contribute to the global properties of the network via changes in the community composition within the network [55]. In our analyses we show that communities may consist of nodes from one resting state network (e.g. community 1: visual) or multiple resting state networks (community 2 and 3). Observing the network organization of the brain within communities may reveal intrinsic functional connectivity between resting state networks. The present study shows that brain regions typically involved in pain processing are differentially assigned to communities between patients with varying levels of clinical pain.

Third, the current study shows that intrinsic functional brain networks are reorganized in FM. It is important to note that differences in the organization of networks are not completely random, but instead there are specific changes in connectivity or network organization, consistent with previous studies [21, 49]. We show that the variations in community structure in FM is associated with clinical pain in FM. Higher clinical pain is associated with lower NMI in FM patients notably contributing to the within group FM having lower NMI. A second factor potentially contributing to lower NMI in FM patients may be that there are multiple ways of engendering pain in FM. Patients may have increased connectivity in pronociceptive regions, insufficient anti-nociceptive activity, or a combination of both[8, 56]. Together these differences could increase variability in the FM group and lower within FM NMI outcomes. The lack of difference in modularity and number of communities suggest that the composition of communities is associated with the difference in functional network architecture.

Fibromyalgia may be an example of maladaptive reorganization of brain networks in which changes in the network composition are a result of, or response to, persistent pain among patients [44, 57]. In both the discovery and replication dataset, our phi analyses revealed only nodes that were more variable in the community assignment in FM, relative HC. The nodes that are different in their community assignment across both datasets were brain regions affiliated with classic pain and sensory processing pathways. How and when these changes occur are still unknown and require both longitudinal studies to understand the transition from acute to chronic pain, along with innovative network analyses to understand how networks change over time [58].

The current study has a number of limitations. The cross-sectional design limits our ability to directly test whether the reorganization of the subject networks causes pain or whether pain causes network reorganization, or perhaps not related at all. Longitudinal studies will provide the opportunity to determine whether intrinsic network organization contributes to state or trait measures of chronic nociplastic pain. Secondly, previous studies have shown that patients with Major Depression Disorder have altered intrinsic network architecture [48]. Additionally, many individuals with FM have multiple comorbid clinical diagnoses including anxiety and depression [59, 60]. While the differences in network architecture in the

current study are not affected by comorbid depression and anxiety, these symptoms may contribute to the intrinsic reorganization of the brain and could be explored further in future studies.

Conclusion:

Intrinsic functional brain networks are more variable among FM patients compared to HC. Furthermore, changes in community composition of intrinsic brain networks varies between patients with high and low levels of clinical pain. This work suggest that mesoscale community structure may be of use as a diagnostic tool for patients with FM.

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Chapter 3 : Anterior Insula Connectivity and Brain Network Community Structure During an Aversive Visual Task is Related to Clinical Pain in Fibromyalgia

Introduction

Fibromyalgia (FM) is a chronic nociplastic pain condition characterized by persistent widespread pain, fatigue, sleep problems and cognitive dysfunction [1-3]. In addition, FM patients show hyperalgesia and allodynia to mechanical and thermal stimuli as well as increased sensitivity to stimuli that bypass peripheral noiciceptors (e.g., sounds, odors, and lights) [4-7]. These findings of a generalized perceptual amplification of sensory input suggest that dysreguated multisensory processing and integration within the central nervous system (CNS) may be a key underlying mechanism of FM.

Heigthened sensitivity due to altered function of pain and more general sensory processing is a common trait observed in FM and other chronic nociplastic pain conditions [8-10]. Application of low pressure stimuli produce high levels of pain across patients with FM, and increased activity in brain regions associated with pain processing such as the somatosensory cortex, superior temporal gyrus, cingulate cortex, medial frontal gyrus, and insular cortex [11]. The anterior insula plays an integral role in sensory processing, conscious experience and pain [12-16]. During the presentation of innocuous visual, auditory, and tactile stimuli, FM patients exhibit greater insula activity compared to healthy controls [17]. In addition, we previously showed that FM patients rated an aversive visual stimulus as significantly more unpleasant than healthy controls and this visual stimulation elicited stronger insula activation in individuals with FM, which correlated positively with the level of self-reported clinical pain [4].

Our group and others have reported increased insula functional connectivity in FM patients at rest [11, 18, 19]. Extending this work to the whole brain network, we have found that the insula is a highly connected and influential hub node in FM patients but not healthy controls [20]. We also found that the community structure of the whole brain network is more variable in FM patients and that these differences are related to clinical pain, namely, patients with high clinical pain levels have more variable community network structure. Interestingly, the community assignment of the insula was more stable within patients displaying high levels of clinical pain [21]. While these findings are related to community structure at rest, how functional connectivity and network organization differ in FM during a task such as a visual stimulus is unknown. Understanding the relationship between the insula and the whole brain network during visual stimulation may shed light onto the aberrant sensory processing in these patients.

Here we explore differences in the functional organization of the brain during aversive visual stimulation between FM and healthy control participants (HC). The changes in functional organization were examined across multiple spatial scales – from the level of individual circuits (i.e. seed to whole brain connectivity of the insula) to connectome-based organization (i.e. insula membership in functional communities). We hypothesized that FM patients would exhibit stronger functional connectivity between the anterior insula and visual cortex during visual stimulation. We predict the community structure of FM networks would be more variable and composed of different nodes during the CHECK and REST (i.e., lower within-group and between-group NMI).

Material and Methods:

Overview of Analyses. Our primary goal was to assess changes in functional connectivity and network organization during presentation of an aversive stimulation (flashing checkerboard, CHECK) in FM compared to HC participants. First, we measured differences in anterior insula functional connectivity during CHECK compared to a resting period (REST). Next, we constructed functional brain networks composed of nodes (i.e. brain regions) and edges (i.e. functional connections). We tested whether there

were differences in network organization between groups during each condition (CHECK and REST) through global (e.g., modularity and community structure) and nodal (e.g., phi) network measures.

Participants. 28 female FM patients (age = 40.8 ± 13.2 years; clinical pain-VAS = 50.3±20) and 19 age- and sex-matched HC (age = 42.5 ±11.1 years) participants underwent an aversive visual stimulation task during a functional Magnetic Resonance Imaging (fMRI) scanning session. Patients were included based on satification of the 1990 American College of Rheumatology criteria for fibromyalgia, duration of disease for more than 6 months, right handed, self-reported clinical pain greater than or equal to 40 mm on a 100mm pain visual analog scale (VAS) at the time of enrollment, willingness to not introduce any new FM treatments for the duration of the study, and capable of giving informed consent. Participants were excluded based on current or history of taking medications for modulating pain, history of substance abuse or positive urine drug screen for drugs of abuse, concurrent autoimmune disease that may cause pain, such as systemic lupus, rheumatoid arthritis, and inflammatory bowel disease, pregnant or nursing, psychiatric illness, contraindications with fMRI procedures or migraine that may affect fibromyalgia. Healthy control participants were age and sex-matched to patients. Healthy controls were excluded if they met the 1990 American College of Rheumatology critera for fibromyalgia, any chronic medical illness including psychiatric disorders, diagnosed with a chronic pain disorder, current pregnancy and contraindications with fMRI procedures. The CHECK versus REST task evoked activation results that have been previously published refs: [4]. Here we extend these findings by examining functional connectivity and brain network organization in this sample. Of note, fibromyalgia does occur in men and children. However, it is more prevalent in women and the focus of these analyses. The Institutional Review Board at the University of Michigan approved the procedures in these studies, and all participants provided informed written consent in accordance with the Declaration of Helsinki.

fMRI data acquisition, preprocessing and quality assessments. During an fMRI scanning session, participants underwent a 3-minute visual stimulation task previously described [4]. In brief, the visual task consisted of a block design of alternating 20-second blocks of a flashing (8 Hz frequency) blue-yellow

annulus checkerboard (CHECK) and a static fixation cross (REST). Visual stimulus intensity remained fixed at 76 lux and participants were asked to keep their eyes open and their attention focused on the screen. Clinical pain ratings were acquired prior to the scanning session.

Data were collected using 2 different scanners across three studies: General Electric 3.0 Tesla Signa Scanner 9.0, VH3 with a quadrature birdcage transmit–receive radio frequency coil (n = 11 participants; FM: n = 7, HC: n = 4), and a General Electric 3.0 Tesla MR 750 Discovery Scanner, DV23.1_VO2_1317.c with a quadrature birdcage transmit–receive radio frequency coil (n = 36 participants; FM: n = 21, HC: n = 15). The visual task fMRI scan sequence was acquired with a T2* reverse spiral gradient echo sequence (repetition time = 2500 milliseconds, echo time = 30 milliseconds, 90-degree flip angle, field of view = 22 cm). Slices were 3-mm thick, with an in-plane resolution of 3.125 × 3.125 mm, acquired at 48 locations parallel to the anterior–posterior commissure plane.

fMRI data were preprocessed and analyzed using Statistical Parametric Mapping (SPM) software package version 12 (Wellcome Department of Cognitive Neurology, London, United Kingdom), and the Conn functional connectivity toolbox (Cognitive and affective neuroscience laboratory, MIT, Cambridge, USA) both running on MATLAB 2014a. Upon collection of fMRI data, physiological artifacts were first removed using RETROICOR algorithm in MATLAB and slice time corrected using FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) software. Preprocessing steps included motion correction, realignment, co-registration to anatomical image, normalization to standard MNI template, and smoothing (FWHM Gaussian kernel of 8mm). Preprocessed fMRI data were entered into the Conn Toolbox and nuisance regression was performed using the CompCor method [22, 23], with six subject-specific motion parameters, the signal from white matter and CSF, and their first order derivatives included as confounds. Lastly, a hipass filter of 0.01 Hz was applied to fMRI data.

Motion assessment. Motion parameters were calculated from the six head realignment parameters (x, y, z, pitch, yaw, roll) during the visual task scan session for each participant. Subjects were excluded at multiple steps in the preprocessing pipeline due to excessive motion. First, participants were excluded from analyses

if parameters exceeded ± 2mm translation or ± 1° rotation in any direction prior to preprocessing. The six motion parameters were combined into a scalar quantity, instantaneous framewise displacement [24]. Mean and maximum framewise displacement (meanFD and maxFD) were calculated across the entire fMRI scan session for each participant (Figure B.1). One FM participant was excluded from the analyses because their meanFD was greater than two standard deviations. After removal of the this participant, meanFD was higher in FM during the CHECK and was included as a covariate of no interest.

Assessment of circuits: Seed based functional connectivity analyses. We previously reported that FM patients had stronger activation in the anterior insula during the visual task and reduced insula activity with pregabalin treatment [4]. We created 6mm spheres based on these insula findings (Table B.1) and measured seed-to-whole brain functional connectivity in the Conn Toolbox. To identify changes in insula functional connectivity during the visual task, subject-specific beta maps that measure functional connectivity for each condition were subtracted (CHECK minus REST) and entered into second level group analysis in SPM12. To identify patterns of insula connectivity that may be associated with clinical pain, we conducted whole brain regression analyses with individual beta maps and clinical pain ratings (Visual analog scale: 1-100), which were collected immediately prior to the scan session.

Assessment of connectome: Network construction. Previous work showed the composition of communities in networks of FM patients generated from resting state fMRI data are more variable than HC, as FM networks had a lower within group NMI (FM-FM, HC-HC). Between-group NMI (FM-HC) was lower than within-group NMI (FM-FM) indicating the community structure in FM networks are different compared to healthy controls [21]. To examine the community organization of functional brain networks during the visual stimulus, we generated subject-specific whole brain networks based on functional correlation matrices for the CHECK and REST conditions separately.

Functional brain networks were defined by 264 non-overlapping nodes (6mm diameter spheres) previously shown to produce consistent functional network topologies at rest and during tasks [25]. Edges were defined by functional connectivity (Fisher z-transformed bivariate correlation) between each pair of

nodes. Using code from the Brain Connectivity Toolbox and custom Matlab scripts [26, 27] [28] network communities were defined via modularity maximization community detection algorithm on the fully connected and weighted correlation matrix over 1000 repetitions. Consensus analyses were used to generate one subject-specific network for each condition and agreement matrices were generated that calculate the number of times two nodes are in the same community across the 1000 iterarions. A variety of thresholds, tau, were applied to each agreement matrix between 0.1 to 0.9, at intervals of 0.1. Tau indicates the proportion of occurences in which two nodes appear within the same community to generate each subject-level partition.

Group consensus networks were constructed during REST and CHECK from subject network partitions. at each threshold for the FM and HC group. In this framework, an agreement matrix measures how consistent two nodes appear within the same community across subjects within the FM and HC group, separately. Group consensus partitions or networks were based on tau thresholds between 0.1 to 0.9, at intervals of 0.1.

Global network measure: Modularity and Normalized Mutual Information. Whole brain network structure was assessed using modularity and normalized mutual information. Modularity (Q*) quantifies the strength of connectivity of nodes within a community relative to other communities in the network [27]. Brain networks with higher modularity values have stronger or more dense connections within a community compared to other communities [29, 30]. Differences in modularity were assessed between FM and HC networks during REST and CHECK conditions separately.

The community structure of networks were assessed using normalized mutual information (NMI). NMI quantifies the similarity in the composition of communities between two networks. NMI is higher between two networks with a similar community structure and equal to 1 if two networks are identical [31-33]. Differences in community structure between FM and HC were examined during the REST and CHECK conditions separately. For each participant's network, within-group (FM-FM; HC-HC) NMI is calculated

from the mean pairwise comparison between all subjects within the same group. Between-group (FM-HC) NMI is computed for each FM network as the mean pairwise similiarity between all HC networks.

Nodal network measure: Phi community assignment analysis. To identify which nodes may contribute to differences in community structure between FM and HC during the visual task, we examined the community assignment of each node between FM and HC networks during REST and CHECK, separately. We calculated Pearson's phi coefficient to quantify the similarity of node community assignment between networks as previously described [21, 33, 34]. For each individual network, a binary matrix was generated based on whether a given node was in the same community as all other nodes (1) or in a different community (0). The binary matrices were then used to generate a node similarity measure using Pearson's phi coefficient. The within-group mean phi (phi) was calculated for all subject-by-subject node pairs for each condition separately. Higher phi values suggest the node shares a similar community assignment within the group (i.e. same or similar community across nodes). Lower phi indicates that the node community assignment is variable and frequently shifts allegiance between different communities across participant networks.

To determine the relationship between node community assignment and clinical pain, within FM node phi values during CHECK were correlated with clinical pain ratings acquired immediately prior to the scan session. We only assessed this relationship between nodes consistently more variable in FM during REST and CHECK. A positive relationship suggests similar node community assignment of that node for patients with high clinical pain. To assess which nodes are in the same community as the insula across the FM group, we quantified the frequency (percent) in which a given node appeared in the same community (e.g. co-classification of the nodes) among patients with low and high pain. FM patients were grouped into low and high pain groups based on the median split of clinical pain intensity ratings. Nodes were visualized on the brain using BrainNet viewer.

