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Eleonora Kay Sheppard March 17, 2025

Examining Nociceptive Processing in Warmth-Insensitive Fields

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An abstract of
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Abstract

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Chronic pain is a significant public health issue, affecting approximately 24% of the U.S. population. However, due to its subjective and complex nature, the diagnosis and treatment of chronic pain presents substantial challenges. In their work, researchers Green and Cruz identified warmth-insensitive fields (WIFs) which are regions in the skin that lack warm fibers and thus primarily rely on nociceptors for sensory perception. These WIFs offer a unique opportunity to examine pain perception in isolation from warmth receptors, as both are often activated together. Thus, this study utilized quantitative sensory testing to examine the sensory processing of WIFs in comparison to adjacent, normally-sensitive fields, specifically focusing on heat and heat pain detection thresholds. Results indicated a significant difference between the heat and heat pain detection thresholds of WIFs and adjacent fields. Furthermore, while variability in sensory response was observed within individual WIFs, no statistically significant correlations were found with demographics or external variables. These findings provide clear evidence of warmth detection through nociceptors, highlighting the complex and integrated relationship between heat and pain perception. Further research into the sensory processing of WIFs may offer valuable insights for the development of innovative and targeted chronic pain management.

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Abstract

Chronic pain is a significant public health issue, affecting approximately 24% of the U.S. population (Lucas & Sohi, 2024). However, due to its subjective and complex nature, the diagnosis and treatment of chronic pain presents substantial challenges. In their work, researchers Green and Cruz (1998) identified warmth-insensitive fields (WIFs) which are regions in the skin that lack warm fibers and thus primarily rely on nociceptors for sensory perception. These WIFs offer a unique opportunity to examine pain perception in isolation from warmth receptors, as both are often activated together. Thus, this study utilized quantitative sensory testing to examine the sensory processing of WIFs in comparison to adjacent, normally-sensitive fields, specifically focusing on heat and heat pain thresholds. Results indicated a significant difference between the heat and heat pain detection of WIFs and adjacent fields. Furthermore, while variability in sensory response was observed within individual WIFs, no statistically significant correlations were found with demographics or confounding variables. These findings provide clear evidence of warmth detection through nociceptors, highlighting the complex and integrated relationship between heat and pain perception. Future iterations of this project will focus on investigating WIFs in chronic pain patients to evaluate how their sensory processing differs from that of healthy individuals. By evaluating nociceptive processing in these regions, this research aims to further understand the mechanisms underlying pain perception and contribute to the development of innovative and targeted chronic pain management.

Introduction

From an early age, painful experiences, such as touching a hot stove, teach us to avoid harm, demonstrating pain's crucial role in protecting the body. Pain is a fundamental biological

function that signals potential or actual tissue damage, an essential tool that influences survival and behavior. However, when pain persists beyond its protective or informative role, it transforms into a debilitating condition. Chronic pain, defined as pain that lasts more than three months, is a serious disorder that affects the daily life of an estimated 24.3% (or approximately 63 million people) of U.S. adults (Lucas & Sohi, 2024). Chronic pain conditions are also commonly linked to serious comorbidities including depression, Alzheimer's and other dementias, and substance misuse while costing billions of dollars annually in terms of direct healthcare costs and lost productivity (Rikard et.al, 2023; Gaskin & Richard, 2012). Due to the subjectivity of the symptoms and the complex neurobiological causes, chronic pain is incredibly difficult to diagnose and treat, leaving many individuals suffering and contributing to the overprescription of opioids. This is a prevalent public health issue that needs to be addressed, starting with a better understanding of somatosensation and the underlying mechanisms of pain processing. This study aims to achieve this by evaluating warmth-insensitive fields (WIFs), regions of the skin lacking low-threshold thermal receptors, to gain a clearer understanding of how nociceptors contribute to heat and pain detection without the influence of warm fibers.

Somatosensation and Thermal Perception

Somatosensation is the body's ability to sense touch, temperature, pain, and proprioception, and it is distinct from the special senses such as vision or hearing since its receptors are distributed throughout the body rather than localized in specific organs. Each somatosensory modality has dedicated receptors in the skin that process information via peripheral sensory neurons and transmit these signals to the central nervous system, primarily to the somatosensory cortex in the postcentral gyrus. There are several kinds of thermal receptors

present in the skin, but the most crucial type are transient receptor potential (TRP) channels, a group of ion channels innervated at the periphery of free nerve endings that respond to a wide range of temperature (Caterina & Pang, 2016).

TRP channels are broadly categorized based on their temperature sensitivity. Warm-sensitive TRPs include TRPV1, TRPV2, TRPV3, and TRPV4, which are activated by temperatures above physiological body temperature. TRPV1, the most well-characterized, is activated by noxious heat (>43˚C), making it a key player in inflammatory pain (Kashio & Tominaga, 2022). Furthermore, the temperature sensitivity of TRPV1 can become elevated under inflammatory conditions meaning that it fires at lower temperatures that would typically be considered innocuous warmth (Wang & Siemens, 2015). This highlights its potential role in peripheral nociceptive sensitization, a key component of our research question that is exploring the possible responsibility of this kind of sensitization in the development of chronic pain. TRPV3 and TRPV4, on the other hand, detect innocuous warmth (>27˚C) and are expressed in both sensory neurons and skin keratinocytes, where they contribute to the perception of mild warmth (Wang & Siemens, 2015).

In contrast, cold-sensitive TRPs include TRPM8 and TRPA1. TRPM8 is activated by temperatures below 27˚C and menthol, mediating cool sensations and providing an analgesic effect in pain conditions (Kashio & Tominaga, 2022). TRPA1 has been controversially linked to noxious cold, with some studies suggesting it detects temperatures below 15˚C, though its primary role may be in detecting irritant chemicals rather than cold alone (Kashio & Tominaga, 2022). Clearly, there are many different classes of thermoreceptors that are responsive to various ranges of temperature, ensuring an effective response to environmental temperature changes.

Neurobiology of Pain

Transitioning specifically to nociceptors, this introduction will explore the neurobiological mechanisms underlying pain perception, focusing on peripheral transduction, central processing, and overall modulation. Pain transduction begins when specialized sensory neurons, nociceptors, convert noxious stimuli into electrical signals that can be processed by the nervous system. This process is mediated by ion channels that, upon activation, generate action potentials that propagate along nociceptive fibers towards the spinal cord (Mertens et al., 2015). Nociceptors are specialized pain receptors responsible for detecting a variety of painful stimuli including thermal, mechanical, and chemical inputs. The two major types of nociceptive fibers are Aδ-fibers, myelinated fibers responsible for the fast conduction of sharp pain, and C-fibers, unmyelinated nerves responsible for slower, dull feelings of pain (Pace et al., 2006). The terminals of these sensory neurons are widely distributed throughout the skin while their cell bodies typically reside in dorsal root ganglia or trigeminal ganglia, clusters of sensory nerves located along the spinal cord. Both classes of nociceptors transmit signals to laminae I and II of the dorsal horn of the spinal cord, but some Aδ-fibers also project to lamina V depending on the specific pathway involved (Middleton et al., 2021).

