MANUSCRIPT

Neural Correlates of Chronic Pain: Functional Connectivity and Neuroinflammation

Cheyenne McBane
Independent Researcher

Chronic pain is a long-term neurological disorder that affects millions of people each year and can be described as the experience of pain persisting longer than the expected timeline for healing of the tissue itself. Although extensive research has looked at chronic pain, the mechanisms that drive the pain through the central nervous system are still poorly understood. However, there is emerging research that suggests that chronic pain is related to disrupted functional connectivity of the central nervous system in pain processing network areas such as the prefrontal cortex (PFC), anterior cingulate cortex (ACC), insula, thalamus and somatosensory cortices in addition to neuroinflammatory signaling through microglia and astrocytes. The hypothesis is that chronic pain results from a maladaptive interaction between PFC inhibitory control and a persistent neuroinflammatory process that leads to increased nociceptive processing and central sensitization. I will test the hypothesis using a multimodal approach that combines resting state and task-based fMRI, MRS and TSPO-PET imaging to achieve a comprehensive understanding of functional connectivity, the excitatory/inhibitory neurotransmitter balance and the burden of neuroinflammation, as determined by neuroinflammatory markers. In tandem with the above-described studies, preclinical studies in animal models will be conducted to examine causal relationships between modulation of the prefrontal network, neuroinflammation and pain behavior. Ultimately, this integrative methodology will help identify convergent neural and inflammatory biomarkers to provide insight into potential network therapeutic strategies.

Chronic pain
Functional connectivity
Neuroinflammation
Central sensitization
Prefrontal cortex (PFC) dysfunction

INTRODUCTION

Chronic pain is often described as pain that lasts more than three months. It can also refer to pain that lasts longer than expected based on how long it typically takes for that type of tissue damage to heal. Acute pain serves a protective purpose, but chronic pain typically occurs and persists regardless of an injury, and is now being described as the result of problems in both the peripheral and central nervous system. As a result, chronic pain has been found to be associated with changes in neural networks involved in sensory processing, emotion regulation, and cognition regarding pain. Neuroimaging studies have shown that pain processing and modulation involve a variety of areas in the brain, including: anterior cingulate cortex, insula, thalamus, prefrontal cortex, and somatosensory cortices (the region responsible for perceiving the sensory aspects of pain). These areas work together to create both the sensory-discriminative and affective aspects of pain perception.

Persistent and potentially altered functional connectivity within these areas has been demonstrated in multiple chronic pain populations, including people with fibromyalgia, individuals with neuropathic pain, and patients who experience chronic back pain. There is abundant evidence to suggest that neuroinflammation plays an essential role in the pathophysiology of chronic pain via the activation of microglia and astrocytes in the central nervous system (CNS). When activated, both microglia and astrocytes release pro-inflammatory cytokines (e.g. interleukin-1β [IL-1β], tumor necrosis factor-alpha [TNF-α], and interleukin-6 [IL-6]) in the CNS. Signaling molecules have the potential to increase neuronal excitement, change how synapses work, and help cause the onset of central sensitization. Therefore, chronic pain might be explained via understanding how this form of pain arises from faulty neural networks combined with continual neuroinflammation.

Although substantial evidence links both altered brain connectivity and neuroinflammation to chronic pain, the relationship between these mechanisms remains poorly understood. Most studies have examined network dysfunction or inflammatory processes independently, making it difficult to determine how these mechanisms interact to sustain persistent pain states. The majority have investigated functional abnormalities or inflammatory processes alone making it difficult to establish whether the two of these mechanisms interact to create an ongoing state of chronic pain through one of more than one way. It is also possible that the neuro-inflammatory signals affecting other parts of the brain responsible for how we deal with and cope with pain, may be contributing to ineffective communication among those regions, thereby perpetuating the ongoing regulation of neural circuits that lead to further development of inflammatory activity due to maladaptive neural plasticity in those regions as well as. Understanding how various processes interact with one another will provide critical insight into the biological process associated with maintaining chronic pain and developing better treatment options that target the mechanism causing chronic pain, rather than just treating the symptoms associated with it.

THE HYPOTHESIS

The central hypothesis of this study is that chronic pain results from a maladaptive interaction between altered pain-processing networks and persistent neuroinflammatory signaling. Specifically, disruptions in functional connectivity between the prefrontal cortex and key pain-processing regions are believed to impair the brain's ability to modulate nociceptive input, thereby contributing to the maintenance of chronic pain. More directly, it is believed that there are disruptions in connectivity between the prefrontal cortex and the major areas of pain processing (i.e., anterior cingulate, insula, thalamus, and somatosensory cortices) that are caused by the ongoing or more chronic effects of the changes to these systems in a person, thereby reducing their ability to modulate nociceptive input at a higher level.