Statistical Analyses. For seed to whole brain functional connectivity analysis, two sample t-test's were used to determine differences in anterior insula connectivity between FM and HC during the visual task (CHECK minus REST). Results were deemed significant with a false discovery rate (FDR) cluster-level

corrected P value < 0.05 derived from a voxel-wise uncorrected p-value < 0.001. To identify differences in insula seed functional connectivity associated with clinical pain intensity, whole brain regression analysis of contrast images (CHECK minus REST) were correlated with clinical pain ratings. Significant relationships were deteremined based on the false discovery rate (FDR) cluster corrected P value < 0.05 derived from a voxel-wise uncorrected p-value < 0.001. Group differences in modularity between FM and HC were determined by two sample t-tests. Differences in modularity between REST and CHECK conditions were assessed by paired t-test for FM and HC separately. Statistical differences in NMI and phi graph measures were determined by permutation tests. Permutation test significance was determined by the number of occurrences after the 1,000 random permuted group differences in NMI were more extreme than the observed difference. Age and motion (meanFD) were included as nuisance regressors. Post hoc pearson correlations were conducted between within-group phi values during CHECK and clinical pain and deemed significant at $p < 0.05$.

Results:

FM patients have increased insula functional connectivity during the visual stimulus.

FM patients had greater right insula connectivity to the primary visual cortex (lingual gyrus, p = 0.006 FDR; cuneus, p=0.005 FDR), bilateral middle frontal gyrus (left mid frontal p = 0.034 FDR; right mid frontal p = 0.006 FDR), and medial orbitofrontal cortex (mOFC, p = 0.032 FDR) during visual stimulation (CHECK minus REST) (Figure 3.1a and Table 3.1). We found higher left insula connectivity during CHECK minus REST to the middle cingulate cortex (p = 0.011 FDR) in FM compared to HC. No group differences in insula connectivity were observed from the other ROIs.

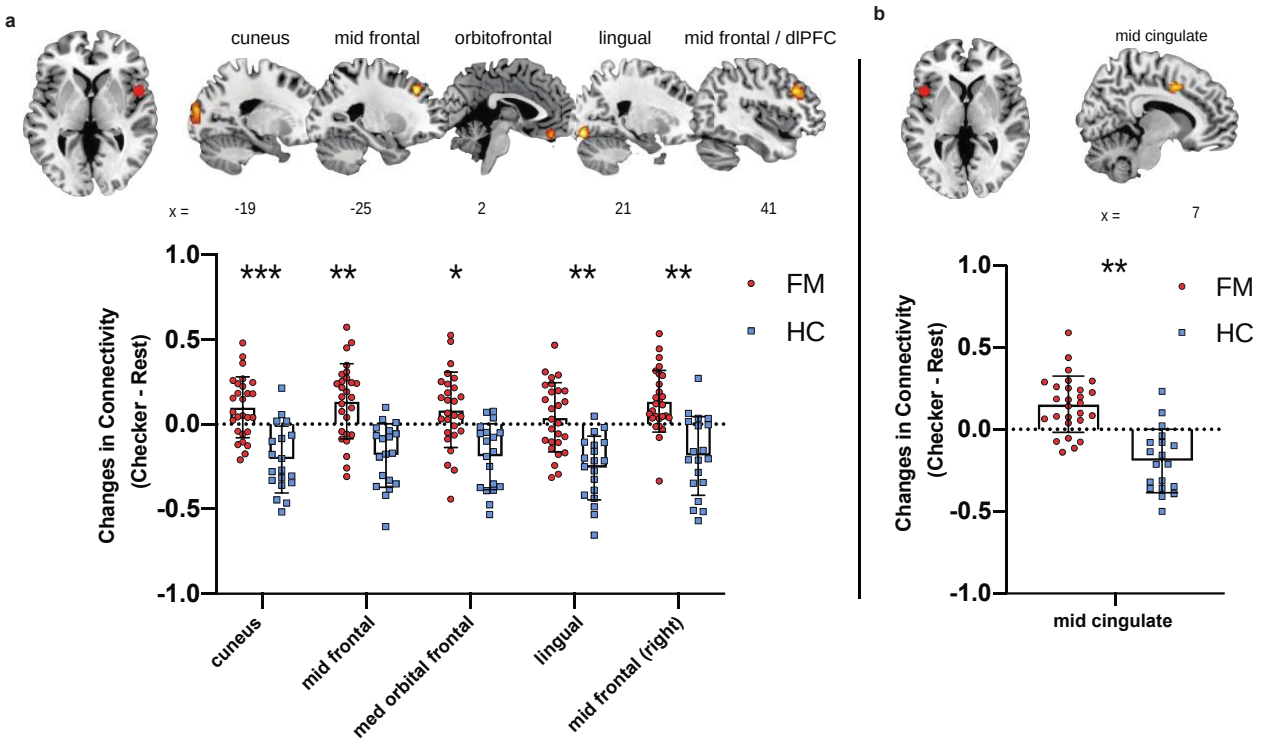


*Figure 3.1 Anterior insula connectivity is greater in FM during an aversive visual stimulus. (a) Increased connectivity between the right anterior insula and visual (e.g. cuneus, and lingual) and prefrontal (e.g. bilateral mid frontal gyrus, medial orbital frontal) in FM during an aversive visual stimulus. (b) During the visual stimulus, left anterior insula functional connectivity to the middle cingulate cortex was higher in FM. Results based on contrast image (checker - rest). FM - red circles, HC - blue squares Legend: * Indicates p < 0.05 FDR; ** indicates p < 0.01 FDR, *** indicates p < 0.005 FDR*

Table 3.1 Seed to whole brain functional connectivity results

Two sample t-test: FM vs. HC		Size	Location			p
	Side	(mm^3)	X	Y	Z	(FDR)
[4] Right anterior Insula:						
Lingual Gyrus	R	242	23	-83	-11	0.005
Dorsolateral PFC	L	129	-25	31	39	0.034
Dorsolateral PFC	R	218	41	27	31	0.006
Cuneus, mid occipital	L	280	-23	-93	19	0.005
Medial orbitofrontal cortex	R	139	1	39	-19	0.042
[3] Left anterior Insula:						
Middle cingulate gyrus	L		-11	11	43	0.001

[#] – denotes which insula seed used as region of interest (ROI) from Supplemental table 1

Whole brain regression analyses revealed that changes in left anterior insula connectivity to the thalamus was positively associated with clinical pain of FM patients (medial dorsal nucleus: p = 0.025 FDR; r = 0.79; Figure 3.2 and Table 3.2). Increased (CHECK minus REST) insula connectivity to the middle occipital gyrus and superior frontal gyrus was negatively associated with clinical pain of FM patients, (middle occipital gyrus p = 0.008 FDR; r = -0.82; p = 0.048 FDR, r = -0.76).

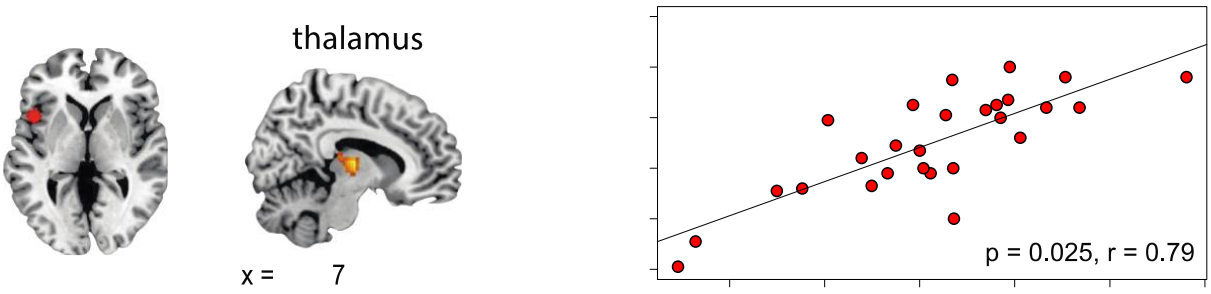


Figure 3.2 Increased FM anterior insula connectivity to the thalamus during an aversive visual stimulus is positively correlated with clinical pain. Change in left anterior insula functional connectivity to the thalamus was positively correlated with clinical pain in FM (regression: p= 0.025 FDR, r =0.79). Results based on whole brain regression using VAS prior to scan and contrast image (CHECK - REST) in FM only. Connectivity values from the same cluster were extracted from HC. FM - red circles; HC - blue squares; VAS - Visual analog scale

Table 3.2 Insula connectivity during the visual task is associated with clinical pain

Clinical pain (VAS)		Location			p	Corr
	Side	X	Y	Z	p (FDR)	(r)
[3] Left anterior Insula						
Positive Correlation with VAS						
Medial dorsal nucleus - thalamus	R	9	-13	7	0.024	0.79
Negative Correlation with VAS						
Middle occipital gyrus	R	39	-83	17	0.008	-0.82
[2] Right anterior Insula						
Negative Correlation with VAS						
Superior Frontal Gyrus - mPFC	R	7	63	1	0.048	-0.76

[#] – denotes which insula seed used as region of interest (ROI) from Supplemental table 1

Network organization during the visual stimulus is different between FM and HC.

Consensus networks suggested group differences in network community structure during visual stimulation task. (Figure 3.3, Figure B.2, Table B.2 and B.3) Both FM and HC group consensus networks consisted of five communities at REST and six communities during CHECK. At REST, all of the nodes affliated with the Visual Network were in community 1 in the FM network, whereas nodes within the Visual Network were split between community 1 (55%), community 2 (6%), community 3 (3%), and community 5 (35%) in the HC network.

During CHECK, visual nodes were split between community 1 (48%), community 4 (3%), and community 5 (48%) in FM, while in the HC network, these nodes were between community 1 (74%) and community 5 (26%). Nodes affiliated with the Salience Network were split across each community in the HC network at REST and CHECK. In FM, these nodes were split between community 2 (50%), community 3 (44%), and community 4 (28%) at REST, and between community 2 (22%), community 3 (33%), community 4 (17%), and community 6 (28%) during CHECK.

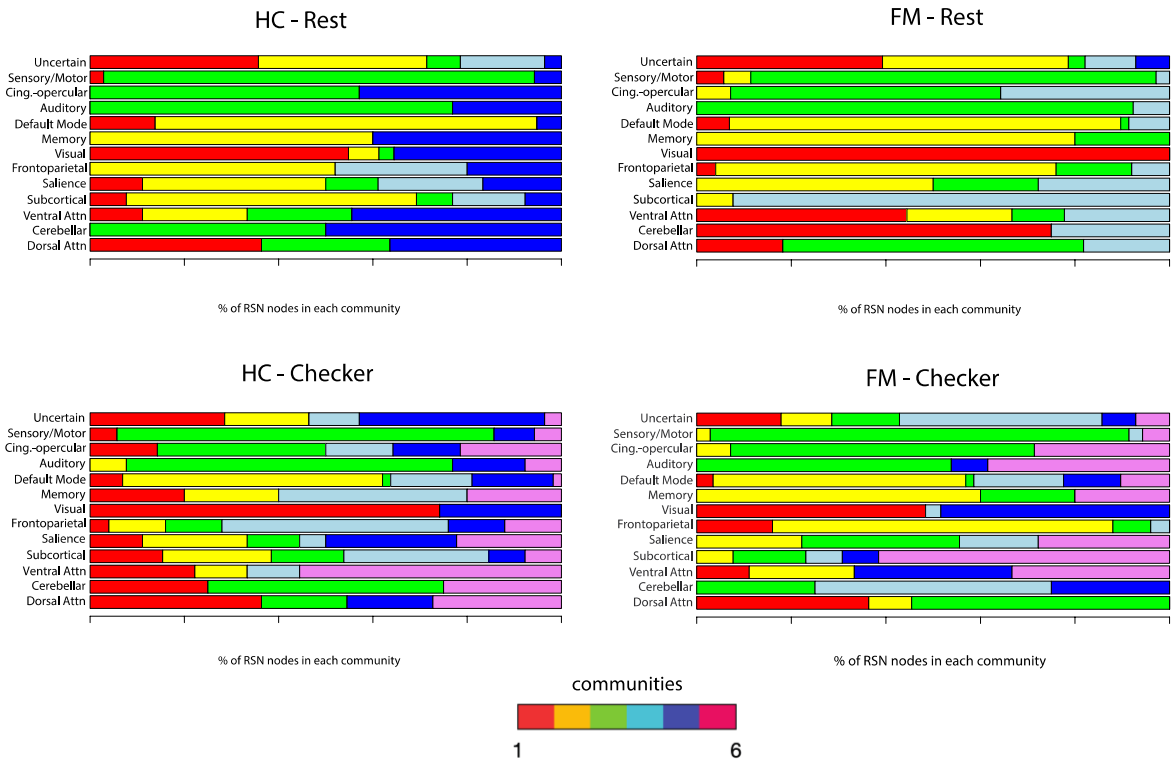


Figure 3.3 Group consensus networks reveal differences in network organization during a visual stimulus. Consensus networks were constructed from individual FM and HC networks at tau = 0.50 during rest and checker. FM and HC networks were both composed of five communities at rest and six communities at checker. Between group differences in node community assignment may be associated with network reorganization into communities during the visual stimulus.

We found no difference in modularity between FM and HC networks during CHECK or REST conditions, (Figure B.3). However, the community structure of FM networks were more variable and different from HC networks during CHECK and REST. Within-group NMI (FM-FM, HC-HC) was significantly lower in FM compared to HC during both rest and checker (Permutation test, $p < 0.0001$; Figure 3.4a-c). Between-group NMI (FM-HC) was significantly lower than within-FM NMI (Permutation test, $p < 0.0001$, not shown). Lastly, between-group NMI was significantly lower during CHECK, compared to REST (paired t-test, $p < 0.0001$, Figure 3.4d), and FM networks were more modular during CHECK compared to REST (paired t-test $t(26)=-4.4429$, $p = 0.00014$, Figure B.3). Group differences in within-group NMI were consistent across

several thresholds (Figure B.4). Moreover, differences in between-group NMI were consistent between tau threshold 0.1 to 0.7.

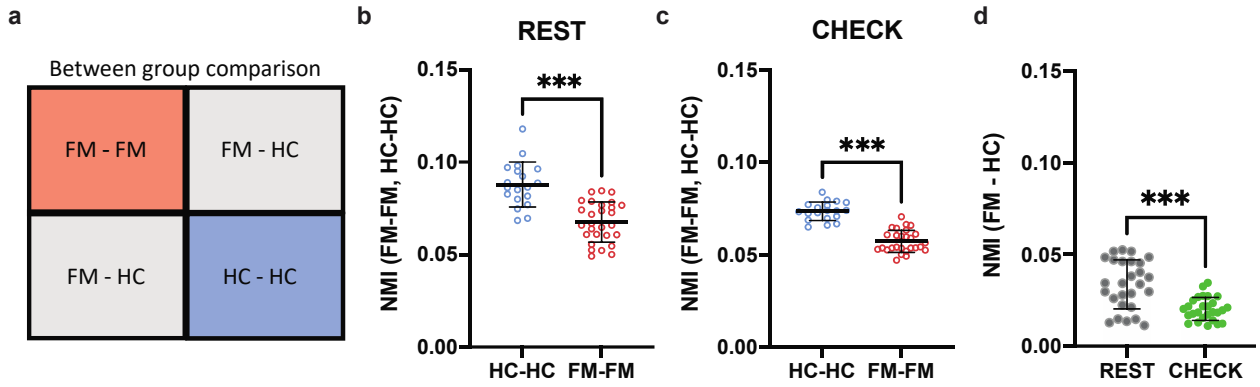


*Figure 3.4 FM network community structure is variable during a visual stimulus as compared to HC. FM network community structure is more variable compared to HC during visual stimulus. Comparison of global community structure of whole brain networks were assessed using NMI during REST and CHECK. (a) Within group NMI (FM-FM; HC-HC) measured similarity of community structure across networks within the same group. Between-group (FM-HC) NMI measured similarity of community structure between FM and HC networks at REST and during CHECK. Within-group NMI was significantly lower for FM than HC during (b) REST and (c) CHECK conditions. Between-group NMI was significantly lower than within-group NMI during REST and CHECK (not shown). (d) Between group (FM-HC) NMI was significantly lower during CHECK compared to REST. Legend: Within FM - NMI - red; Within HC - NMI - blue; Between FM-HC – NMI, REST - gray; Between FM-HC – NMI, CHECK- green REST-NMI (Statistical test based on Permutation test, *** - $p < 0.001$)*

Insula node variability during the visual stimulus is associated with clinical pain in FM

During CHECK, 139 nodes were significantly different in their community assignment between FM and HC networks. The left inferior parietal lobule (Permutation test, $p = 0.004$), right supramarginal/angular gyrus (Permutation test, $p = 0.007$), and superior frontal gyrus (Permutation test, $p = 0.034$) had higher within-group phi in FM compared HC. The community assignment of these three nodes are more stable across networks of individuals with FM compared to HC (Table 3) . The remaining 136 nodes had lower phi values across networks of individuals with FM compared to HC, indicating the community assignment of most nodes are more variable in FM during CHECK. During REST, the phi values of 113 nodes were significantly different between FM and HC networks. Seventeen of the 113 nodes had higher phi values in FM compared to HC.