Beyond thermal nociception, described above, there is also the conversion of mechanical stimuli into nociceptive signals by mechanosensitive ion channels. Two key channels that have been identified are Piezo2 and Piezo1. Piezo2 is predominantly expressed in sensory neurons of the dorsal root ganglia and is essential for detecting light touch, proprioception, and mechanical pain, including tactile allodynia which is a condition where non-painful stimuli cause pain (Fernández-Trillo et al., 2024). This ion channel is particularly involved in low-threshold mechanosensation and contributes to pain hypersensitivity following nerve injury (Xu et al.,

2024). In contrast, Piezo1 is more commonly found in non-neuronal tissues, such as blood vessels and epithelial cells as it is known for its regulation of vascular tone and blood flow (Fang et al., 2021). While Piezo1 has a more limited role in nociception, studies suggest it may contribute to inflammatory pain signaling and the induction of cell apoptosis (Savadipour et al., 2023). The structural and kinetic properties of Piezo channels allow them to rapidly open and close in response to mechanical force, making them essential for sensing pressure in various physiological contexts (Fang et al., 2021). Additionally, acid-sensing ion channels (ASICs) respond to mechanical stimuli in acidified environments, which is particularly relevant in inflammatory conditions where extracellular pH decreases (Mertens et al., 2015).

Following injury or inflammation, these peripheral nociceptors become sensitized, meaning that they require a lower threshold to be activated. This increased excitability occurs when inflammatory mediators and neurotrophic factors enhance the responsiveness of nociceptors, contributing to hyperalgesia and chronic pain. Cytokines, such as TNF-α, IL-1, and IL-6, play a key role in this process by promoting inflammation and immune cell recruitment, which increases pain signaling (Pace et al., 2006). Furthermore, prostaglandins, chemical messengers also involved in the immune response, sensitize nociceptors by increasing the activity of voltage-gated sodium channels, amplifying the response to painful stimuli (Mertens et al., 2015). Nerve Growth Factor is particularly important in this sensitization, as studies have linked it to hyperalgesia through mechanisms like mast cell degranulation and increased activity in capsaicin-sensitive neurons. NGF binds to its high-affinity receptor, TrkA, which is found on approximately 50% of nociceptors and leads to the activation of TRPV1 receptors, resulting in heat hyperalgesia (Pace et al., 2006). Furthermore, NGF alters nociceptor gene expression by increasing levels of pain-related molecules such as TRPV1, BDNF, and substance P, which may

contribute to long-term sensitivity as the NGF-TrkA complex is transported back to the nucleus (Pace et al., 2006). Finally, neurotrophic factors not only support neuronal growth but may also act as neuromodulators during inflammation, making spinal cord neurons more excitable. In cases of neuropathic pain, pain as a result of nerve damage, damaged nerves recruit macrophages that release growth factors and chemokines, leading to altered activity in surrounding sensory neurons. As a result, even intact nerves can transmit abnormal pain signals, and some damaged nociceptors begin firing spontaneously, further driving chronic pain states (Pace et al., 2006).

Moving towards the central processing of pain, once nociceptive signals are transduced in the periphery, they travel to the dorsal horn of the spinal cord before ascending to higher brain regions. The dorsal horn, particularly laminae I and II (substantia gelatinosa) and occasionally lamina V, serves as the first major hub for pain processing. Here, primary afferent nociceptors release neurotransmitters such as glutamate, substance P, and CGRP, which activate postsynaptic neurons and propagate the pain signal (Mertens et al., 2015). The intensity and quality of pain perception are shaped by interactions between excitatory and inhibitory mechanisms within the spinal cord. Inhibitory interneurons release GABA and glycine, dampening pain transmission, while excitatory interneurons amplify nociceptive signaling which can contribute to central sensitization when hyperactive (Tracey & Mantyh, 2007).

From the spinal cord, nociceptive information ascends to the brain through three major pathways. The spinothalamic tract (STT) is the most well-known, transmitting pain signals to the thalamus, the major sensory relay center, and sending inputs to the somatosensory cortex (S1 and S2) for localization and intensity perception. The spinomesencephalic tract projects to the periaqueductal gray and is involved in descending pain modulation, while the spinoreticular tract connects to the brainstem and reticular formation, integrating the emotional and autonomic

aspects of pain (Haggard et al., 2013). Once in the brain, processing occurs in what is referred to as the “pain matrix”, a highly connected network of brain regions involved in the perception of pain. Mainly, the somatosensory cortex is involved for sensory discrimination, the anterior cingulate cortex for emotional processing, the insular cortex for autonomic and affective responses, and limbic structures like the amygdala and hippocampus for pain memory and emotional associations (Tracey & Mantyh, 2007). This complex integration of sensory, cognitive, and emotional inputs explains why pain perception is highly subjective and modulated by both physical and emotional factors.

In fact, it is critical to acknowledge that the terms ‘nociception’ and ‘pain’, while often used interchangeably, represent fundamentally different processes. Nociception is a physiological mechanism where sensory neurons detect potentially harmful stimuli and trigger a cascade of responses to prevent damage, a process that occurs without conscious perception. In contrast, pain is a subjective, conscious experience that arises when the brain interprets nociceptive signals as threatening (Apkarian, 2018). For example, nociceptive activity can persist without causing pain while under anesthesia, and conditions such as phantom limb pain demonstrate the experience of pain without any nociceptive stimuli (Apkarian, 2018). Similarly, chronic pain can sometimes persist without ongoing nociceptive input, a phenomenon known as nociplastic pain.

Nociplastic Pain and Fibromyalgia

In 2017, the International Association for the Study of Pain introduced a third mechanistic pain descriptor, nociplastic pain, in addition to the two prevalent terms, nociceptive and neuropathic pain (Kosek et al., 2021). Nociceptive pain is caused by the physical damage or ongoing inflammation of tissues, while neuropathic pain results from peripheral nerve damage or

central nervous system disorders. The more modern definition of nociplastic pain is defined as "pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease of the somatosensory system causing the pain" (Kosek et al., 2021; Fitzcharles et al. 2021). Nociplastic pain is thought to be associated with widespread pain that is not localized to a specific region as well as central sensitization, an overall heightened response to pain and external stimuli.

The introduction of this term aligns with the evolving understanding of chronic pain as a distinct disorder, not merely a symptom. This recognition is crucial as chronic pain is a deeply complex and subjective experience, with patients often presenting with 'pain states' that involve multiple interacting mechanisms. Nociplastic pain is typically present in chronic pain conditions such as complex regional pain syndrome, irritable bowel syndrome, and fibromyalgia, which is the condition this research project will explore (Kosek et al. 2021, Fitzcharles et al. 2021).