Under normal circumstances, the prefrontal cortex plays an integral role in modulating the perception of pain through descending inhibition by controlling the amount of nociceptive information reaching the brain. However, in patients suffering from chronic pain, the changes in connectivity between the prefrontal cortex and the pain processing regions may have eroded this descending inhibition, thus resulting in magnified sensory and emotional responses to pain. This could cause an increase in the level of activation of the anterior cingulate cortex and insula, thereby increasing the perception of pain intensity and the associated emotional weight.

Both microglial and astroglial cell activation promotes a neuroinflammatory environment with increased levels of interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). These inflammatory mediators enhance synaptic excitability, resulting in central sensitization and destabilization of the functional neural networks with regard to the regulation of pain. This impaired inhibitory control interacting with neuroinflammation could create a self-perpetuating cycle of neuroinflammation that contributes to persistent chronic pain, despite resolution of the underlying injury.

This research study hypothesizes that patients who suffer from chronic pain will exhibit not only a reduction in the functional connectivity between their prefrontal cortex (PFC) and those brain regions that process pain; but also an increase in the presence of neuroinflammatory markers when compared to normal controls. Additionally, the amount of disruption in connectivity should directly correlate with the level of pain severity, the amount of time the person has experienced chronic pain, and the amount of neuroinflammatory burden that the person experiences. If inflammation at the cellular level contributes to the disruption of a neural network's functional operation, then chronic pain patients that exhibit the greatest amount of glial cell activation and pro-inflammatory cytokine signaling will also exhibit the most severe levels of impairment in descending pain modulation. Thus, chronic pain is likely to be the result of not one sole pathological process, but rather an interaction between a dysfunctional neural network and ongoing inflammatory signaling, both of which lead to and contribute to maintenance of the chronic pain condition.

EVOLUTION OF HYPOTHESIS

Based on accumulating data demonstrating that chronic pain is correlated with alterations in the organization of pain networks in the brain along with the presence of ongoing neuroinflammatory processes, the hypothesis has changed in scope to encompass recent neuroimaging and molecular neuroscience findings. Much of the early work on chronic pain was focused primarily on peripheral tissue injury and desensitization due to nociceptor sensitization. As imaging technology and understanding of neuroanatomy continues to evolve, it has become clear that central nervous system (CNS) mechanisms contribute to the maintenance of pain.

To explore the mechanisms of chronic pain, we will recruit individuals diagnosed with chronic pain disorders that include fibromyalgia and chronic low back pain (n = 50) as well as age- and gender-matched healthy controls (n = 30). Individuals who currently have serious psychiatric diagnoses, neurodegenerative diseases, or individuals receiving opioid medication within six months prior will be excluded from the study to limit confounder effects. A multimodal imaging protocol will be used to evaluate functional connectivity, neurochemical alterations, and neuroinflammatory burden.

A resting state fMRI scan will evaluate connectivity between the prefrontal cortex, anterior cingulate cortex, insula, thalamus, and somatosensory cortices; mean blood oxygen level dependent (BOLD) across these same brain regions during the presentation of standardized noxious stimulation will be assessed using task-based fMRI; changes in the concentrations of glutamate, GABA, and myo-inositol will be examined using magnetic resonance spectroscopy to assess balance between excitatory and inhibitory neurotransmitters; and the 18kDa translocator protein (TSPO) will be measured with positron emission tomography (PET) to provide an indirect measure of activation of glial cells and the presence of neuroinflammation. Graph-theoretical methods will be used to examine functional connectivity. Relationships between imaging data, neurochemical markers, and self-reported measures of pain (such as VAS) and pain assessments (such as McGill's) will be assessed via multivariate analyses. Age, gender, and medication use will be used as covariates.

Two established rodent models (the spared nerve injury model for neuropathic pain and the Freund's adjuvant model for inflammation) will be used to augment human studies. fMRI imaging will be employed to measure connectivity within homologous circuits involved in pain processing across species. The inflammatory changes associated with pain will be measurable through the use of immunohistochemistry of microglial (Iba1) and astrocytic (GFAP) markers and cytokine (e.g.: IL-1β, TNF-α and IL-6) assays. Results from these experiments will elucidate the mechanisms through which dysfunction in neural networks relates to neuroinflammation.