These nodes include visual brain regions, like the cuneus and lingual gyrus, and were stable in their community assignment in FM compared to HC at rest.

Within-FM phi values were consistently lower for sixty-seven nodes when compared to the HC, indicating they were more variable in their community assignment during REST and CHECK (Figure 3.5b). These nodes included brain regions involved in pain processing such as the anterior, mid and posterior insula; precentral and post central gyrus; medial orbital frontal; and the dorsal and middle cingulate. We found that the within-FM phi during CHECK was correlated with clinical pain for nine nodes: the right precentral ($r = -0.531$, $p = 0.004$), and postcentral gyri ($r = .585$, $p = 0.001$), precuneus ($r = 0.459$, $p = 0.016$), left anterior insula ($r = 0.522$, 0.005, Figure 3.5c), right caudate ($r = 0.520$, $p = 0.005$), and superior temporal gyrus ($r = -0.390$, $p=0.044$). (Table 3.4). Positive correlations suggest that node community structure was similar across of individuals with high clinical pain during CHECK relative to low pain. Negative correlations indicated that node community assignment is more consistent across networks for low pain, relative to high pain.

Table 3.3 Nodes with higher phi values in FM compared to HC REST and CHECK

		Location			Observed difference	p
ROI #	Name	X	Y	Z	(mean-phi)	(FDR)
Node phi: FM > HC - CHECK						
Node68	L IPL	-50	-34	26	0.0251	0.004
Node99	L SFG	-16	29	53	0.0164	0.034
Node130	R supramarginal angular	47	-50	29	0.0214	0.007
Node phi: FM > HC - REST						
Node16	R SMA mid cingulate	10	-2	45	0.0532	<0.001
Node74	L MTG	-41	-75	26	0.0209	0.047
Node78	L Sup orb frontal	-18	63	-9	0.0282	0.042
Node85	R IFG	27	16	-17	0.0184	0.023
Node93	R precuneus	15	-63	26	0.0326	0.014
Node103	L SFG	-10	55	39	0.0220	0.015
Node104	L SFG	-20	45	39	0.0364	<0.001
Node140	occip lingual	8	-91	-7	0.0222	0.018
Node147	L mid occip	-28	-79	19	0.0269	0.023
Node150	R parahipp lingual	27	-59	-9	0.0241	0.015
Node152	L cuneus calcarine	-18	-68	5	0.0331	<0.001
Node159	R cuneus	15	-77	31	0.0238	0.007
Node164	L mid occip	-42	-74	0	0.0186	0.047
Node165	R fusiform lingual	26	-79	-16	0.0243	0.046
Node168	L inf occip	-40	-88	-6	0.0418	<0.001
Node169	R mid occip	37	-84	13	0.0300	0.035
Node171	L mid occip	-26	-90	3	0.0202	0.049

Table 3.4 Nodes showing significant correlation between within-FM phi values correlated with clinical pain during CHECK consistently more variable in FM compared to HC during REST and CHECK

Node phi x Clinical pain (VAS)		Location			Pearson Corr.	Sig.
ROI #	Name	X	Y	Z	(r)	(p)
Node 26	R postcentral	50	-20	42	0.585	0.001
Node 44	R pre postcentral	51	-6	32	-0.531	0.004
Node 136	R precuneus	4	-48	51	0.459	0.016
Node 208	L anterior Insula	-35	20	0	0.520	0.005
Node 233	R caudate	15	5	7	0.522	0.005
Node 237	L superior temporal gyrus	-55	-40	14	-0.390	0.044

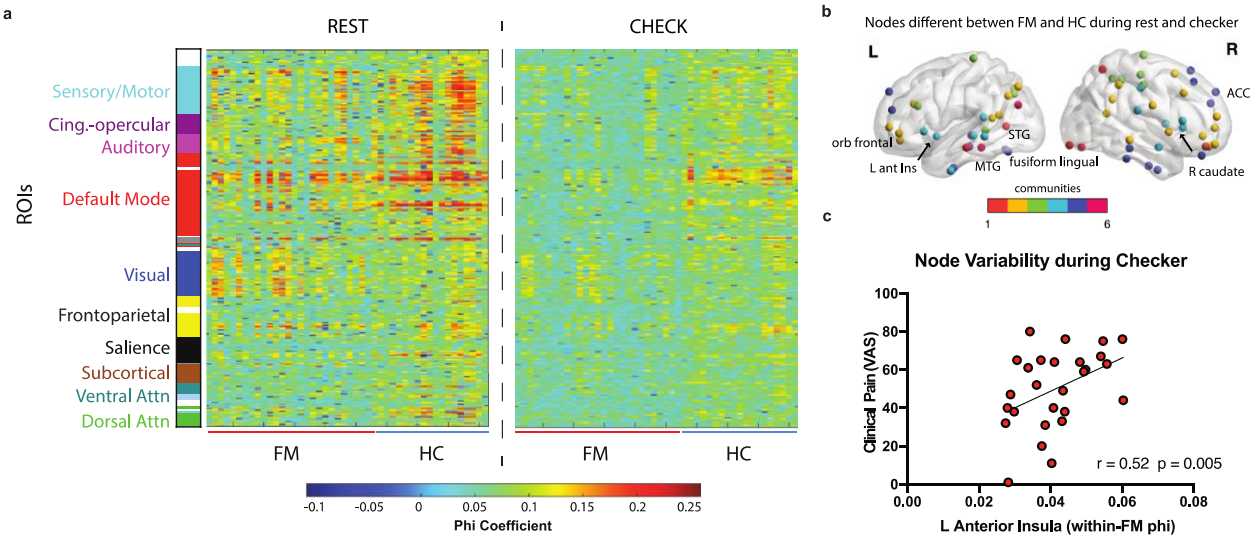


Figure 3.5. Phi analyses during rest and checker. (a) Within-group phi values were assessed during the visual stimulus to measure nodal community structure within each group. Nodes with higher phi correlation coefficients suggest similar community structure and is indicated by warmer colors. 113 nodes were significantly different during rest and 139 were significantly different during checker. Phi values were significantly higher in FM for seventeen nodes during REST and three nodes during CHECK. (See Table 3). (b) 68 nodes (including the anterior insula) were consistently different in their node community assignment between FM and HC networks during the visual stimulus. (c) Node variability measures by within-FM phi for the left anterior insula is positively correlated with clinical pain in FM. This suggest similarity in insula node community assignment among FM patients with high pain. Legend: community 1 - red; community 2 - yellow; community 3 - green; community 4 - light blue; community 5 - blue; community 6 – pink

Lastly, to identify the nodes in the same community as the anterior insula among individuals with high pain, relative to low pain, we quantified the number of times each node was in the same community as the anterior insula node in individuals with FM with low and high pain separately (Figure 3.6a, Table B.4 and B.5). We find the middle (62%) and posterior (54%) insula, superior temporal gyrus (77%), medial dorsal thalamus (62%) and cuneus (69%) are in same community as the anterior insula in FM networks from patients with high pain. Nodes in the mid frontal orbital (64%), along with nodes in the mid insula, precunues/PCC, dorsal ACC, and caudate (all at a 57%) were in the same community as the anterior insula across low pain networks. Of note, nodes in the middle temporal gyrus were found in both low and high pain networks at least 50% of the time. However, nodes in the occipital-inferior temporal cortex were in the insula community in at least 50% of the low pain networks during CHECK (Figure 3.6b).

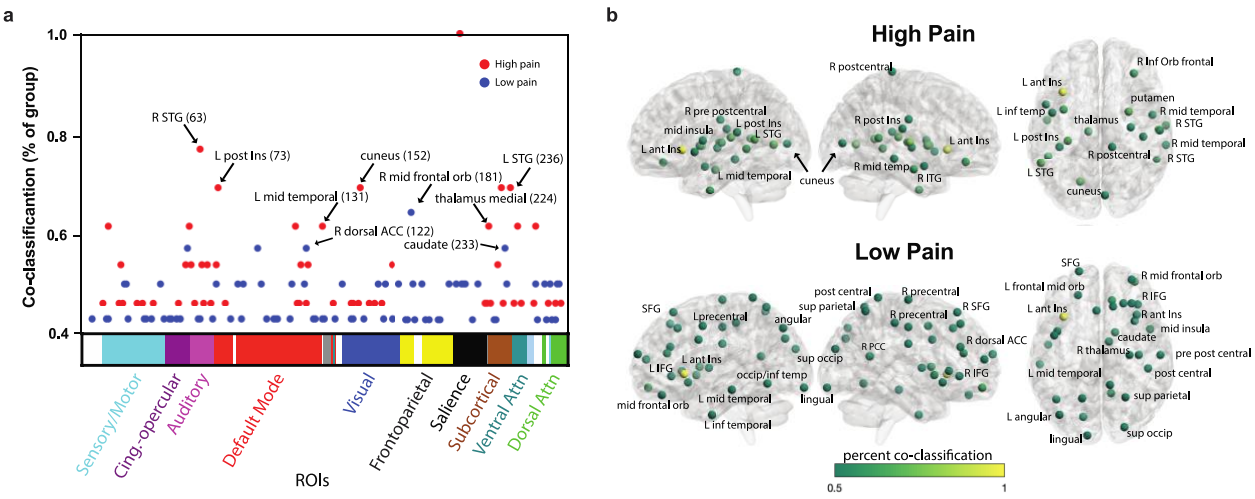


Figure 3.6 Visualization of anterior insula community assignment in FM during a visual stimulus (a) Insula community structure between FM networks with low and high clinical pain were assessed by the frequency in which each node was assigned (i.e. co-classified) to the insula community within each group. (b) Nodes with at least 50% co-classification within each group were identified and labeled. The cuneus (69%), superior temporal gyrus (77%), and thalamus (62%) were among the nodes in the same community as the L anterior insula across high pain networks. Nodes in the orbital frontal (64%) dorsal anterior cingulate cortex (57%), and caudate (57%) were in the same community as the insula in low pain networks. (See Appendix B. Table B.4 and B.5 for full list of nodes). Legend – Co-classification: Low Pain - blue, High pain – red; Node color – percent co-classification

Discussion

Here we sought to examine functional connectivity between the insula and other brain regions in FM patients during an aversive visual task. Overall we found increased insula connectivity to visual and attention brain regions, including the cuneus and prefrontal cortex. Furthermore, whole brain regression analyses revealed that stronger functional connectivity between the insula and thalamus was associated with greater clinical pain. Community structure of FM networks are more variable compared to HC networks during both REST and flashing checkerboard (CHECK) conditions. While there were no differences in modularity between FM and HC networks, FM networks were more modular during CHECK compared to REST. We also found that insula connectivity and node variability in community assignment was associated with enhanced clinical pain. These findings suggest that FM brain networks are more variable than HC networks while viewing the aversive visual stimulus. This work supports the hypothesis that functional connectivity to the insula is different in FM at rest and during a visual task.

Our results are consistent with prior findings demonstrating the insula plays a key role in the integration of multiple sensory modalities [11, 13, 17, 35, 36]. We show that insula connectivity to visual cortex is stronger in FM compared to HC. Increased insula functional connectivity to frontal cortex and mid cingulate may reflect a functional role of insula in integrating low-level sensory information with higher order sensory processing associated with attention and modulatory processes [20, 37-39]. Specifically, visual information is processed through the ventral visual network, the insula may act as a hub to route this information to both the cingulate and the prefrontal cortices. Indeed, it has recently been demonstrated that the anterior insula, which is positioned in a neurocognitive hierarchy between primary sensory cortex and higher-order association cortices, serves as a cortical "gate" for sensory information to be consciously accessed [39]. While the cingulate and orbitofrontal cortex are involved in modulation of visual processing, aberrant activity or we show decreased functional connectivity between these higher order visual regions may further suggest dysregulated modulatory processes in general sensory and pain processing in FM.

The current study is the first to show large scale network interaction of brain regions involved in pain and visual processing, potentially through similar or shared mechanisms. Previous studies have manipulated levels of evoked laser stimuli, visual (object recognition) and working memory (N-back) to measure how pain modulates visual processing [40, 41]. Moreover findings in FM demonstrate that activity in the insula is associated with heightened sensory perception or sensitivity (e.g. clinical pain)[4]. Together our findings strengthen these results to show that increased insula and salience network connectivity play an integral role in pain processing potentially via sensory integration. We show that heightened insula connectivity to the thalamus was associated with elevated clinical pain. The thalamus is a key regulator of gating

sensory information into the cortex and cortical dynamics across the brain emphasizing that connectivity between this structure and the insula could be an important modulator of clinical pain [42-49]. Furthermore, although the thalamus gates the transfer of sensory information to primary sensory cortex, the insula has been shown to gate the access of this information by higher-order association areas [39].

Interestingly we find that increased functional connectivity between the insula and mid occipital cortex connectivity was associated with lower clinical pain. In HCs– anticorrelations were present between the insula and visual cortex regions. We speculate this further highlights the difference in response to aversive visual stimulation between our groups and the interaction between nociplastic pain and sensory sensitivity in FM patients.

In addition to altered connectivity between the insula and visual encoding brain regions, we found broader alterations in the functional organization of the brain network during presentation of a visual stimulus in patients with FM. This extends our previously published work, also in FM, which examined community structure at rest [21]. Using normalized mutual information (NMI), a metric assessing brain network architecture, we found that FM brains were more variable than HCs. The increase variability in FM during the visual task may reflect the difference in brain response to the visual stimulus among individuals with FM. This was true for both REST and CHECK conditions, and moreover the differences between groups were stronger during the visual task. Together, this indicates that network structure is even more aberrant in FM when the brain is processing a visual stimulus. This has certain implications for conscious access of visual stimuli in these individuals, in that they may have altered cognition or conscious access of visual information.

FM patients with elevated clinical pain may also lack engagement of anti-nociceptive circuits, resulting in a more intense perception of visual stimuli. Some individuals with FM display altered conditioned pain modulation, wherein painful conditioning stimuli facilitate or enhance pain from a test stimulus [50-53]. This is typically opposite of what is seen in HCs where the conditioning stimulus inhibits pain from the test stimulus. Although not shown here, our results lead us to speculate that enhanced sensitivity to visual stimuli may also enhance pain in FM patients via altered connectivity between the insula and other brain regions. If true, this might reflect a type of cross-modal facilitation of conditioned pain modulation wherein a different sensory modality (e.g. visual stimulation) may exacerbate pain or increase sensitivity [4].