Fibromyalgia is a complex disorder characterized by widespread pain commonly accompanied by fatigue, unrefreshing sleep, and other functional symptoms which cannot be explained by structurally defined causes. These comorbidities often include cognitive dysfunction, memory issues, hypersensitivity to various external stimuli, anxiety and depression. In many cases, there are also autonomic disturbances that affect the entire body such as dry mouth, blurred vision, and Raynaud phenomenon, the discoloration and numbing of the fingers and toes. Beyond the generalized pain itself, 20-30% of fibromyalgia patients also report paraesthesia, tingling or pins-and-needles sensations, in the upper body (Sarzi-Puttini et al., 2020). Fibromyalgia can be conceptualized as a 'centralized pain' state implying that the nature of this disorder stems from dysregulation of the central nervous system. This understanding of fibromyalgia does not discount the potential role of peripheral nociceptive input, but simply

emphasizes that the brain and spinal cord are most likely responsible for this hyperreactivity to stimuli and amplification of pain (Clauw, 2014).

Fibromyalgia is one of the most frequent musculoskeletal conditions, behind lumbar pain, rheumatoid arthritis and osteoarthritis, and its occurrence increases with age. While the prevalence of fibromyalgia varies according to which set of diagnostic criteria used, it appears to affect approximately 2-4% of the world population with a female to male ratio of 2:1, similar to other chronic pain disorders (Sarzi-Puttini et al., 2020). There is often a significant discrepancy between self-reported cases of fibromyalgia and its administrative prevalence based on physician diagnosis, as the disorder remains controversial and continues to spark debate and discussion within the clinical community (Häuser, 2015). Fibromyalgia is notoriously difficult to diagnose as there are no biomarkers or visible clinical signs associated with the conditions; blood tests and physical examinations tend to be unremarkable except for the possible presence of tender points along the body. There is a growing area of research dedicated to investigating biomolecules that might aid the diagnostic process for this disorder, and while some studies have been promising, this is still a very novel field (Sarzi-Puttini et al., 2020).

Fibromyalgia should be suspected in individuals experiencing widespread pain that cannot be explained by peripheral injury or inflammation, and a fibromyalgia diagnosis is commonly based on the exclusion of other chronic pain or rheumatic diseases (Clauw, 2014). Many individuals diagnosed with fibromyalgia commonly have a long history of chronic pain throughout their entire body and typically present with comorbid conditions including migraines, temporomandibular joint disorder, irritable bowel syndrome, and other regional pain conditions. Furthermore, chronic pain frequently occurs within families, with first-degree relatives being

eight times more likely to also be diagnosed with fibromyalgia or a similar chronic pain condition (Clauw, 2014).

Due to the complex pathophysiology of the disorder, the diagnostic criteria for fibromyalgia have evolved over the years, reflecting ongoing controversy over its classification. Some clinicians refer to it simply as “chronic widespread pain” (CWP), while others classify it as a rheumatic disease. Those who recognize fibromyalgia as a distinct disorder often view it as a central sensitization syndrome, emphasizing the central amplification of pain (Häuser, 2015). Given this complexity, many different versions of diagnostic criteria have been proposed, each attempting to further refine its approach and improve patient identification.

The earliest widely accepted criteria were established by the American College of Rheumatology (ACR) in 1990, which defined fibromyalgia based on the presence of CWP and tenderness in at least 11 out of 18 specific tender points. However, this method faced criticism for excluding other common symptoms and disproportionately diagnosing women, as they were more likely to exhibit increased tenderness (Clauw, 2014). In response, the 2010 and 2011 modifications removed the tender point examination (TPE) and introduced more inclusive criteria that acknowledged fatigue, sleep disturbances, and cognitive dysfunction (Sarzi-Puttini et al., 2020). These changes balanced the sex ratio of diagnosed individuals to approximately 2:1 female-to-male, consistent with other chronic pain conditions (Clauw, 2014). The TPE was replaced by the Widespread Pain Index (WPI), which scores pain across 19 body regions, alongside a symptom severity scale evaluating the four categories of fatigue, unrefreshing sleep, cognitive issues, and other somatic symptoms. These items are combined into a 0-31 score index, forming the basis of the current Fibromyalgia Survey Questionnaire (FSQ), widely used in both clinical and research settings (Häuser, 2015). This shift toward self-report surveys not only

provides a more comprehensive picture of fibromyalgia but also conceptualizes pain as a spectrum, allowing for a multidimensional assessment of the disorder.

Sex Differences in Pain

As research on pain has advanced, increasing attention has been given to sex differences in pain perception, with studies consistently showing greater pain sensitivity in women. While biological mechanisms such as hormonal and genetic influences contribute to these findings, social constructs and expectations also play a significant, yet often overlooked, role. The most prevalent finding across a large number of qualitative studies is the higher occurrence of pain in women relative to men across various conditions. Firstly, chronic pain is significantly more common in women across many geographical regions (Bartley & Fillingim, 2013 ; Sorge & Totsch, 2017). While chronic pain affects approximately 20% of the world's population, a study examining pain across 17 countries found a prevalence of 45% in women and 31% in men (Keogh et al., 2024). Furthermore, while women have been found to report widespread chronic pain more frequently than men, they also exhibit a higher prevalence in particular disorders including fibromyalgia, migraines, irritable bowel syndrome, and temporomandibular disorders (Bartley & Fillingim, 2013; Keogh et al., 2024). Beyond chronic pain, more acute forms of pain such as postoperative sensitivity are also reported more frequently by women (Keogh et al., 2024).

These data align with the consistent experimental findings that women have lower pain thresholds and tolerances across multiple pain modalities including heat, cold, pressure, and ischemic pain. This sex difference, however, can also vary depending on the specific pain modality as a stronger sex difference was reported for pressure pain compared to ischemic pain

(Keogh et al., 2024). These experimental methods provide insight into potential pain mechanisms underlying these sex differences. For example, men were found to exhibit greater conditioned pain modulation, a form of inhibition, while women experienced stronger temporal summation or the amplification of pain (Keogh et al., 2024).

While many factors are at play, past research has placed a greater emphasis on evaluating hormonal influences in modulating sex differences. A few studies notably found that sex differences in pain perception do not appear before puberty, strengthening the argument that hormonal fluctuations are key drivers in these sex differences (Keogh et al., 2024). Furthermore, studies indicate that women experience variations in pain sensitivity across the menstrual cycle, with higher pain sensitivity occurring during phases of low estrogen. This potentially highlights an important mechanism involving the interaction of estrogen with opioid receptors. Lower estrogen levels are associated with decreased opioid receptor activity, which may heighten pain perception (Morgan et al., 2024). On the other hand, testosterone is linked to reduced pain sensitivity and greater pain tolerance since it appears to have an analgesic effect by modulating nociceptive stimulation and reducing inflammatory responses (Morgan et al., 2024). Beyond hormonal differences, however, there are also potential neurobiological and genetic contributions. Brain imaging studies have found that women show greater activation in the insula, thalamus, and anterior cingulate cortex during pain perception (Melchior et al., 2016). There also appear to be significant differences in opioid receptors with men exhibiting stronger opioid system activation thus contributing to greater pain inhibition. However,the MC1R gene, associated with fair skin and red hair, affects this lowered opioid response specifically in women with both variant alleles leading to greater analgesic effects (Wiesenfeld-Halliin, 2005).