HYPOTHESIS TESTING

The chronic pain which is the target of the proposed hypothesis will display both neuroinflammatory signaling and altered connectivity throughout multiple pain-regulatory networks. More specifically, chronic pain patients should have decreased functional connectivity from the prefrontal cortex to structures associated with nociceptive processing and should also have higher levels of glial activation and production of inflammatory cytokines. To investigate these mechanisms, multimodal imaging techniques have been employed. Functional MRI allows researchers to examine large-scale networks associated with regulating and perceiving painful stimuli, while magnetic resonance spectroscopy (MRS) measures alterations within these networks in terms of excitatory and inhibitory neurotransmitter systems. TSPO-PET imaging gives a molecular marker of neuroinflammatory activity; all of these methods will help researchers find convergent biomarkers that can capture the complexity and heterogeneity of chronic pain.

To evaluate if prefrontal dysfunction/activation can cause chronic pain, animal models will be used wherein optogenetic stimulation of the prefrontal cortex will be performed. The behaviour (mechanical sensitivity to von Frey hair and thermal nociception) of these animals will be evaluated pre and post-stimulation to determine whether enhancement of prefrontal cortex activity decreases the frequency of pain-related behaviours and reduces neuroinflammatory signalling; this would support a direct link between disrupted prefrontal regulation and the maintenance of chronic pain.

In addition to examining each measure independently, relationships among functional connectivity, neurochemical balance, neuroinflammatory burden, and behavioral pain outcomes will be analyzed to determine whether these variables converge on a common mechanism underlying chronic pain. If the proposed hypothesis is correct, individuals exhibiting the greatest reductions in prefrontal connectivity should also demonstrate elevated neuroinflammatory markers and report higher levels of pain severity. Similarly, animal models showing the strongest evidence of glial activation and central sensitization should display the greatest pain-related behavioral changes. Identifying these associations would strengthen the argument that chronic pain arises from the combined effects of network dysfunction and neuroinflammation rather than from either process alone.

IMPLICATIONS

The results may considerably improve the current understanding of chronic pain as a disorder based on both network dysfunction and neuroimmune mechanisms. By identifying specific altered patterns of connectivity and neuro-inflammatory activity, these studies will contribute to developing objective biomarkers for the diagnosis and progression of chronic pain — especially considering the subjective nature of pain measurement in clinical settings.

Moreover, establishing a mechanism linking prefrontal dysfunction with underlying neuro-inflammation will help identify potential therapeutic targets. Future interventions aimed at restoring network functions in the prefrontal cortex, decreasing activation of glial cells, or altering inflammation-modulating pathways could offer alternatives to the traditional pharmacological treatments that focus primarily on pain reduction. Finally, incorporating both molecular and neuroimaging biomarkers will enable the development of personalized treatment plans based on individual differences in neurobiology.

In addition to identifying novel treatment targets, these results could help physicians better forecast a patient's response to treatment as well as their progression of chronic pain. Most chronic pain treatments today involve an extended period of trial and error before determining which treatment will work best, leading to varying results and lengthening the time patients report having debilitating chronic pain. If certain functional connectivity or neuroinflammatory patterns are linked with specific pain phenotypes, then physicians may use these markers for selecting and managing different types of therapies for patients or determining the likelihood that a patient will face long-term chronic pain after sustaining an injury or developing a new medical condition.

This proposed research could have ramifications for issues outside of chronic pain as well. Changes in functional connectivity and neuroinflammatory signaling have been linked to many neurological and psychiatric disorders, such as depression & anxiety (major depressive disorder, generalized anxiety disorder) in addition to Alzheimer's disease and multiple sclerosis. Accordingly, a better understanding of how neural network dysfunctions interface with immune signaling in chronic pain may yield important insights regarding broader neurobiological mechanisms of disease progression across a range of central nervous system disorders. Such an interdisciplinary approach may facilitate the development of therapies focusing on the shared pathological processes rather than solely the disease-specific symptoms.

CONCLUSION

Increasing recognition of chronic pain as a complex neurological disorder with disrupted neural network organization, and associated with persistent underlying neuroinflammation, has led to the development of a working hypothesis whereby decreased inhibitory output from the prefrontal cortex, combined with continuing inflammation as mediated by glial cells, results in a self-perpetuating cycle of nociceptive amplification and central sensitization.

The following studies will utilize functional neuroimaging, neurochemistry, molecular neuroimaging, and experimental animal models in an effort to define the relationship between pain-related neural connectivity and pain-related neuroinflammation. Through the identification of convergent biomarkers and the characterization of putative causative mechanisms underlying each type of chronic pain disorder, this research will enhance both the scientific and clinical knowledge base with respect to chronic pain. Ultimately, these findings may facilitate the

development of targeted and efficacious interventions directed towards the biological underpinnings of chronic pain disorders, rather than simply providing symptomatic treatment for chronic pain disorders.