During the visual task, network modularity was higher in FM, indicating stronger connectivity within communities than between in FM networks. Through measuring the frequency of co-classification of nodes, the relationship between altered global and nodal community structure within a network may extend functional connectivity to larger systems of functional re-organization. Increased co-classification of two nodes may be associated with increased or stronger connectivity between brain regions and resting state networks within a group. Indeed, during the visual stimulus we find that insula node communities are more stable among FM patients with high pain. Consistent with our functional connectivity findings, we show that nodes in the visual cortex and thalamus are in the same community as the insula in individuals with high pain.

This study has limitations. First, our sample of 28 FM patients compared to 19 HC is relatively small. Second, this study was limited to women with FM and future studies should examine if there are sex differences in visual hypersensitivy in FM and similar chronic pain conditions. In addition, it is unknown if our findings are generalizable to other chronic nociplastic pain conditions. It remains unknown whether these changes in connectivity observed here are also

present for other sensory stimuli such as odors and sounds. Lastly, as previous analyses report changes in insula activation during the visual stimulus were reduced with pregabalin treatment, future research studies seek to assess changes in connectivity or network structure with treatment.

In conclusion, we demonstrated that functional connectivity between the anterior insula and visual/medial frontal brain regions was higher in FM patients compared to healthy controls during an aversive visual stimulus. Increased insula connectivity to the thalamus was associated with greater clinical pain in FM patients. Network analysis revealed that FM network community structure of the brain was more variable than HC during both rest and visual stimulation, but group differences were stronger with the visual stimulus. Together these findings show that the anterior insula might play an integrative role in pain and sensory processing associated with visual hypersensitivity in FM. Importantly the connectivity patterns we see may also be targets for treatment effects related to reducing sensory hypersensitivity in chronic pain patients.

Acknowledgements

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Chapter 4 : Conclusions and Future Directions

The complex and subjective experience of pain is a function of the intrinsic alert system of the body to signal potential threat to prevent tissue damage. Unfortunately, maladaptive processes that augment central nervous system processing may contribute to the spontaneous and persistent pain, which can result in chronic pain without peripheral, nociceptive drive (e.g., nociplastic pain as seen in FM). Aberrant functional connectivity between several brain regions involved in pain and sensory processing, including the somatosensory and motor cortex, striatum, posterior parietal cortex, cerebellum, prefrontal, and insula cortex, are all involved in chronic pain CNS processes. Here we determined that the functional reorganization of the brain is associated with clinical pain in fibromyalgia (FM), the prototypical nociplastic pain condition. Using graph theory, we examined network mechanisms of sensory processing in chronic nociplastic pain. This approach provides a link between the traditional methods of functional connectivity (e.g., bivariate correlation) and multivariate network analysis. Communities are a collection of nodes or brain regions that are more connected to each other than to other communities [1-4]. Through global and local network measures we demonstrated that intrinsic network organization is more variable in FM at rest and during an aversive visual stimulus. Together these results demonstrate that there are functional relationships between large-scale networks within the brain associated with pain and visual processing in FM. Identification of such network mechanisms provide novel potential targets for the treatment of individuals with chronic nociplastic pain.

Summary of Findings

The primary finding in Chapter 2 is that intrinsic network architecture is altered in chronic pain patients. Using normalized mutual information (NMI), FM patients had significantly lower within- and between-group NMI, suggesting that the composition of communities is more variable across FM networks and significantly different from healthy controls (HC). Changes in resting state network connectivity are associated with this lower NMI in FM. Salience network (SLN) connectivity was negatively associated with brain regions in the default mode (DMN) (mPFC and PCC), salience (ACC), and visual (VIS) network (cuneus). Group consensus analysis revealed that resting state networks spanned multiple communities. Moreover, the community assignment of nodes within a resting state network was different for FM and healthy control networks. Intrinsic community structure also differed between patients with varying degrees of clinical pain. FM patients with high pain had significantly lower within- and between-group NMI, suggesting the community structure was variable and different when compared to the networks of patients with low pain. The community ratio revealed differences of node community assignment across chronic pain intensity.

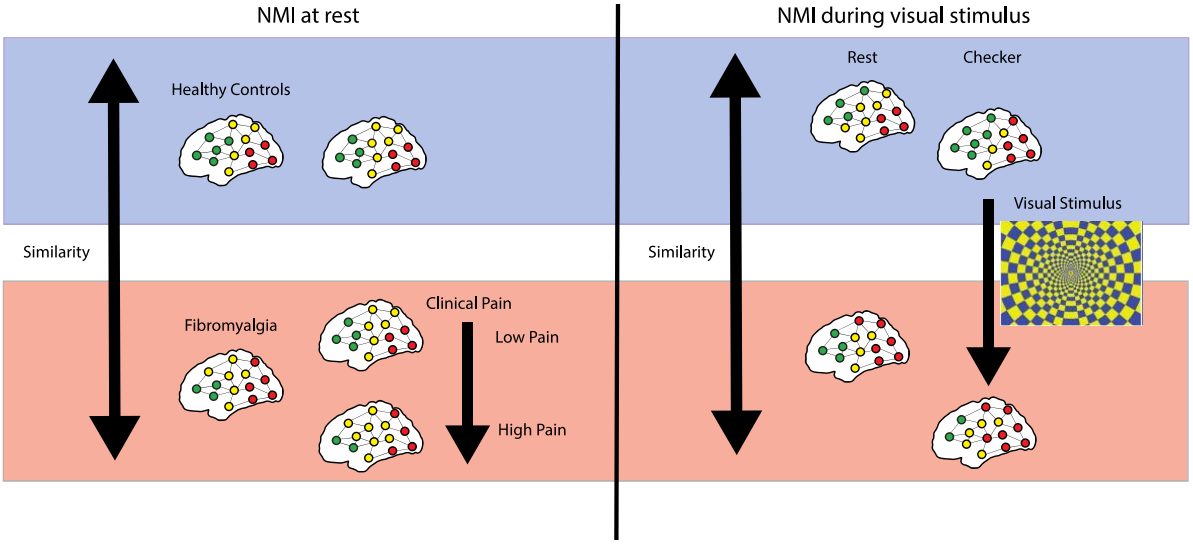


Figure 4.1 Maladaptive reorganization of functional brain networks in FM. Similarity of community structure was assessed using normalized mutual information. Our results show that community structure is more variable in FM compared to HC (Lower

within-FM NMI). Group differences in community structure were modulated with the visual stimulus, previously reported more pleasant in FM compared to HC (Black bi-directional arrows represent the span of network stimiliarity, uni-directional arrow represents the directional shift in similarity community structure)

This variability in network structure in FM was attributed to differences in node community assignment across FM networks with varying degrees of clinical pain. The community ratio revealed one way in which nodes are assigned to different communities between low pain and high pain groups. For example, we found that the anterior insula was more stable in its community assignment among high pain networks compared to low pain networks. Further, the insula had a low alpha ratio among high pain networks suggesting it was assigned to the DMN community among high pain patients. This is consistent with previous results from our group that show that functional connectivity between the insula and DMN is positively associated with clinical pain in FM.

The primary finding in Chapter 3 is that FM patients have increased anterior insula connectivity during the visual stimulus. The insula was more connected to visual cortex, bilateral middle frontal gyrus, and medial orbital frontal cortex during visual stimulation. During the visual stimulus, anterior insula connectivity to thalamus was positively correlated with clinical pain and displayed stronger connectivity in FM patients with higher clinical pain. Here, the thalamus may play a role in intergrating primary sensory information or maintaining ongoing neural processes. FM networks were more variable and different compared to healthy controls during visual stimulation (i.e., lower within-group and between-group NMI). There was no difference in modularity of networks between FM and healthy controls. However, modularity values were significantly higher during visual stimulation compared to rest in FM. Of note, the anterior insula node was again significantly more variable in community assignment in FM compared to healthy controls during visual stimulation. Moreover, insula node phi values were positively correlated

with clinical pain. Lastly, the cuneus and thalamus were assigned to the insula community in over 60% of the high pain networks.

Overall, these findings suggest that the functional organization of the brain is variable and different across individuals with FM at rest and during presentation of an aversive visual stimulus. At rest and during a visual stimulus, we found differences in insula node community assignment in FM associated with clinical pain. The composition of communities across this analysis highlights two main points: functional brain networks are reorganized among FM patients, and salience network function is associated with cross-modal integration and visual hypersensitivity in chronic centralized pain.

Implications and Conclusions

Maladaptive reorganization of the network

Our analysis of the functional organization of the brain reveals that the intrinsic community structure of FM networks is more variable and different from healthy controls. Yet there were no differences in modularity between the two groups. We show that intrinsic functional brain networks are reorganized in chronic nociplastic pain. It is important to note that differences in the organization of networks are not completely random, but instead there are specific changes in connectivity and network organization. Across multiple thresholds we show a group difference in network community structure between indviduals with FM and HC. At each threshold, the within group NMI for was significantly higher than the between group NMI. The average number of communities increased with tau thresholds, yet we found no group difference in community number or modularity between groups.

The lack of difference in modularity and the number of communities suggests that the composition of communities contributes to the difference in network architecture. As nodes

naturally change their communities over time, the brain adaptively reorganizes to optimize its function and efficiency. Changes in the community composition within the network may reflect stable changes in the local information flow occurring at faster timescales and contribute to the global properties of the network [24] [25] [26]. The stability and heterogeneity of node community assignment across patients is associated with FM and their subjective clinical pain report. Nociplastic pain may be an example of maladaptive reorganization of brain networks, in which changes in the network composition result in, or are a response to, persistent pain or stressors among patients [27] [28].

The community structure of functional brain networks is based on the composition of communities and the connections within and between them. While the increased variability of network organization may reflect the heterogeneity of FM, differences in community organization between levels of pain indicate that pain is associated with differences in the composition of communities in the functional brain networks in FM. Moreover, between-group NMI was significantly lower in FM compared to healthy controls during visual stimulation. FM networks were also more modular during the visual stimulus, meaning increased within-community connectivity, or less between-community connectivity during the visual stimulus associated with visual or pain processing. The community assignment of nodes involved in pain processing (i.e., insula, postcentral gyrus, and caudate) are positively correlated with clinical pain in FM. The insula node community structure is similar across networks of high pain patients. Thus the community structure changes, like functional connectivity, with clinical pain and sensory (visual) processing. Of note, node phi was negatively associated with clinical pain for the pre-post central and superior temporal gyrus, indicating more variable community structure among high pain networks. These differences support the notion that FM patients with low pain may have intact or

proper functioning descending modulatory networks during sensory processing, whereas in FM patients with high pain the modular organization of the networks is associated with heightened pain perception or altered pain or sensory modulation.

Our results are consistent with previous findings that show increased intrinsic functional connectivity between the DMN and insula among patients with FM [29, 30]. Specifically, we show that variability in community structure (NMI) was associated with connectivity between the DMN and SLN. Second, nodal analysis of the insula shows that the community assignment of the insula was stable among FM patients with high pain. The community ratio investigates the distribution of nodal community assignment within a group. DMN nodes that were stable among high pain patients had a higher alpha ratio, indicating that these nodes were in the visual and sensorimotor community among FM patients with high pain [31, 32]. Moreover, salience nodes (the insula) that were more stable among high pain patients had a lower alpha ratio, indicating that these nodes are more commonly found in community 2, with other DMN nodes [30, 33] (Chapter 2, Appendix A. Figure). These findings are consistent with previous reports of increased functional connectivity between the anterior insula and default mode network in FM .

Visual hypersensitivity in FM

These results agree with the current body of literature on sensory processing, as the insula is responsive to pressure, heat, sound, and plays a key role in the integration of multiple sensory modalities [34] [35] [36] [37]. We show that insular functional connectivity to the visual cortex is stronger in FM compared to HC. Increased insula functional connectivity to prefrontal cortex and mid cingulate suggests that under certain conditions, the insula can become more involved in integrating low-level sensory information with higher order visual attention and modulatory processes. Our results show that increased insula and salience network (cingulate) activity may

play a maladaptive role in pain processing via heighten sensory integration. In this way, integration may not considered good, and therefore increased functional connectivity between the insula and visual, frontoparietal and attention networks during the visual task is suggestive of aberrant integration of general sensory and pain processing in chronic pain.

Increased functional connectivity between the insula and the cingulate cortex reflect increased salience network connectivity during the visual stimulus in FM. The ventral visual network is associated with object and color processing of visual stimuli. The occipitotemporal network is connected to neostriatum and orbitofrontal brain regions, which further implies higher order visual processing and cross talk between sensory and pain processing engaged modulatory processes during presentation of a stimulus [38]. As the cingulate and orbital frontal cortex/rACC are involved in modulation of visual processing, aberrant activity or decreased functional connectivity between these higher order visual regions may imply an altered modulatory role in sensory and pain processing in FM.

The current study is the first to show the functional role of the insula at both the circuit and network level interactions of pain and visual processing in FM. Insula functional connectivity was negatively correlated with the middle occipital gyrus, a brain region affiliated with the ventral visual network that has previously shown to be modulated by acute pain. We show that during the visual stimulus reductions in functional connectivity between these brain regions are also implicated in clinical pain, lower changes in functional connectivity during the visual task are associated between these regions. It is important to note that in HCs, anticorrelations were present between the insula and visual cortex regions, further highlighting the difference in response to visual stimulus between groups. This modulation, however, may reflect that in FM, sensory

processing among patients with high pain may be associated with persistent, ongoing pain processing.

In FM patients, increased insula functional connectivity may be indicative of altered modulatory processes. We show that increased connectivity between the insula and thalamus was associated with elevated clinical pain. The thalamus is a key regulator of gating sensory information into the cortex, and functional connectivity between two cortical regions emphasizing that connectivity between this structure and the insula could be an important modulator of clinical pain [39] [40] [18, 19, 37, 41-43]. A recent neuroimaging study of both level and content of consciousness supported the hypothesis that insula is a cortical gate to conscious awareness [36]. As such, its dysregulation in FM could plausibly modulate both pain and visual processing and could further play a role in altered conscious processing in this population.

Future Directions

Functional organization of brain communities in FM

From a graph theoretical approach, the functional organization of brain networks is based on the functional connections between the components of the network. Community detection organize multiple brain regions (nodes) into sub-networks that are typically more connected to nodes within the community than between. Whether these communities are functionally relevant remains unknown. However, whether the community is the same for every analysis has yet to be explored. In the present analysis, the community structure of brain networks provides a framework to measure the complex interactions of the brain across individuals, revealing different potential solutions of this problem. [5, 6].

Differences in intrinsic community structure may reveal the large-scale network organization of the brain in which two or more resting state networks are highly connected and

form one community. As the composition of these communities span multiple resting state networks, it is harder to think that the modular organization of intrinsic resting state networks of the brain must directly overlap (1:1) with communities. At least one node within a community (i.e., "connector" hubs) would be labeled between communities or assigned to a community opposite of its resting state network due to its high participation coefficient within global network architecture [7] [8]. This is consistent with the notion that communities represent between-resting-state-network connectivity. For example, nodes affliated with the medial visual network, salience network and frontoparietal network are located in one community may reflect an aspect of visual processing during a cognitive task. Interaction (e.g. functional coupling) between these networks would suggest they play a role in processing and differences may be associated with performance.

During the visual stimulus, resting state networks span multiple communities as well. Nodes affiliated with the visual network based on the atlas used, were in the same community (i.e., community 1) during the REST condition in the FM consensus network. During CHECK, these nodes were distributed across multiple communities in the FM consensus network. This may further suggest that community organization changes dynamically with a task, in which the network is organized into functionally relevant communities associated with information flow or general sensory processing. Moreover the node community organization may capture the functionally relevant processing streams of neural information associated with sensory and pain processing that contribute to the underlying pathology of the brain in chronic nocicplastic pain [9] [10] [11] [12-16].