A final factor that is important to be acknowledged is the role of psychosocial influences on pain perception, especially in regards to stereotyped gender roles and the associated pain coping strategies. Men typically use behavioral distraction and problem-focused coping strategies, which may contribute to a higher pain tolerance as well as a tendency to underreport pain. Conversely, women are more likely to seek social support and use emotion-focused coping mechanisms (Racine et al., 2012). However, women also have a higher tendency to catastrophize, which can heighten pain perception and distress. These differences are shaped by social expectations surrounding masculinity and femininity, where men are expected to be more stoic while it is normalized for women to express their feelings openly. This variation in pain expression becomes even more nuanced across different cultures, with some social norms reinforcing greater suppression of pain in both men and women (Sorge & Totsch, 2017 ; Bartley & Fillingim, 2013). Additionally, early exposure to environmental stress, such as childhood trauma or abuse, can further impact pain sensitivity and reporting in both men and women (Bartley & Fillingim, 2013). Clearly, individual pain experiences are incredibly influenced by a variety of psychological and social experiences in combination with biological and nociceptive mechanisms.

Discovery of Warmth-Insensitive Fields

In attempting to better understand this complex experience of pain, a major turning point in sensory and pain research was the discovery of Warmth-Insensitive Fields (WIFs) by researchers Green and Cruz in 1998. Through their work, these researchers were able to localize fairly large regions of skin, around 5 cm^2 in size, that lacked the ability to detect warmth through thermoreceptors but were still capable of sensing painful heat through nociceptors (pain

receptors). In these fields, heating was not detected until it exceeded 41˚C, the activation threshold of heat-sensitive C-fiber nociceptors and a temperature at which low-threshold warm fibers should exhibit a strong response. Furthermore, these fields exhibit reduced sensitivity to heat pain with heat pain thresholds in WIFs averaging at 2˚C higher than those in normally sensitive fields (Green & Cruz, 1998). Participants also report a lower perceived intensity of heat in these regions, often describing sensations as 'burning', 'pricking', or 'stinging' rather than simply 'warm' or 'hot'. In contrast to heating, cooling sensitivity in WIFs remains comparable to that of adjacent, normally-sensitive fields, indicating that WIFs are not merely areas of the skin with compromised sensory perception (Green & Cruz, 1998).

The existence of these warmth-insensitive fields is largely due to the fact that different receptor types are innervated within the body at varying densities according to their function. Nociceptors are the most widely and densely dispersed thus ensuring broad, whole-body coverage for pain detection (Schmidt et al., 1997). In contrast, innocuous warm fibers responsible for detecting mild increases in temperature are the most sparsely innervated type of receptor (Jänig, 2018). This sparsity allows for certain regions in the body to exist without any capability of warmth perception. Since the sensations of heat and pain are usually associated with each other, these warmth-insensitive fields offer a unique model to study the activation patterns of nociceptors in isolation from thermal receptors (Green & Cruz, 1998). This also provides an opportunity to evaluate the contribution of low-threshold warmth fibers to the perception of heat and heat pain. Understanding nociceptive stimulation could be incredibly significant in the development of more efficient pain management strategies and treatments for individuals suffering from chronic pain.

Thermal Grill Illusion

WIFs present a compelling model for investigating nociceptive mechanisms since the sensations of heat and pain are often closely linked, with one of the most prevalent examples of this connection being the thermal grill illusion (TGI). The TGI, first demonstrated by Thunberg in 1896, is a paradoxical sensation of painful heat elicited by simultaneous application of interlaced warm and cold stimuli to the skin (Craig & Bushnell, 1994). This resulting pain sensation, despite the absence of an actual noxious stimulus, highlights the complex interplay between thermal and nociceptive pathways. The Disinhibition Theory, as demonstrated by Craig and Bushnell, provides a physiological explanation for the paradoxical pain experienced in the TGI. By recording from spinothalamic tract cells in lamina 1 of the dorsal horn, they identified three classes of cells: nociceptive-specific (NS) cells, which respond to painful stimuli; COLD cells, which respond to cooling; and heat-pinch-cold (HPC) cells, which are involved in both thermal and pain processing (Craig & Bushnell, 1994). Their findings revealed that warm stimuli alone did not activate any of these cells while, in contrast, cool stimuli strongly activated both COLD and HPC cells. When the thermal grill was applied, COLD cell activity was significantly reduced while HPC cell activity remained consistent. This suggests that the warm bars of the grill reduced cold-specific signals, effectively unmasking HPC activity and creating signals that resemble the experience of painful heat or cold (Craig & Bushnell, 1994). This illusion exemplifies how innocuous temperature stimuli can interact to produce a painful sensation, emphasizing the influence of thermal receptors on pain perception and further supporting the value of studying warmth-insensitive regions of the skin to isolate and better understand nociception.

Hypothesis and Research Question

This thesis will use these WIFs to evaluate the temperature gap between nociceptor awareness of a noxious stimulus and the conscious perception of pain. I hypothesize that there will be a significant difference between nociceptor activation and the perception of pain, further underscoring the distinction between nociception as a physiological process and pain as a subjective experience. I also hypothesize that this gap between receptor activation and sensory perception is smaller in patients with chronic pain than it is in healthy individuals, and that the temperature threshold for general heat pain is lower in patients with chronic pain than in healthy controls. Future iterations of this project with a larger sample size will continue comparing warmth and heat pain thresholds in WIFs between individuals with chronic pain and healthy controls. This research will hopefully provide more information about nociceptive sensitivity in patients with chronic pain, thus providing a direction for future, case-specific treatment and medication. Overall, the aim of this project is to create a mechanistic understanding of how and when an external stimulus goes beyond receptor awareness to conscious perception, providing novel insight into nociceptive processing.

Methods

In a single study session, participants provided informed consent and completed self-report questionnaires before undergoing a series of sensory assessments. WIFs were first identified through the protocol WIF Mapping to determine regions lacking low-threshold warmth receptors. Quantitative sensory testing was then conducted within these WIFs to evaluate their sensory processing in comparison to adjacent, normally-sensitive fields. The sensory assessments included measurements of warmth thresholds, warmth detection levels, and heat pain thresholds, both in WIFs and control fields. While the forearm served as the primary testing site, alternative locations such as the upper arm or lower leg were used if a WIF could not be located at that site. Study data were collected and managed using REDcap electronic data capture tools (Harris et al., 2009; Harris et al., 2019) hosted at Emory University.

Participants

25 healthy controls and 2 fibromyalgia (FM) patients were recruited through ResearchMatch and campus flyers and successfully screened for inclusion in the study. A recent IRB modification also allowed the recruitment of FM patients from Emory clinics. Healthy controls had no recent history (<5 years) of chronic pain conditions, while FM patients met the diagnostic criteria for fibromyalgia. Participants with conditions that could impact sensory processing, such as severe physical impairments, a history of alcoholism, cardiopulmonary or autoimmune diseases, or Raynaud's diseases, were excluded. Participants were reimbursed up to $330 for completing all the study-related tasks in entirety. All protocols were reviewed and approved by the IRB at Emory University (Protocol #00007822).

Of the 25 healthy controls recruited, 8 were excluded from the final statistical analysis. One participant was removed due to persistent reports of warmth sensations even in the absence of a thermal stimulus. Another participant was excluded for confusion in comprehending the experimental instructions, resulting in inconsistent responses that introduced variability in the data. The remaining six participants were excluded due to the inability to identify a definite WIF during testing.