The integrated multi-level analytic framework contains many levels of analysis, from large-scale neural networks to cellular and molecular mechanisms. It is not likely that chronic pain results from one single pathogenic mechanism, so methods that only study neural activity and or just inflammatory signaling will not be able to account for the complexity of chronic pain. By combining fMRI, neurochemistry, and precision pain stimulus manipulation of neural circuits involved in pain, this research will be able to develop a deeper understanding of how chronic pain develops, is maintained and can be potentially reversed.

The studies to connect the study of neuroscience with working with patients. If there are strong indicators, or biomarkers, related to network problems or neuroinflammation, diagnostic accuracy can be improved and therapies based on mechanisms tailored to the needs of individual patients can be developed and delivered earlier in the treatment cycle. Chronic pain will continue to be a growing issue globally, and understanding what is going on at the neurobiological level will be crucial to mitigating its impact on an individual, society, and economically. As a result of this work over time, investigators will increasingly consider chronic pain not just as a symptom, but rather as a separate neurological condition that requires mechanism-based intervention and management.

REFERENCES

1. Baliki MN, Mansour AR, Baria AT, Apkarian AV (2014) Functional Reorganization of the Default Mode Network across Chronic Pain Conditions. PLOS ONE 9(9): e106133. https://doi.org/10.1371/journal.pone.0106133
2. Baliki, M., Petre, B., Torbey, S. et al. Corticostriatal functional connectivity predicts transition to chronic back pain. Nat Neurosci 15, 1117–1119 (2012). https://doi.org/10.1038/nn.3153
3. Bushnell MC, Ceko M, Low LA. Cognitive and emotional control of pain and its disruption in chronic pain. Nat Rev Neurosci. 2013 Jul;14(7):502-11. doi: 10.1038/nrn3516. Epub 2013 May 30. PMID: 23719569; PMCID: PMC4465351.
4. Farmer MA, Baliki MN, Apkarian AV. A dynamic network perspective of chronic pain. Neurosci Lett. 2012 Jun 29;520(2):197-203. doi: 10.1016/j.neulet.2012.05.001. Epub 2012 May 8. PMID: 22579823; PMCID: PMC3377811.
5. Fiore, N.T., Debs, S.R., Hayes, J.P. et al. Pain-resolving immune mechanisms in neuropathic pain. Nat Rev Neurol 19, 199–220 (2023). https://doi.org/10.1038/s41582-023-00777-3
6. Grace, P., Hutchinson, M., Maier, S. et al. Pathological pain and the neuroimmune interface. Nat Rev Immunol 14, 217–231 (2014). https://doi.org/10.1038/nri3621.

7. Hashmi JA, Baria AT, Baliki MN, Huang L, Schnitzer TJ, Apkarian VA. Brain networks predicting placebo analgesia in a clinical trial for chronic back pain. Pain. 2012 Dec;153(12):2393-2402. doi: 10.1016/j.pain.2012.08.008. Epub 2012 Sep 15. PMID: 22985900; PMCID: PMC3494789.
8. Inoue, K., Tsuda, M. Microglia in neuropathic pain: cellular and molecular mechanisms and therapeutic potential. Nat Rev Neurosci 19, 138–152 (2018). https://doi.org/10.1038/nrn.2018.2
9. Ji, RR., Xu, ZZ. & Gao, YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov 13, 533–548 (2014). https://doi.org/10.1038/nrd4334
10. Kuner R, Flor H. Structural plasticity and reorganisation in chronic pain. Nat Rev Neurosci. 2016 Dec 15;18(1):20-30. doi: 10.1038/nrn.2016.162. Erratum in: Nat Rev Neurosci. 2017 Feb;18(2):158. doi: 10.1038/nrn.2016.158. Erratum in: Nat Rev Neurosci. 2017 Jan 20;18(2):113. doi: 10.1038/nrn.2017.5. PMID: 27974843.
11. Li, T., Chen, X., Zhang, C. et al. An update on reactive astrocytes in chronic pain. J Neuroinflammation 16, 140 (2019). https://doi.org/10.1186/s12974-019-1524-2
12. Malcangio, M., Sideris-Lampretsas, G. How microglia contribute to the induction and maintenance of neuropathic pain. Nat. Rev. Neurosci. 26, 263–275 (2025). https://doi.org/10.1038/s41583-025-00914-5
13. Milligan, E., Watkins, L. Pathological and protective roles of glia in chronic pain. Nat Rev Neurosci 10, 23–36 (2009). https://doi.org/10.1038/nrn2533
14. Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. Neuron. 2007 Aug 2;55(3):377-91. doi: 10.1016/j.neuron.2007.07.012. PMID: 17678852.