During the stimulus node phi was correlated with clinical pain in FM. We show that through node co-classification in the differences in insula community structure was difference in patients with FM with varying degrees of clinical pain. These differences in community structure

may reflect functionally relevant subsystems of network connectivity in the brain. For example, the co-classification of nodes within the caudate, during the visual stimulus in FM, differed between low and high pain networks and overlapped with the cortico-striatal-thalamic circuit within the brain (Figure B.5) [17]. This suggests a role for the engagement of the somatosensory cortex and other brain regions involved in modulatory pain processes among individuals with low pain compared to high pain. High pain networks would then reflect the inability to engage with visual processing, or perceive the visual stimulus as painful. Extending our functional connectivity analyses, co-classification of the thalamus reflects the integrative nature of the thalamus with macroscale connectivity regulating cortico-cortico and thalamocortical interactions[18] [19].

Examining the organization of the brain as a connectome allows the exploration of how changes in community structure may play a role in pathological states. Specifically, we found that FM networks are more variable in their community structure compared to healthy controls. This is consistent with other clinical populations. For example, recent work in schizophrenia, major depressive disorder, and chronic low back pain has identified altered network structure among patients compared to healthy controls [20] [21] [22] [23]. Our research group and others have demonstrated that among patients with chronic centralized pain (FM), the brain regions critical for information processing and integration, called hubs, are pro-nociceptive, suggesting the reorganization of the brain in centralized pain states [8] [23]. The biological significance of these communities remains to be elucidated. They do not map to anatomical or resting state networks and yet, they reflect similar results of functional connectivity analyses. Together this suggests that the increased variability or altered community structure may be a general marker of underlying brain pathology. Integrating global and nodal measures like NMI and phi would require both longitudinal studies to examine the consistency of communities across individuals and their

functional relevance significance. Alternatively, a general distribution of normal NMI values can be determined with adequate sample size and conditions. If so, exploring how community structure varies across clinical populations is imperative. Subsquent analyses to understand how community structure varies over time naturally and with treatment remains a future area of discovery.

Application of network neuroscience

Community structure may reveal how the brain is organized in healthy populations and reorganized in disease. Resting state networks and functional connectivity were initially the largest level of organization of functional brain networks [44]. Here we show that the community organization may reveal how these resting state networks are functionally connected or overlap over time that resemble the functional properities of the brain consistent with our previous connectivity results (DMN - insula) [30] [45]. Communities may reveal functional systems, pathways, or circuits that can inform mechanistic investigation of pain pathophysiology. Yet, the level or threshold in which community structure shows a functional relevance remains unknown. The group differences observed in the current analyses are robust across many thresholds, yet whether 3-6 communities are functional relevant remain unknown.

Network modularity has previously been suggested to be used as a biomarker for pain [46]. Modularity was not different between FM and healthy control networks in both or our analyses. However, the change in modularity during the visual task may reflect changes in functional connectivity in FM (increased functional connectivity positively correlated with increased modularity).

As a biomarker, this may be promising on a few levels. First, the community organization reveals changes in functional connectivity associated with the task. How these communities

change may then reflect specific differences in the response to the stimulus and reveal circuit mechanisms in pain and sensory processing [47] [48] [15]. This would be consistent with previous imaging findings in which changes in task-based connectivity reveal individual phenotypes across subjects [49] [50] [51] [52]. As we observe differences in modularity in response to the task, it may be that at rest the modularity values are the same, however when stressed by a functional task, the FM brain may become more connected in response to the stimulus. This change in network organization may then be associated with pain or sensory processing within the individual patient[53].

Second, NMI may reveal that the composition of communities extends functional connectivity and resting state networks alterations in the brain to capture changes in brain organization at the level of functional system or macro-scale circuits in the brain. Variation in network organization may reflect difference in response to visual processing or ongoing nociplastic pain within these circuits revealed through the phi analysis of networks. This is consistent with previous findings in which patients with FM show failed conditioned pain modulation, or descending facilitation [54]. In this way, personalized medicine could then be used to determine whether changes in an individual's response to the visual stimulus (facilitation or modulation of pain and sensory stimuli) is associated with altered CNS function, to further guide treatment [52] [55] [56].

Comparison of community structure using normalized mutual information may be useful in large cohort studies in which comparison of networks can identify individuals with underlying brain pathology [47] [57]. This may be useful for classifying subgroups of patients within a general group or population or identifying patients with potential risk of developing pain. This would be useful in large, longitudinal cohort studies that track symptoms associated with pain, and

the potential for developing chronic pain or centralized features prior to diagnosis to determine whether the organization of networks are a cause or effect of persistent widespread pain.

Lastly, these analyses show the community organization of the brain, through functional systems or circuits within a network. With use of pharmacological tools, such as anesthetics or therapeutics, further characterization of these systems and their functional role in pain and sensory processing should be identified and assessed [58] [59] [60].

Treatment of Nociplastic Pain

As supported by our analyses here, chronic nociplastic pain is associated with functional reorganization of the central nervous system [61] [28] [27] [45]. Structural changes in the insula have been further associated changes in pain evoked functional connectivity, indicative of a relationship between the neuroplastic changes in the brain and functional connectivity [28]. However, whether the change in functional brain network reorganization in FM is either a cause or an effect remains unknown. The prevalence of nociplastic pain and/or hypersensitivity may vary across chronic overlapping pain conditions, as individuals with the same condition may experience varying combinations of peripheral (e.g., inflammation) and central (visual sensitivity) drivers [62] [63] [64, 65]. Through identification of markers of nociplastic pain, individuals with chronic pain can be treated by interventions targeting nociplastic versus neuropathic or acute nociceptive mechanisms.

Prior studies demonstrate functional connectivity predicts the analgesic response to pharmacological agents and neurostimulation [28, 66-68]. Successful treatment through reducing pain was associated with decreases in pro-nociceptive activity, or increases in engagement of descending modulation system. Harte and colleagues (2016) demonstrate that insula and DMN (IPL) activity during visual stimulation was associated with reductions in clinical pain following

pregabalin treatment. As we extend our understanding of insula activity via functional connectivity and community structure, future analyses will examine changes in functional connectivity or network organization associated with pregabalin treatment. This will be investigated in future analyses.

The origin of nociplastic pain varies at the individual level among chronic overlapping conditions, yet it is associated with increased sensitivity. Through measuring changes in network organization (as an extension of functional connectivity) following treatment through graph metrics like NMI and phi may shed light in the functional systems involved in sensitivity in improve prediction of treatment response across patients with overlapping pain conditions or surgical cohorts. Use of therapeutics that treat chronic pain both pharmacologic and non-pharmacologic may change network organization in which group differences in network metrics may no longer persist following successful treatment. Additionally, anesthetics disrupt functional connectivity or network organization associated with sensory processing and would further identify how changes in mechanisms of sensory processing are associated with pain experience [36, 37, 69] [70, 71]. In any case, successful treatment would be multifaceted, in which changes in the functional connectome will result in reorganization of the brain to resemble decreased sensitivity or normal facilitation thresholds.

Limitations

The current study has notable limitations. Our analyses here were performed on cross-sectional data sets. The lack of longitudinal design limits our ability to test whether community structure is stable within individuals or reflects individual variability across the population. As nodes naturally change their communities over time, the brain adaptively reorganizes to optimize its function and efficiency. Through a test-retest analysis among HC over several days or weeks apart, we can

determine whether communities are consistent and stable within and inbetween individuals. This would further determine whether the differences observed in FM are not random, but also provide a scale for global and nodal network measures like NMI. Second, as the goal of the second aim was to examine the relationship between pain and more general sensory processing, our analysis is limited in that these results are based on one sensory modality, vision. Whether these changes in community structure extend to other supraspinal sensory modalities and reflect more general cognitive processes remain unknown. Additionally, our investigations focused entirely on individuals with FM. As such these results may be limited to nociplastic pain, and not nociceptive or neuropathic pain. Moreover, these findings may not even apply to other nociplastic pain conditions. Extending our analysis to other chronic overlapping pain conditions would further generalize our findings and understanding of brain organization.

Concluding Remarks

Pain is a complex subjective experience often associated with physical threat or insult to the body. Many patients experience chronic widespread pain, in which pain may arise from augmentation of central nervous system connectivity. These aberrant changes in brain function, measured via neural activity and functional connectivity, which we linked to increased sensitivity among patients with chronic nociplastic pain associated with both heightened sensory and pain processing and dysregulated modulatory processing.

The brain is an adaptive complex system, in which the functional connections between multiple brain regions can be studied using multivariate tools such as graph theory. Through studying functional brain organization, we may understand how the brain normally works and what happens when it changes in ways that individuals experience pain without any peripheral insult. It remains unknown how the functional brain organizes to form intrinsic communities within a

network or at what level of the organization of the brain best contributes to cognition or sensory processing. As these analyses show the overlapping mechanisms of pain and sensory processing, future analyses should replicate these findings in larger datasets to assess whether similar or different functional systems exist across other populations, potentially beyond chronic pain. Identifying the causes of individual variability in community structure and understanding how the organization is paired with intrinsic physiology of the brain may shed light on how sensory processing and pain are modulated. Both may required to would be necessary to develop therapeutic approaches. This is an interesting goal for future work and can only improve our understanding of the functional organization of the brain.

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Appendix A : Chapter 2 - Supplemental Figures and Tables

Table A.1. Demographic and clinical characteristics for the discovery and replication datasets

	Discovery				Replication			
	Fibromyalgia (n = 38)	**Healthy (n = 17)**			**Fibromyalgia (n = 32)**	**Healthy (n = 20)**		
	Group Mean (SD)		t-value	*p-value*	Group Mean (SD)		t-value	*p-value*
Age	41.00 (12.11)	43.82 (10.39)	0.83	0.383	43.19 (14.68)	40.50 (14.37)	0.646	0.519
Clinical Pain	54.00 (20.20)	-			57.26 (22.24)	-		
Mean FD[#]	0.063 (0.04)	0.042 (0.02)	2.27	**0.027***	0.068 (0.04)	0.0635 (0.03)	0.502	0.618
Max FD[#]	0.320 (0.24)	0.285 (0.29)	0.49	0.63	0.411 (0.32)	0.427 (0.290)	0.180	0.858
Depression[+]	12	-			19	-		
Anxiety[+]	15	-			4	-		

[#]Motion calculated based on mean or max framewise displacement (meanFD, maxFD)

[+]Possible cases identified based on cut-off values: HADS – Depression: ≥ 8; HADS – Anxiety: ≥ 9; STAI > 40; CESD ≥18. *(Aparicio et al., 2013; Julian, 2011; Smarr and Keefer, 2011)*

* $p < 0.01$;

Table A.1 Demographic and clinical characteristics for the discovery and replication datasets. Clinical variables (pain, depression and anxiety) ratings for each participant were acquired prior to the scan session. Clinical pain was assessed on a visual analog scale (0 – 100), with 0 being "no pain" and 100 being "worst pain imaginable" prior to the scan. Mean framewise displacement (meanFD) was calculated as the average instantaneous framewise displacement across the entire fMRI scan session. In order to compare clinical variables across all subjects, cutoff values for depression and anxiety were used to split patients into two groups: possibly depressed or anxious, or not.

Table A.2. Table of scan parameters.

		Participants				Resting State fMRI Parameters						
		Fibromyalgia Mean(SD)		**Healthy Mean(SD)**							**Voxel size(mm)**	
Dataset	**Study**	**N**	**Age**	**N**	**Age**	**Scanner**	**TR**	**TE**	**volumes**	**slices**	**x y**	**z**
Discovery	Study 1	15	39.2(11.5)	-	-	3.0T	2000	30	180	43	3.13	4
Discovery	Study 2	13	45(12.67)	-	-	3.0T	2000	30	180	43	3.12	3
Discovery	Study 3	10	38.5(12.07)	17	43.82(10.39)	3.0T	2000	30	180	43	3.12	3
Replication	Study 4	18	41.88(15.03)	12	38.92(15.62)	3.0T	2000	30	180	43	3.13	4
Replication	Study 5	14	44.86(14.59)	8	42.88(12.88)	3.0T	2000	30	180	43	3.12	3

Table A.2 Demographic variables and MRI acquisition parameters for the 5 studies included in the analysis.

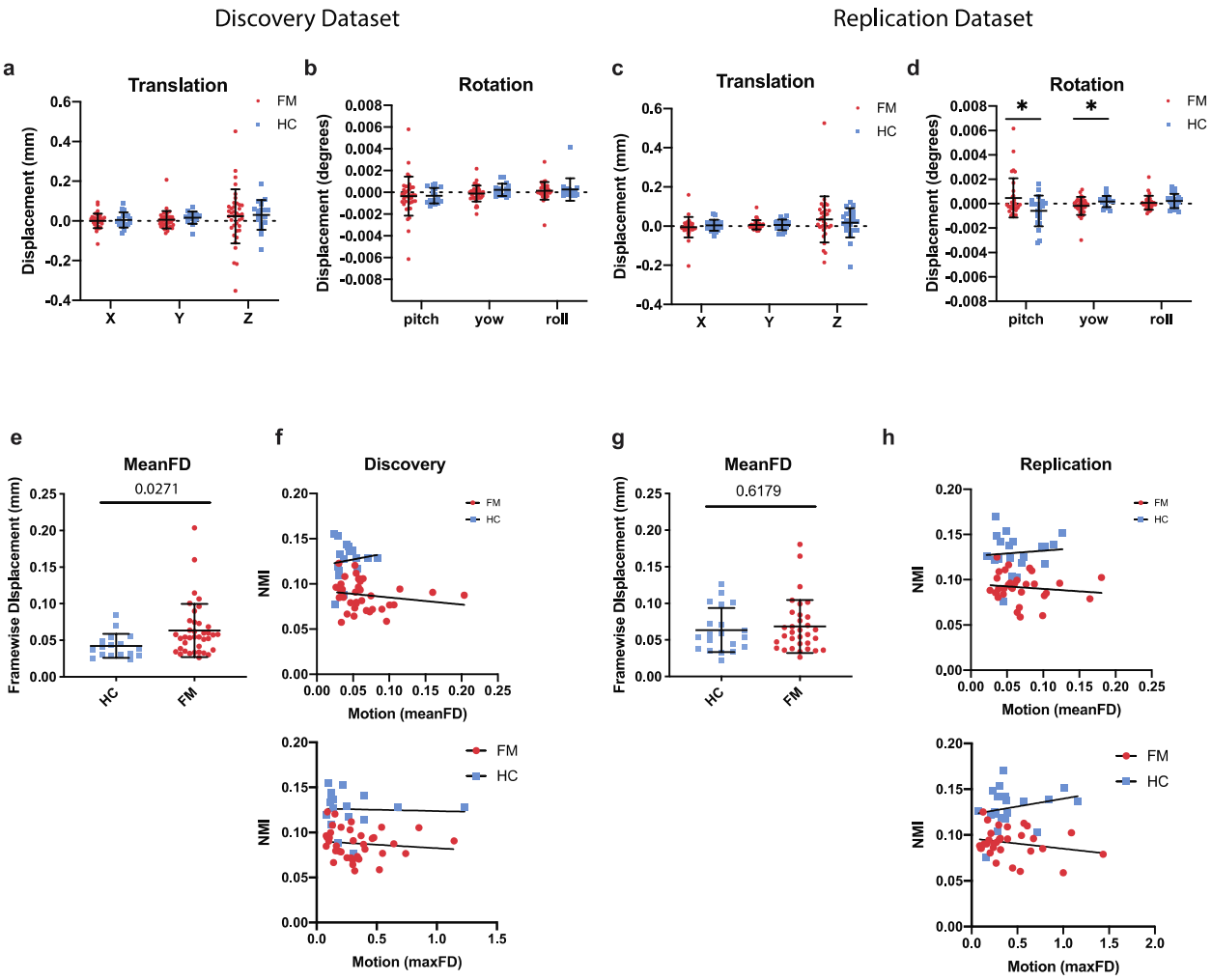


Figure A.1 Motion parameters calculated from fMRI data. Framewise displacement was calculated using 6 motion parameters (x,y,z, pitch, yaw, and roll). (a-d) There were no significant differences in the mean translation and rotation between FM and HC in the discovery dataset. In the replication dataset, we found no difference in mean translation but significant group differences in displacement in both pitch and yaw rotation parameters between FM and HC (e) There was a significant group difference in the mean framewise displacement (meanFD) in the discovery dataset. (f) However, there was no correlation between motion and within-group NMI measures for FM or HC. (g) There were no group differences in meanFD in the replication dataset. (h) There were no significant correlations between motion parameters (meanFD and maxFD) and within-group NMI values in the replication dataset. MeanFD was used as a covariate in all permutation tests of group differences for graph metrics to control for effects due to motion. (designates $p < 0.05$, ** designates $p = 0.005$).*