Participant Questionnaires

Prior to the experimental session, participants completed a series of self-report questionnaires designed to assess demographics, various aspects of their pain experience and related emotional factors. Participants provided information on their sex, age, race/ethnicity, education, employment status, as well as relevant medical history, including any chronic pain diagnoses or treatments and routine use of narcotic analgesics or stimulants. Participants also listed all current medications and were instructed to refrain from taking certain medications (opioids, tramadol, sedatives, NMDA receptor antagonists, eugeroics) for a wash-out period and as-needed basics (analgesics, muscle relaxants, nasal decongestants) for a minimum of 8 hours before the testing session to minimize potential confounding effects. This process typically took less than an hour and included assessments of pain intensity, the presence of comorbidities and emotional well-being, providing a comprehensive overview of the participant's individual pain experience and any related symptoms.

Pain Catastrophizing Scale - Short Form

The Pain Catastrophizing Scale: Short Form (McWilliams, Kowal, & Wilson, 2015) is a shortened version of the original Pain Catastrophizing Scale (Sullivan, Bishop, & Pivik, 1995) and assesses the extent of negative thoughts related to an individual's pain experience, as well as their perceived control over symptoms and coping mechanisms. It consists of 6 items on a 7-point frequency Likert scale (0 = Never, 6 = Always) to quantify cognitive biases about pain. In clinical settings, it is used to examine the relationship between negative thought patterns and overall post-treatment satisfaction.

Positive and Negative Affect Scale

The Positive and Negative Affect Scale (PANAS) (Watson, Clark, & Tellegen, 1988) consists of 20 emotion-related items rated on a 5-point intensity Likert scale (1 = Very Slightly or Not At All, 5 = Extremely) to assess the extent to which participants have experienced each affective state within the week prior to their session. Within this questionnaire, there are 10 positive items and 10 negative items. The total score is calculated by finding the sum of these two sets of items separately, yielding two subscale scores that range from 10 to 50, with higher scores indicating greater levels of positive or negative affect, respectively.

Fear of Pain Questionnaire-III

The Fear of Pain Questionnaire-III (FPQ-III) (McNeil & Rainwater, 1998) contains 30 items rated on a 5-point intensity Likert scale (1 = Not At All, 5 = Extreme) that assesses an individual's fear of pain in both clinical and non-clinical situations. The total score ranges from 30 to 150 with higher scores associated with more prevalent fear. Within this total score, there

are also three subscale scores each consisting of 10 items that can be derived: Severe Pain, Minor Pain and Medical Pain.

PainDETECT

The PainDETECT questionnaire (Freynhagen et al., 2006) is a screening tool developed to detect neuropathic pain components in individuals as treatment choices can be directed at more specific pain mechanisms if correctly identified. This questionnaire consists of multiple components each designed to assess specific aspects of the participants' pain experience. The first set includes 3 items on a numeric rating scale, where 0 represents no pain and 10 represents the maximum pain, asking participants to rate their current pain and average pain experienced over the past 4 weeks. Additionally, the questionnaire features two diagrams to help participants describe the location of their pain, including whether it radiates, and to indicate the pattern of their pain. Finally, there are 7 items with an intensity 6-point Likert scale (0 = Never, 5 = Very Strongly) to assess components of neuropathic pain such as tingling, prickling, numbness, and allodynia. A score of 19 or higher on this questionnaire is a significant score that indicates a strong probability of a neuropathic pain component.

Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) assesses the severity of anxiety and depression symptoms in individuals, consisting of 12 items divided into two subscales: 6 items assessing anxiety and 6 items assessing depression. Each item is rated on a 4-point frequency Likert scale (1 = Not At All, 4 = Nearly All The Time), reflecting the frequency of symptoms experienced in the past week (Zigmond & Snaith, 1983). While not a diagnostic tool, the HADS

is widely used to assess the emotional well-being of individuals, with higher scores indicating greater prevalence of anxiety and depression symptoms.

Complex Medical Symptoms Inventory

The Complex Medical Symptoms Inventory (Wolfe et al., 2010) is a screening tool used to identify conditions commonly associated with functional syndromes or chronic pain disorders. This study uses the 2010 version from the American College of Rheumatology diagnostic criteria for fibromyalgia which contains two subscales. The Widespread Pain Index (WPI) is a body diagram to identify areas where participants have felt pain or tenderness in the past week. The Symptom Severity (SS) score contains 6 prompts designed to capture a broad range of symptoms associated with chronic conditions, including fatigue, cognitive dysfunction, and depression. A WPI score of 7 or more, combined with an SS score of 5 or more, or a WPI score between 3 and 6 with an SS score of 9 or more is considered to be a significant indicator of widespread pain, especially in the context of fibromyalgia.

Central Sensitization Inventory

The Central Sensitization Inventory (Meyer et al., 2012) is used to assess the severity of central sensitization, a condition where the central nervous system becomes hypersensitive to pain and other somatosensory signals. Part A is a 25-item questionnaire scored on a 5-point frequency Likert scale (0 = Never , 4 = Always) with different score ranges representing different intensities of central sensitization (from subclinical to extreme). Part B determines if the participant has a history of being diagnosed with chronic pain or related disorders. It includes questions regarding diagnoses for 10 conditions, such as chronic fatigue, fibromyalgia,

migraines, and others. A score of 40 or above indicates the presence of central sensitization symptoms.

Brief Pain Inventory

The Brief Pain Inventory (Cleeland, 1991) measures two aspects of pain experience: the intensity of pain and its impact on daily functioning. To assess pain intensity, participants are asked to rate their pain over the past 24 hours using a numeric rating scale from 0 to 10, with 4 prompts addressing different contexts. To evaluate the interference with activities, 7 additional items are rated on the same numeric scale. Finally, this inventory also includes questions about current pain treatments and their level of satisfaction. The subscale scores are calculated by averaging the ratings from each set of questions, and an average score of 7 or above is considered clinically significant.

Experimental Setup

There were three protocols used to obtain the data for this study, all relying on the use of a Medoc TSA2 which is a medical instrument that comes with variable temperature probes used for quantitative sensory testing (Medoc Ltd., 2019).

WIF Mapping - Piloting

Before beginning experimental sessions with recruited subjects, we prioritized the optimization of the protocol for locating WIFs, WIF Mapping, through a piloting phase to ensure reproducibility in future studies. The current procedure stencils a 5x10 grid on the subject's non-dominant arm where a 40°C (upper limit of innocuous warm) thermode is placed on each

square and the subject is asked to rate their thermosensation from 0-100 (0 being none, 100 being the most severe imaginable). Our piloting began with a phase that tested different body locations, including the upper arm, lower leg, and back. The results indicated comparable reliability across these sites, allowing for flexibility in selecting an alternative location if WIFs could not be identified on the forearm. Piloting also involved evaluating the protocol at various temperatures to account for the possibility that individuals with chronic pain may have lower heat pain thresholds than healthy controls. Through this process, we determined that 38˚C and 40˚C provided reliable detection of WIFs.