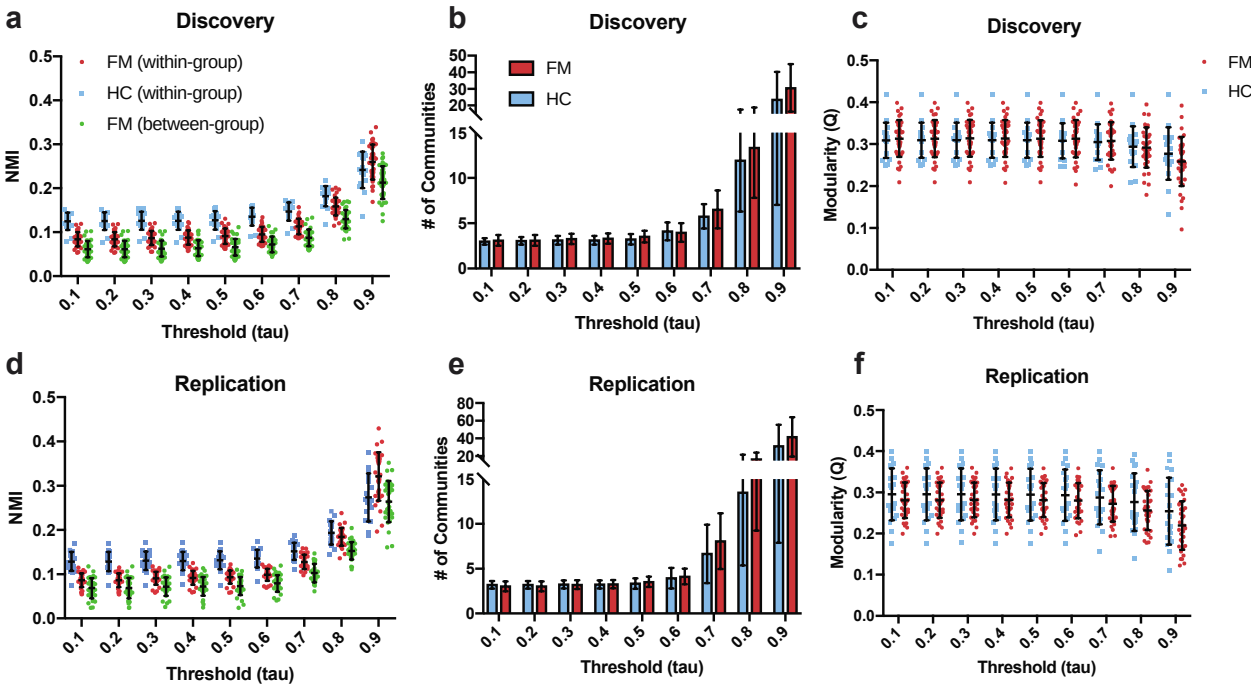


Figure A.2 Robustness of group difference in network architecture NMI differences were observed across multiple thresholds (tau = 0.1 – 0.8) in both the discovery (a) and replication (d) dataset. While the number of communities increased, there no differences in community number or modularity across thresholds (b,c,e,f).

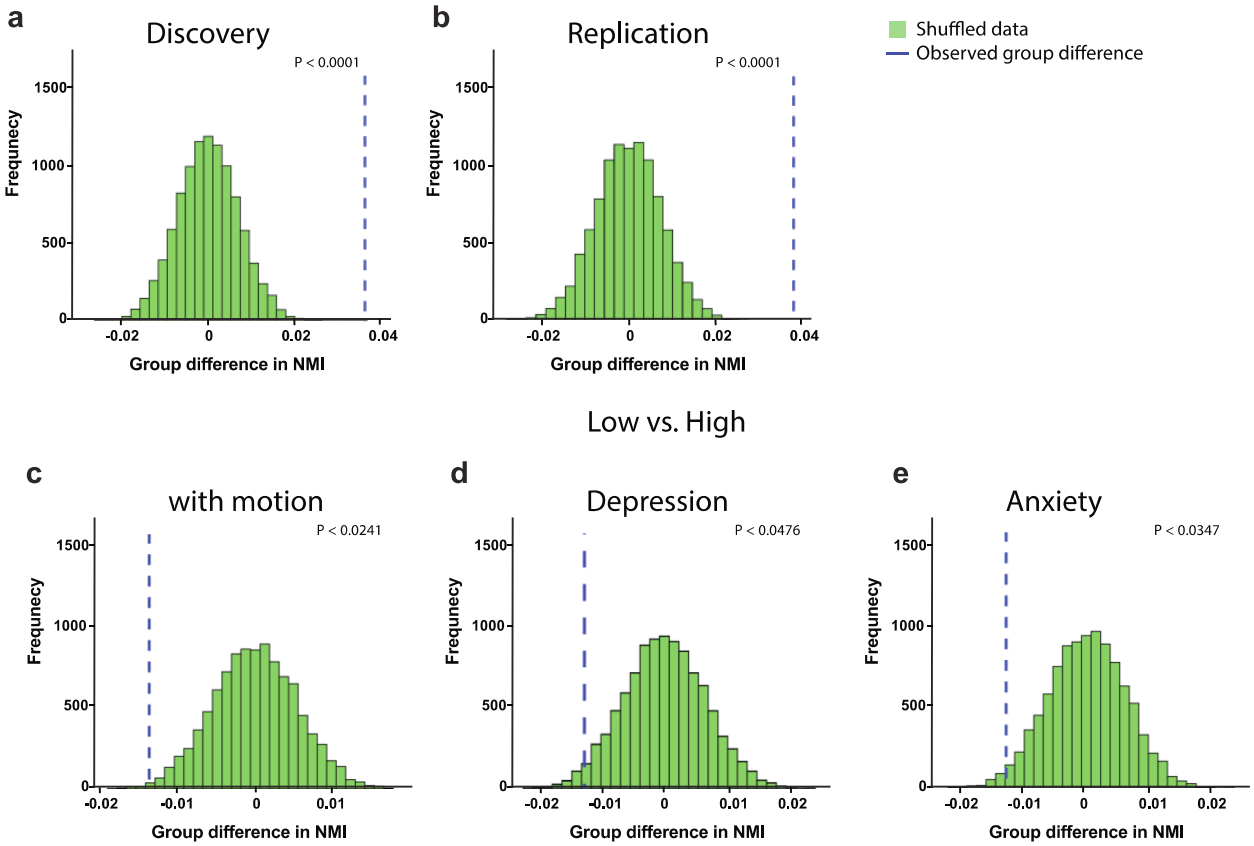


Figure A.3 Robustness of group difference in community structure to motion and clinical variables. Group differences in community structure were determined based on permutation tests with motion parameters. (a, b) Discovery and Replication dataset group differences in community structure for subject-level networks generated at tau = 0.40 with motion (meanFD and maxFD) included as a regressor of no interests. (c-e) Group differences in community structure between low and high pain patients were determined based on permutation tests with motion as a regressor of no interests. Group differences in NMI persist when (d) comorbid depression or (e) anxiety were included as regressors in the final model.

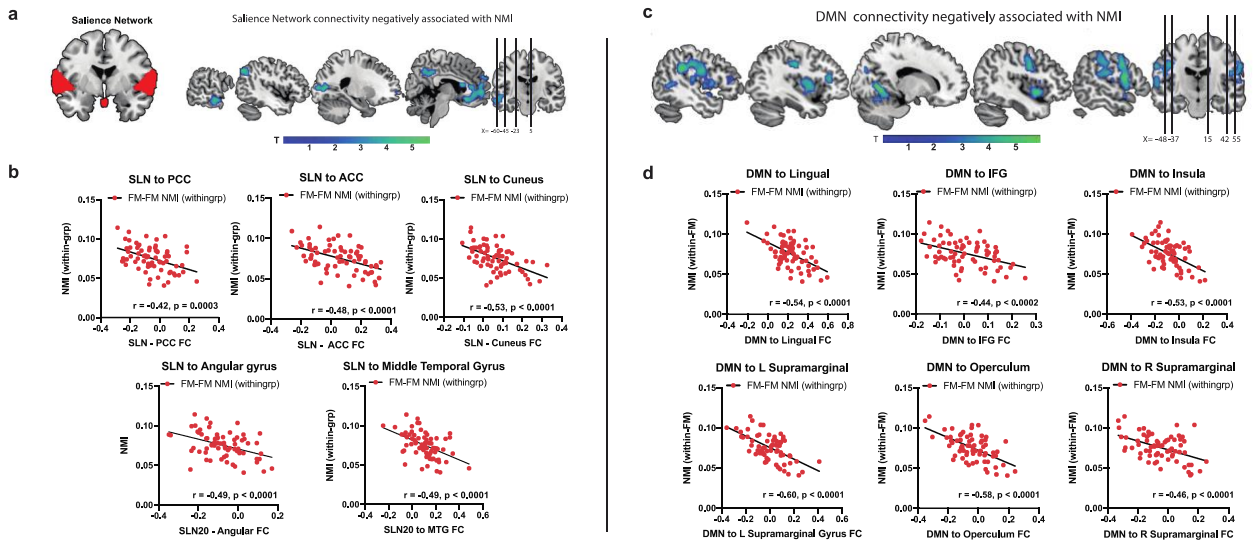


Figure A.4 Resting state network connectivity is associated with NMI. Whole brain regression analysis correlating NMI with changes in functional connectivity of salience network in FM. (a and b) Salience connectivity between the posterior and anterior cingulate/subcallosal gyrus, cuneus, middle temporal gyrus and angular gyrus were negatively associated with NMI. (c and d) Whole brain regression analysis correlating NMI with changes in functional connectivity of default mode network in FM. Functional connectivity between the insula cortex, lingual, inferior frontal, supramarginal, and precentral gyri were negatively associated with NMI in FM. (NMI relationship is based on within group NMI: FM-FM, HC-HC)

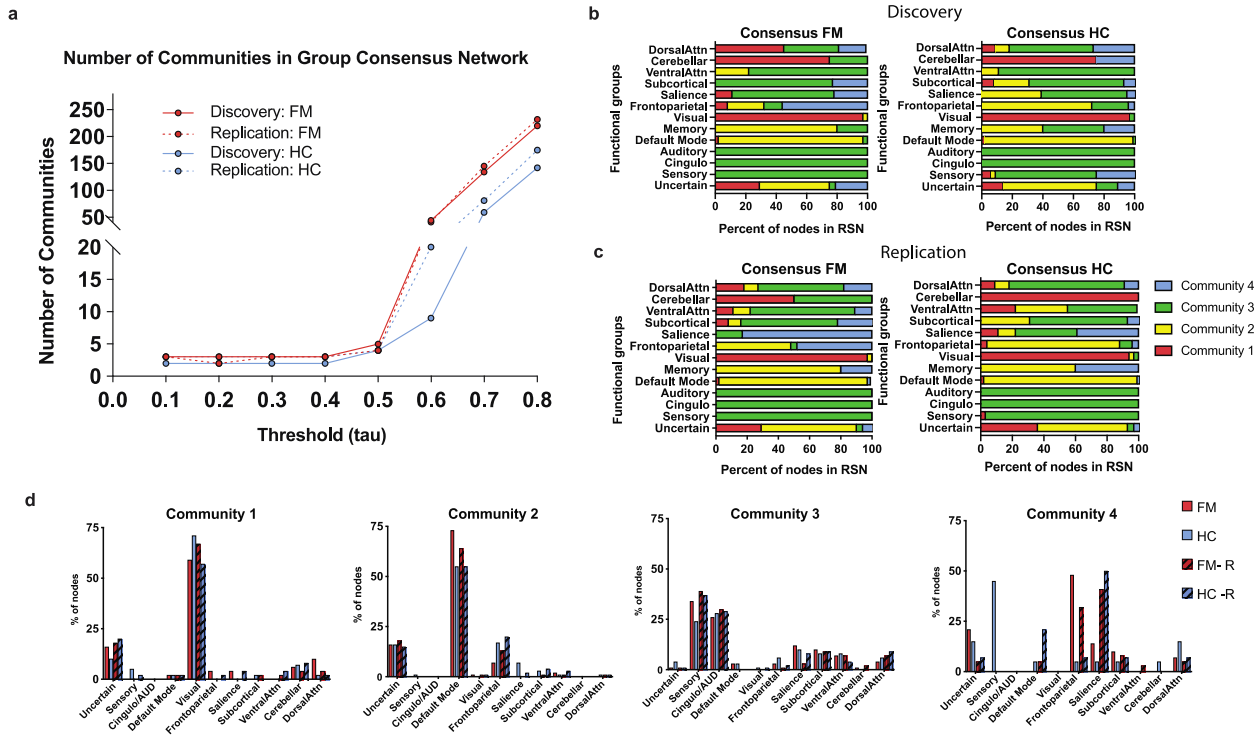


Figure A.5 Group Consensus reveals differences in community organization between FM and HC. Agreement matrices were generated from subject networks at each threshold tau. To identify group consensus networks, a tau threshold was applied at intervals of 0.1 from 0 to 1 to the group agreement matrix. For subject partitions at tau=0.40, the number of communities between FM and HC networks increased as thresholds increased. (a) The number of communities increased in the consensus analysis across multiple thresholds. We report findings at 50% agreement, based on consistent numbers of communities between groups and datasets (a and b).

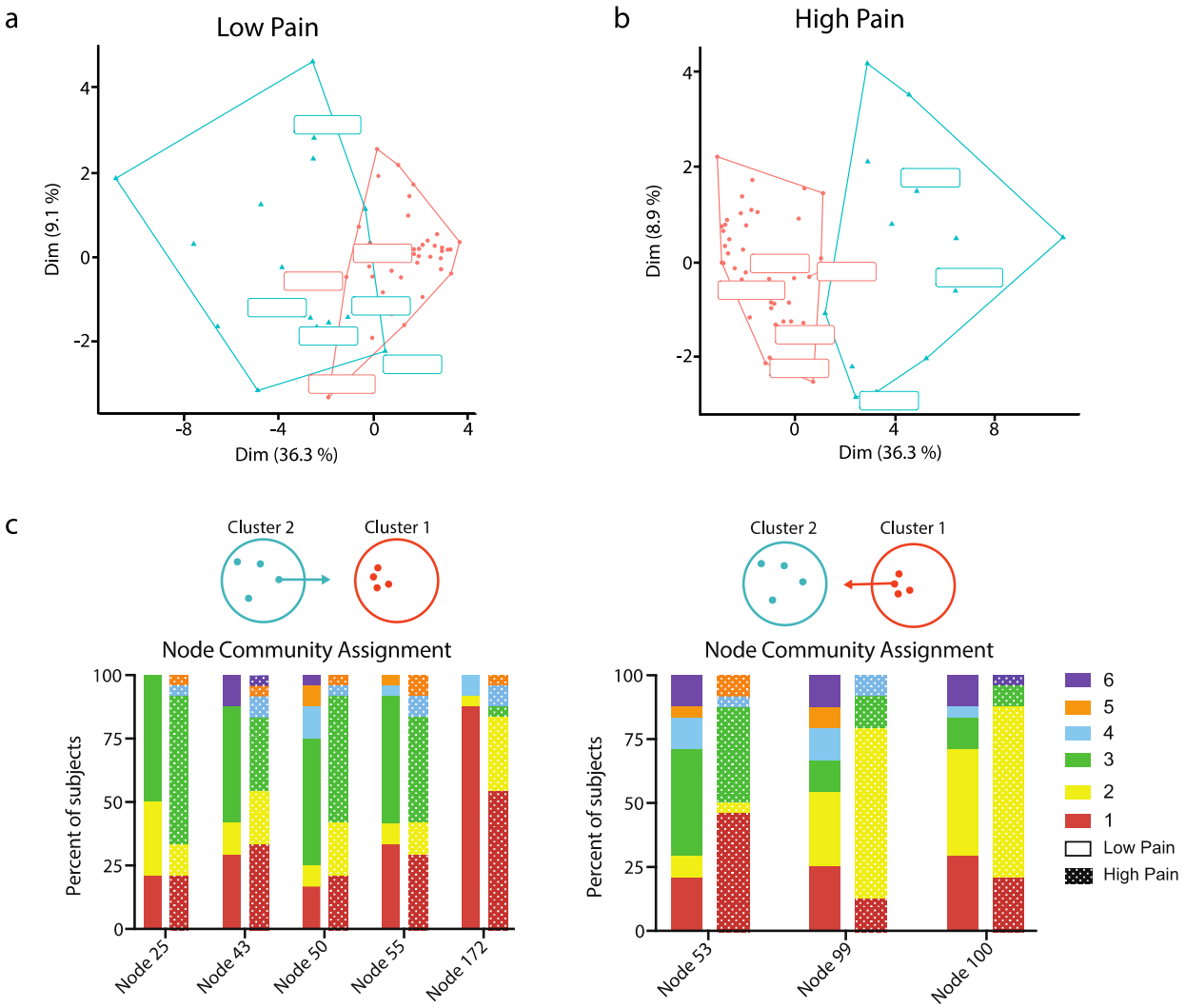


Figure A.6 Nodes that are significantly more variable in FM networks form clusters organization. Using the nodes that were more variable in FM compared to the HC in both the discovery and replication dataset, partition around medoids clustering analysis revealed two cluster solution in (a) low and (b) high pain. (c) Differences in the solutions revealed that nodes that move from Cluster 1 to Cluster 2 become more stable in their community assignment, while nodes that move from Cluster 2 to Cluster 1 may become more variable in their community assignment with the high pain group.

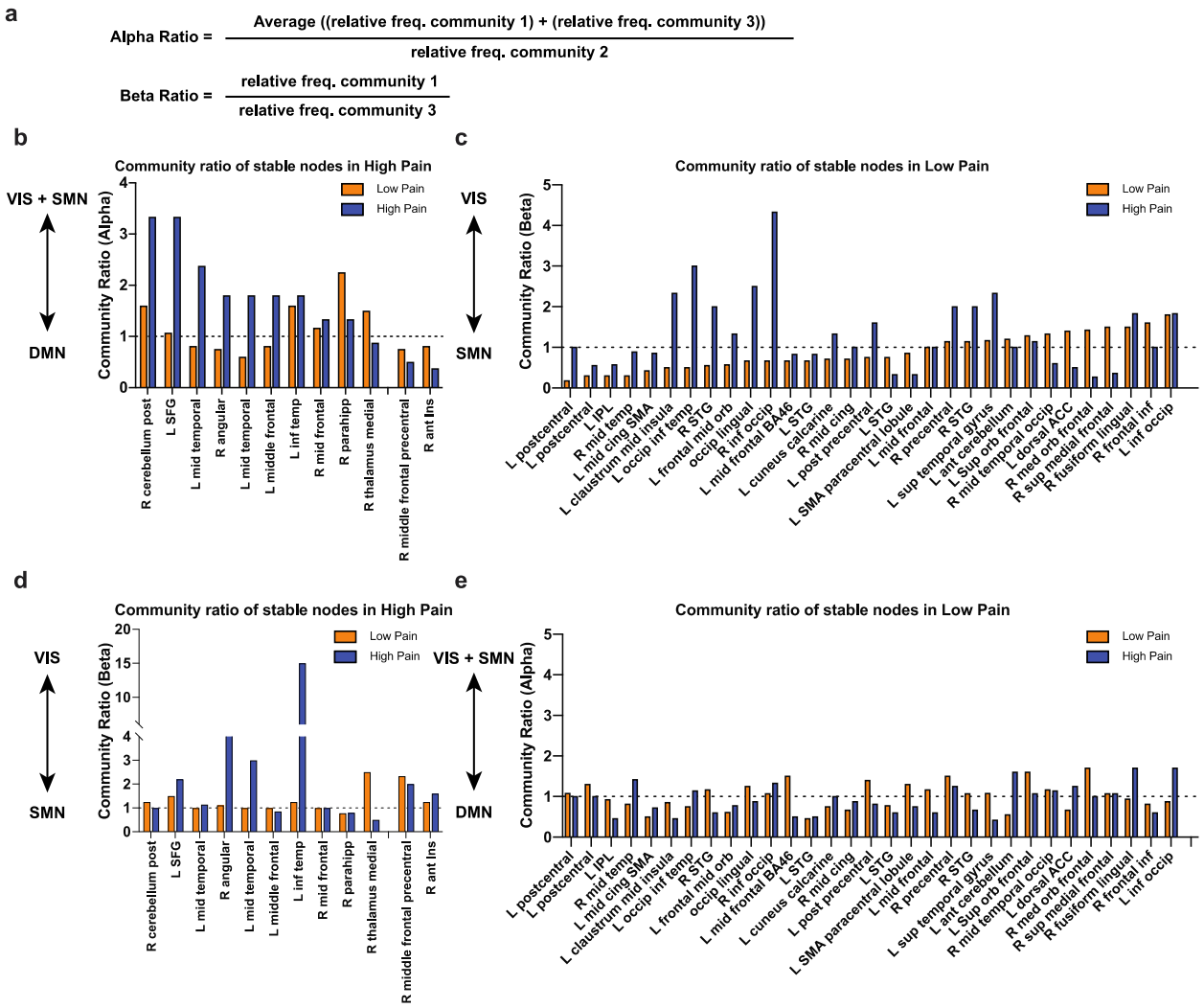


Figure A.7 Community ratio reveals differences in node community assignment between patients with low and high pain networks. (a) Based on the frequency distribution of node community assignment, the Alpha ratio quantifies the average frequency nodes were assigned to community 1 and 3 relative to community 2. The Beta ratio quantifies by the frequency nodes were assigned to community 1 relative to community 3. (b and c) To observe how a node differs in their community assignment between low and high pain networks, we calculated the alpha ratio for nodes stable in high pain and the beta ratio for nodes stable in low pain. (d and e) We also calculated the beta ratio for nodes stable in high pain and the alpha ratio for nodes stable in low. (Low pain – orange; High pain – blue.)

Appendix B : Chapter 3 - Supplemental Figures and Tables

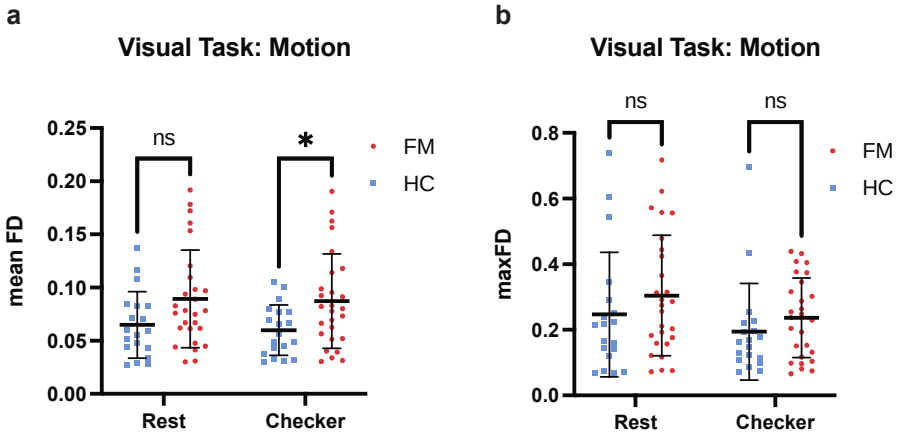


Figure B.1 Assessment of motion during the visual stimulus. Motion parameters were calculated from the six head realignment parameters (x, y, z, pitch, yaw, roll) during the visual task session for each participant. The six motion parameters were combined into a scalar quantity, instantaneous framewise displacement. Mean and maximum framewise displacement (meanFD and maxFD) were calculated across the entire fMRI scan session for each participant. FM patients had a higher meanFD during checker compared to HC (two-sample t-test: $p > 0.042$). meanFD was used as a nuisance regressor in group comparisons.

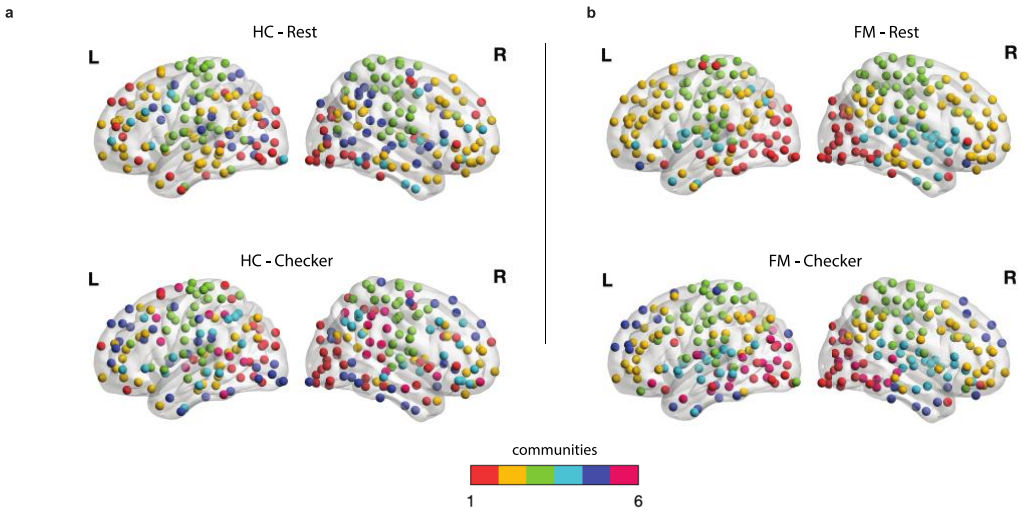


Figure B 2 Visualization of group consensus networks reveal differences in network organization during visual stimulus. Consensus networks were constructed from individual FM and HC networks at tau = 0.50 during rest and checker. FM and HC networks were both composed of five communities at rest and six communities at checker. Between group differences in node community assignment may be associated with network reorganization into communities during the visual stimulus. Legend: community 1 - red; community 2 - yellow; community 3 - green; community 4 - light blue; community 5 - blue; community 6 – pink

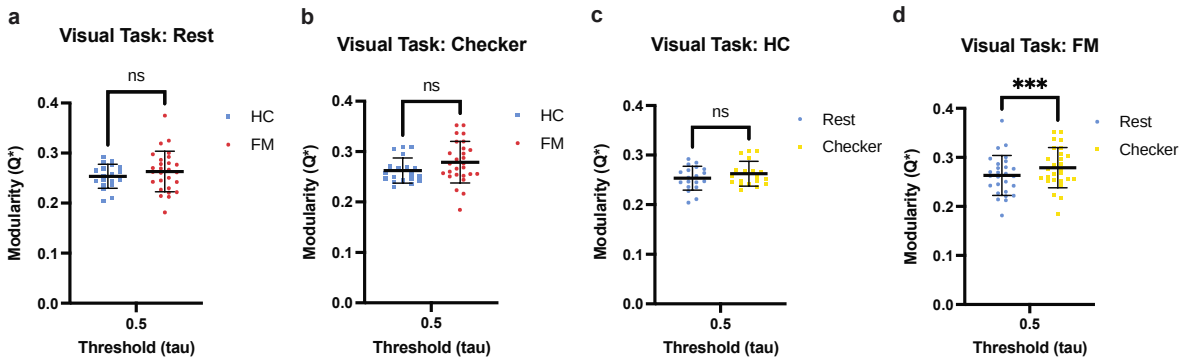


Figure B.3 Modularity during the visual stimulus. There were no differences in modularity of networks between FM and HC during (a) rest or (b) checker. During the visual stimulus (c,d), only FM networks show a significantly higher modularity during the CHECK stimulus (Paired-t-test: $p < 0.001$). This suggests stronger (or dense) connectivity within communities than between communities for FM networks during CHECK.