While 40˚C is commonly used as the upper limit for innocuous warm sensations, beyond which pain typically begins, 38˚C is slightly below this threshold. This lower temperature offers a lower margin for chronic pain patients who may experience heightened sensitivity, enabling more accurate assessments of thermosensation. An earlier version of this protocol included testing at 35˚C, but it was excluded as it was too close to baseline body temperature, making it unreliable for measuring thermal sensation. In contrast, 38˚C was found to be sufficiently distinct to activate low-threshold warm fibers while remaining below the heat pain threshold. Furthermore, to ensure clarity and consistency in participant instructions, scripts were developed and standardized for all evolving protocols (see Appendix A for full scripts). These scripts were structured to provide comprehensive understanding of the experimental setup while avoiding leading language that could influence participant responses.

WIF Mapping - Experimental

The experimental phase involved applying the standardized WIF mapping procedure, using the described stenciled grid and sensory ratings, initially on the forearm at 38˚C and 40˚C.

If WIFs could not be identified in this site, alternative locations were tested. This protocol was critical for locating the Warmth-Insensitive Fields in each subject that would next be evaluated with quantitative sensory testing. To locate WIFs, thermosensation was first evaluated at 38˚C, as previously described, with the participant giving a numerical rating from 0-100 of their thermal sensation. The 10 regions with the lowest sensation ratings were then retested at 40˚C. For these regions, subjects also provided sensation qualities such as “Neutral”, “Stinging”, or “Warm” to better characterize the kind of sensation they were receiving from the thermode. Once a region with little to no warmth sensation is found, it was designated as a WIF. Regions were considered WIFs when given a thermal rating of 5 or under, accompanied with neutral sensation qualities that do not indicate perception of heat. The goal was to identify two WIFs per participant; if none were found on the forearm, other body locations such as the biceps or lower leg may be tested. For each WIF, an adjacent field with normal sensation was also selected to serve as a control for subsequent testing.

Quantitative Sensory Testing

After locating subjects’ WIFs, the Medoc was used in a randomized series of thermal sensation studies to evaluate the nociceptive sensitivity of each participant. For all protocols, the skin temperature of each subject at the location of the testing was taken and recorded to determine a baseline. The first set of stimulations included Warmth Thresholds and Heat Pain Thresholds. For Warmth Thresholds, the temperature of the thermode applied to these fields was incrementally increased from 32˚C at a rate of 1˚C per second until the subject reported a sensation of warmth. Heat Pain Thresholds followed the same protocol but continued increasing the temperature until the subject perceived pain. For all thresholds, the protocol was repeated

three times, and the average of the results was calculated to determine the overall threshold. In the adjacent, normally sensitive fields, these tests determined the activation temperatures of thermoreceptors (warmth threshold) and nociceptors (heat pain threshold). In the WIFs, which lack thermoreceptors, these tests identified the temperatures at which nociceptors first respond to a stimulus (warmth thresholds) and subsequently elicit pain (heat pain thresholds). The next set of studies involved Cool Thresholds and Cold Pain Thresholds, using a similar protocol but with the thermode's temperature decreasing from 32˚C.

In the third set of studies, levels testing was performed to further characterize warmth and cold detection. During this process, the thermode's temperature either increased or decreased in small increments of 0.5˚C until the participant reported perceiving a change in temperature. This pattern continued, with the temperature progressively increasing until a change was detected, followed by a gradual decrease until the participant again perceived a shift. This alternating temperature modulation was repeated multiple times to determine the smallest temperature change reliably detected by the participant, therefore establishing their individual perception threshold. For adjacent fields, warmth levels testing began at 32˚C to capture responses from thermoreceptors while for WIFs it began at 39˚C due to the absence of warm fibers. For cold levels, both fields began at 32˚C. The final sensory test, Prolonged Noxious Stimulation, investigated the effects of sustained heat on pain sensitivity. Participants were exposed to a heat stimulus set 1˚C above their individual heat pain threshold for a period of 10 seconds for eight trials. After each trial, the participant rated their pain on a scale of 0-100 and underwent a round of threshold testing to assess changes in sensitivity from repeated noxious exposure. All these tests, except for the prolonged noxious stimulation, were performed in both the WIF and the adjacent fields to allow for comparison.

Data Analysis

The final statistical analysis included 21 WIFs across 17 healthy controls and was conducted using IBM SPSS (Version 29) software. Temperature data from the experimental sessions were analyzed to assess differences in sensory thresholds between WIFs and adjacent fields, as well as variations within WIFs themselves. Paired-sample *t*-tests were performed to compare warmth thresholds, heat pain thresholds, and warmth detection levels between WIFs and their respective adjacent fields. Furthermore, a paired-sample *t*-test was also performed to compare warmth and heat pain thresholds within individual WIFs. Pearson's correlation analysis was conducted to further examine the relationship between WIF warmth and heat pain detection. These statistical tests were used to evaluate significant differences in the sensory processing and activation patterns within WIFs and surrounding areas. Finally, Welch's t-tests and Pearson's correlation analyses were also performed between each WIF's difference score, which is the temperature difference between its warmth and heat pain thresholds, and participant age, sex, and questionnaire scores. This was done to see if cognitive or demographic factors were modulating the individual variability found within WIF thresholds.

Results

Identification of Warmth-Insensitive Fields

WIFs were successfully found in 68% of the healthy controls screened. A total of 21 Warmth-Insensitive Fields were identified and analyzed across 17 healthy controls. Statistical analyses were conducted using paired-sample *t*-tests with standardization based on the corrected standard deviation of the difference. This analysis revealed a statistically significant mean difference of 6.90˚C between WIF and adjacent warmth thresholds ($p < 0.001$) (Figure 1). Pearson correlation analysis indicated a moderate positive relationship between these warmth thresholds between WIFs and adjacent sites ($r = 0.481$, $p = 0.027$) (Figure 2). Furthermore, the difference between WIF and adjacent warmth detection using levels testing was also statistically significant with a mean difference of 7.28˚C ($p < 0.001$) (Figure 3). A moderately positive correlation was found for warmth levels between WIFs and adjacent sites ($r = 0.452$, $p = 0.045$) (Figure 4). Finally, there was also a significant mean difference of 3.00˚C between WIF and adjacent heat pain detection ($p < 0.001$) (Figure 5). Pearson correlation analysis demonstrated a strong positive relationship between these heat pain thresholds between sites ($r = 0.637$, $p = 0.002$) (Figure 6).

Sensory Processing Within Warmth-Insensitive Fields

Threshold data was then used to compare respective heat and heat pain detection within the individual WIFs of the 17 healthy controls, revealing a statistically significant difference of -2.63˚C ($p < 0.001$) (Figure 7). Pearson's correlation analysis demonstrated a strong positive correlation between these thresholds ($r = 0.663$, $p < 0.001$) (Figure 8). There was considerable variability between subjects in the difference between heat and heat pain detection within their

WIFs, highlighting individual differences in sensory processing. Figures 9 and 10 visually represent this variability, illustrating the extent of individual variation present in the data. These figures emphasize that while WIFs all share the quality of lacking warmth-detection fibers, their sensory processing is not uniform across subjects and is potentially modulated by other factors.