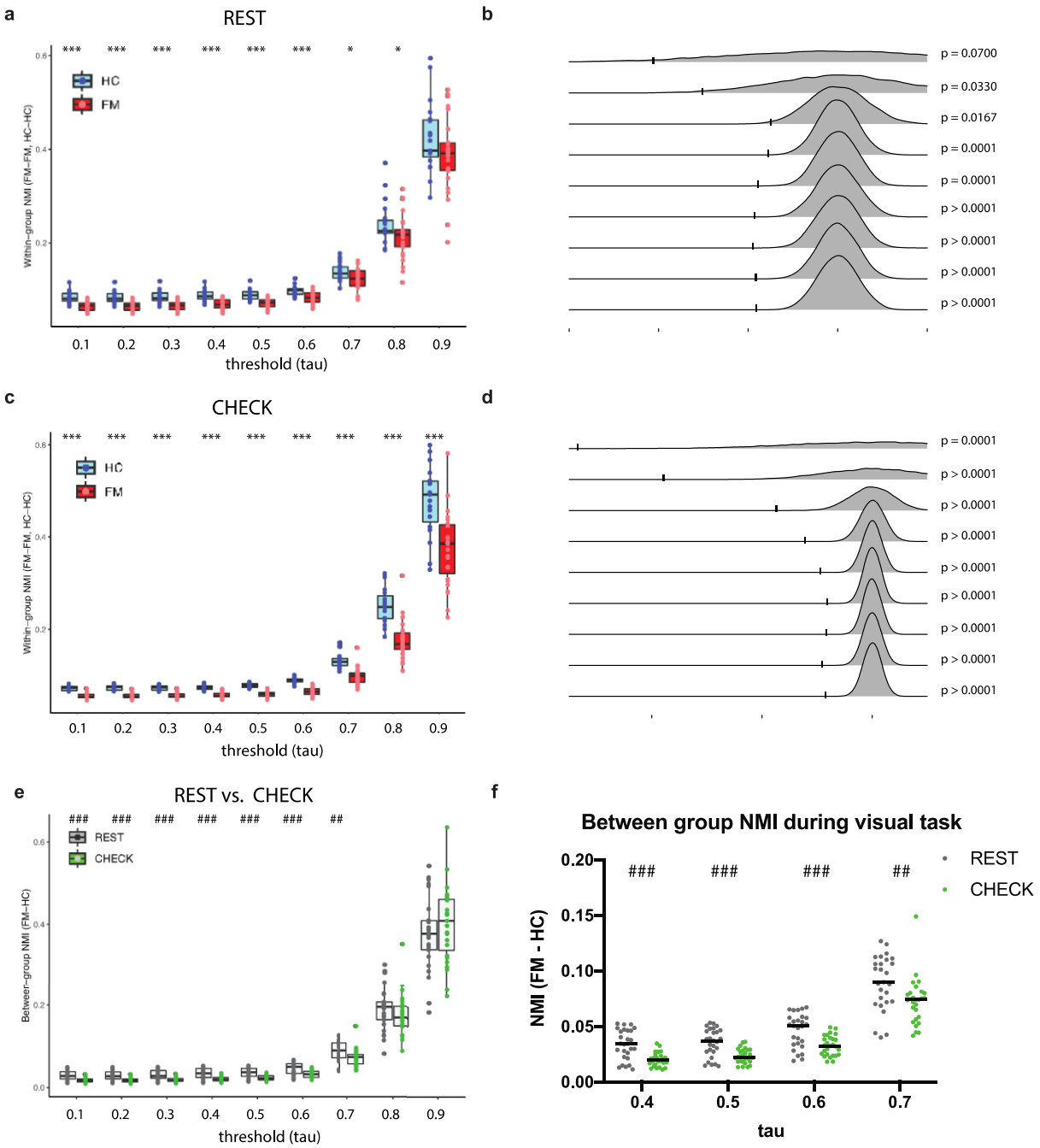


*Figure B.4 Robustness of community structure differences across tau (0.1 – 0.9). Within-group NMI (FM-FM, HC-HC) was lower during (a) REST and (b) CHECK across multiple tau thresholds. (c,d)Significance was determined by the number of random permutations more extreme than the observed difference. (e) Between group NMI (FM-HC) was significantly lower during CHECK compared to REST suggesting more altered network community structure during visual stimulation in FM as compared to HCs. (f) The between-group NMI during REST and CHECK across tau (0.4-0.7). (Statistical test based on Permutation test, * - p < 0.05; *** - p < 0.0001; paired t-test ## - p < 0.001### - p < 0.0001)*

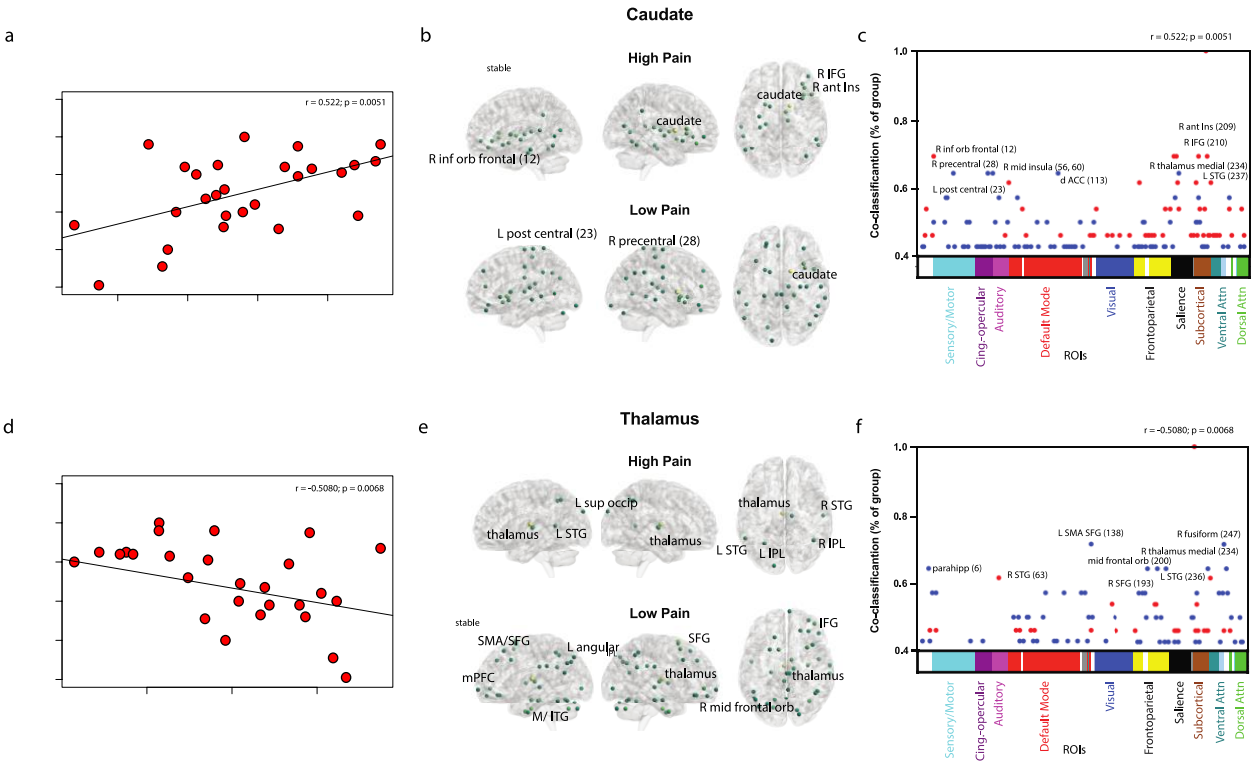


Figure B.5 Phi co-classification of caudate and thalamus nodes during visual stimulus in FM. Node variability of the caudate and thalamus during the visual stimulus were correlated with clinical pain in FM. The positive relationship between node phi and clinical pain, suggest the community assignment of this node was similar among patients with high pain. Node co-classification shows differences in community assignment may be associated with nodes in the precentral and postcentral gyrus. The negative association between node phi and clinical pain, suggests the community assignment of this node was more similar among networks of low pain patients compared to high pain. Of note, the community of thalamus in the low pain network was more integrative in nature.

Table B.1 Seed ROI for insula connectivity based on results from Harte et al 2016

	ROIs	MNI Coordinates
1	Right anterior insula	[45, 21, 5]
2	Right anterior insula	[42, 20, 4]
3	Left anterior insula	[-46, 14, 2]
4	Right anterior insula	[50, 8, 2]
5	Right anterior insula	[36, 30, 4]

Table B.2 Distribution of RSN nodes across communities of group consensus network during REST at tau threshold of 0.5

HC - REST

RSN	nodes in RSN	Community 1	Community 2	Community 3	Community 4	Community 5
Uncertain	28	10(36)	10(36)	2(7)	5(18)	1(4)
Sensory	35	1(3)	0(0)	32(91)	0(0)	2(6)
Cingulo	14	0(0)	0(0)	8(57)	0(0)	6(43)
Auditory	13	0(0)	0(0)	10(77)	0(0)	3(23)
Default Mode	58	8(14)	47(81)	0(0)	0(0)	3(5)
Memory	5	0(0)	3(60)	0(0)	0(0)	2(40)
Visual	31	17(55)	2(6)	1(3)	0(0)	11(35)
Frontoparietal	25	0(0)	13(52)	0(0)	7(28)	5(20)
Salience	18	2(11)	7(39)	2(11)	4(22)	3(17)
Subcortical	13	1(8)	8(62)	1(8)	2(15)	1(8)
Ventral Attn	9	1(11)	2(22)	2(22)	0(0)	4(44)
Cerebellar	4	0(0)	0(0)	2(50)	0(0)	2(50)
Dorsal Attn	11	4(36)	0(0)	3(27)	0(0)	4(36)
	264	44 (17)	92(35)	63(24)	18(7)	47(18)

FM - REST

RSN	nodes in RSN	Community 1	Community 2	Community 3	Community 4	Community 5
Uncertain	28	11(39)	11(39)	1(4)	3(11)	2(7)
Sensory	35	2(6)	2(6)	30(86)	1(3)	0(0)
Cingulo	14	0(0)	1(7)	8(57)	5(36)	0(0)
Auditory	13	0(0)	0(0)	12(92)	1(8)	0(0)
Default Mode	58	4(7)	48(83)	1(2)	5(9)	0(0)
Memory	5	0(0)	4(80)	1(20)	0(0)	0(0)
Visual	31	31(100)	0(0)	0(0)	0(0)	0(0)
Frontoparietal	25	1(4)	18(72)	4(16)	2(8)	0(0)
Salience	18	0(0)	9(50)	4(22)	5(28)	0(0)
Subcortical	13	0(0)	1(8)	0(0)	12(92)	0(0)
Ventral Attn	9	4(44)	2(22)	1(11)	2(22)	0(0)
Cerebellar	4	3(75)	0(0)	0(0)	1(25)	0(0)
Dorsal Attn	11	2(18)	0(0)	7(64)	2(18)	0(0)
	264	58(22)	96(36)	69(26)	39(15)	2(1)

Table B.3 Distribution of RSN nodes across communities of group consensus network during CHECK at tau threshold of 0.5

HC - CHECK

RSN	nodes in RSN	Community 1	Community 2	Community 3	Community 4	Community 5	Community 6
Uncertain	28	8(29)	5(18)	0(0)	3(11)	11(39)	1(4)
Sensory	35	2(6)	0(0)	28(80)	0(0)	3(9)	2(6)
Cingulo	14	2(14)	0(0)	5(36)	2(14)	2(14)	3(21)
Auditory	13	0(0)	1(8)	9(69)	0(0)	2(15)	1(8)
Default Mode	58	4(7)	32(55)	1(2)	10(17)	10(17)	1(2)
Memory	5	1(20)	1(20)	0(0)	2(40)	0(0)	1(20)
Visual	31	23(74)	0(0)	0(0)	0(0)	8(26)	0(0)
Frontoparietal	25	1(4)	3(12)	3(12)	12(48)	3(12)	3(12)
Salience	18	2(11)	4(22)	2(11)	1(6)	5(28)	4(22)
Subcortical	13	2(15)	3(23)	2(15)	4(31)	1(8)	1(8)
Ventral Attn	9	2(22)	1(11)	0(0)	1(11)	0(0)	5(56)
Cerebellar	4	1(25)	0(0)	2(50)	0(0)	0(0)	1(25)
Dorsal Attn	11	4(36)	0(0)	2(18)	0(0)	2(18)	3(27)
	264	52 (20)	50(19)	54(20)	35(13)	47(18)	26(10)

FM - CHECK

RSN	nodes in RSN	Community 1	Community 2	Community 3	Community 4	Community 5	Community 6
Uncertain	28	5(18)	3(11)	4(14)	12(43)	2(7)	2(7)
Sensory	35	0(0)	1(3)	31(89)	1(3)	0(0)	2(6)
Cingulo	14	0(0)	1(7)	9(64)	0(0)	0(0)	4(29)
Auditory	13	0(0)	0(0)	7(54)	0(0)	1(8)	5(38)
Default Mode	58	2(3)	31(53)	1(2)	11(19)	7(12)	6(10)
Memory	5	0(0)	3(60)	1(20)	0(0)	0(0)	1(20)
Visual	31	15(48)	0(0)	0(0)	1(3)	15(48)	0(0)
Frontoparietal	25	4(16)	18(72)	2(8)	1(4)	0(0)	0(0)
Salience	18	0(0)	4(22)	6(33)	3(17)	0(0)	5(28)
Subcortical	13	0(0)	1(8)	2(15)	1(8)	1(8)	8(62)
Ventral Attn	9	1(11)	2(22)	0(0)	0(0)	3(33)	3(33)
Cerebellar	4	0(0)	0(0)	1(25)	2(50)	1(25)	0(0)
Dorsal Attn	11	4(36)	1(9)	6(55)	0(0)	0(0)	0(0)
	264	31(12)	65(25)	70(27)	32(12)	30(11)	36(14)

Table B.4 Left anterior insula– node co-classification across high pain networks during CHECK

Node (208) phi: High pain - CHECK		Location			Node co-classification
ROI #	Name	X	Y	Z	(percent)
Node12	R Inf Orb frontal	34	38	-12	0.62
Node19	R postcentral	13	-33	75	0.54
Node55	L precentral operculum	-45	0	9	0.54
Node57	L claustrum mid insula	-34	3	4	0.62
Node58	L sup temporal gyrus	-51	8	-2	0.54
Node63	R STG	58	-16	7	0.77
Node64	L posterior insula operc	-38	-33	17	0.54
Node67	R post insula	43	-23	20	0.54
Node72	R pre postcentral	59	-17	29	0.54
Node73	L post insula	-30	-27	12	0.69
Node116	R ITG	65	-12	-19	0.62
Node119	R mid temp	65	-31	-9	0.54
Node123	R mid temp	52	-2	-16	0.54
Node131	L mid temporal	-49	-42	1	0.62
Node152	L cuneus calcarine	-18	-68	5	0.69
Node170	cuneus calcarine	6	-81	6	0.54
Node208	L ant Ins	-35	20	0	1.00
Node224	L thalamus medial dorsal	-10	-18	7	0.62
Node229	R putamen	31	-14	2	0.54
Node231	R putamen	29	1	4	0.69
Node236	L STG	-56	-50	10	0.69
Node240	R STG	56	-46	11	0.62
Node250	L inf temp	-50	-7	-39	0.62

Table B.5 Left anterior insula – node co-classification across low pain networks during CHECK

Node (208) phi: Low pain - CHECK		Location			Node co-classification
ROI #	Name	X	Y	Z	(percent)
Node21	R precentral	29	-17	71	0.50
Node22	R postcentral precuneus	10	-46	73	0.50
Node41	R precentral	38	-17	45	0.50
Node56	R mid insula	49	8	-1	0.57
Node72	R pre postcentral	59	-17	29	0.50
Node84	L mid temporal	-58	-26	-15	0.50
Node87	L angular	-39	-75	44	0.50
Node95	R precuneus PCC	11	-54	17	0.57
Node97	R middle frontal sup	23	33	48	0.50
Node114	L SFG	-20	64	19	0.50
Node121	R SFG	13	30	59	0.50
Node122	R dorsal ACC	12	36	20	0.57
Node142	L lingual occip	-12	-95	-13	0.50
Node162	R sup occip	24	-87	24	0.50
Node166	L precuneus	-16	-77	34	0.50
Node174	L precentral	-44	2	46	0.50
Node181	R mid frontal orb	34	54	-13	0.64
Node182	L frontal mid orb	-21	41	-20	0.50
Node187	L IFG precentral	-41	6	33	0.50
Node202	L medial sup frontal	-3	26	44	0.50
Node206	R mid frontal	31	33	26	0.50
Node208	L ant Ins	-35	20	0	1.00
Node209	R ant Ins	36	22	3	0.50
Node210	R IFG	37	32	-2	0.50
Node211	R ant Ins	34	16	-8	0.50
Node218	R sup middle frontal	31	56	14	0.50
Node233	R caudate	15	5	7	0.57
Node234	R thalamus medial	9	-4	6	0.50
Node250	L inf temp	-50	-7	-39	0.50
Node255	R postcentral	47	-30	49	0.50
Node258	R sup parietal	25	-58	60	0.50
Node262	L occip inf temp	-42	-60	-9	0.50
Node263	L sup parietal	-17	-59	64	0.50