Examining Confounding Variables

This variability in WIF heat and heat pain detection was further analyzed in correlation with external factors, such as demographics and pain questionnaire responses. For these correlations, there was a number of 17 Warmth-Insensitive Fields analyzed, as 3 of the total WIFs were from earlier pilot sessions and thus do not have questionnaire responses. Furthemore, the difference score of 8.1 from participant 17 was excluded as an outlier based on the interquartile range (IQR) method. This method defines outliers as values exceeding 1.5 times the IQR above the third quartile or below the first quartile.

In the healthy control group, there were 7 WIFs found in male participants (8 including the outlier) and 10 found in female participants. The average threshold difference score for the total sample was 2.74, with the male controls showing a higher average difference of 3.41 compared to the female controls' score of 2.20 (Figure 11). A Welch's *t*-test, which accounts for unequal sample sizes, was conducted between the WIF warmth thresholds, heat pain thresholds, levels, and difference scores of males and females, but a statistically significant difference was not found for any of these tests (Table 1). Additionally, individuals were divided into three age groups: below 30 (n=10), 30-45 (n=4), and above 45 (n=3). The average difference scores for each group were 2.38, 3.00, and 1.81, respectively (Figure 12). However, due to the limited sample size and largely unequal distribution across age groups, the data lacked sufficient

statistical power to conduct meaningful analyses comparing these demographic differences. Future studies with larger sample sizes could conduct further statistical studies, including independent *t*-tests or a two-way ANOVA to examine the potential effects of age and sex on thermal perception.

Considering the role of cognitive factors in sensory perception, Pearson's correlation analysis was done between the individual difference values of WIF warmth and heat pain thresholds and individual scores to the various pain questionnaires. Aspects of surveys that were not scored on a Likert or numeric scale (for example, the body diagrams representing pain) were excluded from this analysis.While there were some strong correlation coefficients, none of these correlations were statistically significant. These analysis results are summarized in Table 2.

Fibromyalgia Patients

Due to time constraints, only two fibromyalgia patients were recruited and tested, preventing statistical analysis. However, questionnaire scores and sensory thresholds are presented in Tables 3-5. As shown in Table 3, subject 26(FM) exhibited clinically significant scores on both subscales of the CMSI. This finding aligns with the patient's fibromyalgia diagnosis, as the CMSI is a fibromyalgia screening tool. Additionally, the patient's score on the CSI further supports the presence of potential nociplastic pain, as it is commonly associated with central sensitization. Interestingly, despite these elevated scores, the sensory threshold data presented in Table 5 indicate no substantial differences from those observed in healthy controls. However, as this result is based on a single patient, no definitive conclusions can be drawn.

Subject 28 (FM) also exhibited significant clinical indicators of pain and central sensitization. As shown in Table 4, the subject's painDETECT score suggested a neuropathic

pain component, while their CMSI and CSI scores confirmed fibromyalgia and central sensitization, respectively. Additionally, the BPI score indicated severe pain, and the FPQ-III score was also substantially high. In terms of sensory processing, Table 5 demonstrates that the gap between warmth and heat pain thresholds was remarkably small, approximately 0.1˚C, in both WIFs and normally-sensitive fields. This minimal difference suggests a heightened sensitivity to thermal stimuli and impaired differentiation between warmth and pain, consistent with central sensitization.

As this is an ongoing project, further recruitment and testing of fibromyalgia patients is underway. Subsequent analysis will aim to provide a more comprehensive understanding of nociceptive processing in fibromyalgia and identify any significant differences from healthy controls.

Discussion

Thermal Perception and Nociception

The primary goal of this study was to further investigate the sensory processing within Warmth-Insensitive Fields, especially the temperature difference between the detection of heat and heat pain. Since these fields lack warmth-responsive fibers, these thermal perceptions are primarily driven by the activation of nociceptors, which offers a unique opportunity to study this process in isolation. The results demonstrated significant differences in how these WIFs respond to temperature stimuli in comparison to normally sensitive fields. Additionally, within WIFs themselves, there was a significant temperature gap between when heat was first detected and heat pain was perceived.

The first series of results validates the identification of WIFs as regions of the skin that lack innocuous warmth receptors. Figures 1 and 3 demonstrate that warmth detection thresholds are significantly higher in WIFs than in normally sensitive fields, confirming the absence of warmth-sensitive fibers in these regions as they require substantially higher temperatures to detect heat. This finding is consistent with the original work of Green and Cruz (1998), whose groundbreaking research first established the concept of warmth-insensitive fields. As confirmed by our results, WIFs do not detect heat until it surpasses noxious temperatures, typically around 41˚C, at which point the sensation is most likely mediated by C-fiber polymodal nociceptors. Similarly, there was also a significant difference in heat pain thresholds, further supporting the distinction between WIFs and adjacent fields in sensory processing (Figure 5). These results, combined with Green & Cruz' work, further support the emerging notion that low-threshold warm fibers contribute to the perception of both heat and heat pain. While previously thought to only encode sensations of warmth, the significant heat pain threshold difference indicates the

potential role of warm fibers in heat pain detection as well (Green & Cruz, 1998; Green, 2004). The key implications of these findings are that nociceptors are not independent detectors of pain, but, instead, work together with warm-sensitive fibers to encode the full sensation of heat pain.

As mentioned previously, the thermal grill illusion (TGI) provides a compelling example of how warm fibers can contribute to the encoding of pain, not just warmth. Furthermore, studies have shown that the pain intensity experienced during the TGI is not simply the sum of individual warm and cool sensations. Instead, the degree of perceived pain is often dependent on the difference between the warm and cold temperatures (Bach et al., 2011; Bouhassira et al., 2005). Larger temperature differences tend to produce more intense pain sensations, and, notably, this effect is driven more by the contrast between stimuli than by proximity to thermal pain thresholds (Bouhassira et al., 2011). This role of warmth fibers in pain detection aligns with our findings where WIFs have significantly higher heat and heat pain detection in comparison to areas with intact warmth perception. The higher heat pain thresholds in WIFs suggest that nociceptors alone cannot account for pain modulation, as they are present in both WIFs and adjacent fields. Further investigating the role of low-threshold warmth receptors in pain modulation could provide valuable insights into chronic pain conditions. Studies have determined that thermal receptors, such as TRPV3 and TRPV4 channels, play a role in pain modulation by influencing nociceptive pathways. TRPV3 and TRPV4 channels are both low-threshold warmth fibers activated by innocuous warm temperatures, but have also been found to be involved in amplifying pain perception in inflammatory conditions (Laing & Dhaka, 2015). TRPV3 releases mediators such as nitric oxide and prostaglandin E2, which sensitize nearby nociceptors and enhance pain signaling while TRPV4 is implicated in both mechanical and inflammatory pain (Laing & Dhaka, 2015). These findings on the role of warmth receptors

in pain modulation provide a valuable context for understanding the elevated heat pain thresholds we found in WIFs. Since WIFs lack low-threshold warmth fibers and elicited reduced pain perception, this supports the important contribution of thermal receptors to pain sensations. Further exploration could inform the development of targeted therapies that modulate warmth receptor activity, offering more effective pain management strategies.

The next set of results focuses on sensory processing within individual WIFs to explore potential confounding variables that may modulate their inherent variability. A significant difference was found between the heat detection and heat pain detection thresholds within individual WIFs, emphasizing that these are two distinct sensations elicited by nociceptors (Figure 7). A Pearson's analysis revealed a strong positive correlation ($r = 0.663$, $p < 0.001$) between WIF warmth detection and heat pain threshold, suggesting that the warmth and heat pain senses are connected and may be influenced by specific characteristics (Figure 8). To further investigate this variability, each WIF was analyzed using its difference score, calculated as the difference between its heat pain detection and warmth detection threshold. The extent of individual variability within WIFs is demonstrated in Figures 9 and 10, emphasizing that WIFs are not identical in their nociceptive activation. Finally, a series of additional variables including age, self-reported sex, and pain questionnaire scores were examined to determine whether they might serve as potential modulators of this variability.

In terms of demographics, the expected results were lower detection thresholds in females, reflecting greater sensitivity, and higher thresholds in older adults, reflecting a reduction in perception (Keogh et al., 2024, Guergova & Dufour, 2011). The relationship between age and pain perception is complex, with ongoing debate in the field. First, many studies report that heat pain thresholds remain relatively unchanged with age while pressure pain thresholds often

decrease significantly in older adults, suggesting increased sensitivity to mechanically-evoked pain (El-Tumi et al., 2017, Lautenbacher et al., 2005). Conversely, other research has found the opposite pattern, showing a significantly positive correlation between heat pain thresholds and age, with no notable changes in pressure pain thresholds (Zhi et al., 2024).

Lower thermal perception is typically expected in older adults due to reductions in warm fiber density as the skin ages. This progressive decline in perception is largely attributed to structural and functional changes in the peripheral nervous system (Guergova & Dufour, 2011). Studies have shown that older adults exhibit decreased thermal detection due to factors such as reduced thermoreceptor density, decreased skin blood flow, and alterations in nerve conduction velocity (Guergova & Dufour, 2011). Additionally, neuroanatomical changes, including degeneration of cutaneous free nerve endings and impairments in the spinothalamic pathway, contribute to diminished temperature sensitivity. These declines follow a distal-to-proximal pattern, with the extremities being more affected than central regions (Lautenbacher & Strian, 1991; Guergova & Dufour, 2011). Interestingly, preliminary findings from our study do not align with these studies and showed slightly decreased warmth and heat pain thresholds in the oldest cohort (>45), suggesting a mild increase in perception with age. However, the middle-aged cohort (30-45) exhibited slightly increased thresholds (Figure 12).

A possible explanation for the increased thermal perception observed in older adults in our study is the differential effects of aging on nociceptive fibers. Research suggests that while Aδ fibers experience significant age-related decline, C fibers may remain relatively preserved. Older adults may rely more heavily on C-fiber input for thermal and pain perception, leading to heightened sensitivity when Aδ fiber function is diminished (Chakour et al., 1996). Additionally, differences in experimental methodologies, body sites tested, and psychophysical techniques

contribute to the variability observed across studies (Guergova & Dufour, 2011). Finally, given that our sample is predominantly composed of individuals under 30, it may be expected that our findings do not align with the ongoing discussion surrounding age and pain perception. Future studies with a more diverse age range are necessary to provide future insight and contribute meaningfully to this debate.

In addition to age-related differences, sex biologies have also been shown to influence warmth and pain thresholds. Numerous studies have consistently reported that females tend to have lower heat pain thresholds compared to males, indicating greater sensitivity to heat-induced pain (Keogh et al., 2024; Averbeck et al., 2017). This heightened sensitivity is often attributed to a combination of biological, hormonal, and psychosocial factors. In terms of sex, our findings did not align with the expectations present in current literature. Both male and female cohorts had similar heat pain thresholds while the male group appeared to have a lower warmth detection threshold (Figure 11). Furthermore, none of the Welch's t-tests comparing male and female thresholds were significant (Table 1). However, studies investigating heat and heat pain sensitivity across sex differences have produced inconsistent results, and the variability of our findings may be attributed to differences in testing methodologies. For instance, in one study, assessments using the method of limits yielded no sex differences while the method of levels elicited lower thresholds for females (Fillingim, Maddux, & Shackelford, 1999). While the demographic observations of this study are not statistically significant due to the small sample size, they provide initial insights that future iterations of this study with larger, more diverse participant groups can use to draw more definitive conclusions regarding age and sex-related changes in thermal and pain perception.

Psychological Factors of Pain

The next series of results examines potential correlations between individual variability of WIFs and pain questionnaire scores. Given the well-established relationship between pain, anxiety, and depression in the research community, it was hypothesized that higher pain sensitivity would correlate with increased scores of the Pain Catastrophizing Scale, HADS, and FPQ-III. Psychological factors are known to heavily influence pain perception, making it not solely a physiological experience. Factors such as attention, interpretation, emotions, and coping strategies can amplify or mitigate pain experiences (Linton & Shaw, 2011). For instance, catastrophizing and negative beliefs can worsen pain perception, a concept assessed using the Pain Catastrophizing Scale in this study. Additionally, negative emotions such as fear, anxiety, and depression, determined by the HADS, often form a feedback loop that intensifies experiences of pain. Fear-avoidance is a typical example where fear of pain leads to avoidance behavior and overall heightened pain, measured using the PANAS and FPQ scales in this study (Linton & Shaw, 2011).

Furthermore, anxiety has been shown to enhance pain perception through a mechanism known as anxiety-induced hyperalgesia. In a study by Ploghaus et al. (2001), healthy volunteers underwent fMRI while receiving noxious thermal stimuli. Under high-anxiety conditions, where participants faced uncertainty about the severity of pain, the same moderate pain was perceived as significantly more intense than in low-anxiety conditions (Ploghaus et al., 2001). This heightened pain perception correlated with increased activity in the entorhinal cortex, supporting the Gray-McNaughton theory (Gray & McNaughton, 2000), which posits that the hippocampus amplifies aversive events in response to anxiety. This theory provides a framework for understanding how anxiety can exacerbate pain.

While the current study focuses on healthy, pain-free individuals, future iterations of this ongoing project will investigate these relationships in chronic pain patients. It is hypothesized that stronger correlations between pain questionnaire scores and pain thresholds will be evident in this population as chronic pain and anxiety disorders are often comorbid, with evidence suggesting shared risk factors and bidirectional causality (Gureje, 2008). Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and neurotransmitter systems, particularly serotonin and norepinephrine, are common in both conditions. Other factors including childhood trauma, genetic vulnerabilities, and neurobiological changes may further predispose individuals to developing both anxiety and chronic pain disorders (Romano & Turner, 1985; Gureje, 2008). Similarly, the relationship between chronic pain and depression is well-documented, with numerous theoretical models offering explanations. Beyond biological models emphasizing dysregulation in the nervous system, psychodynamic models focus on negative disposition and maladaptive coping strategies that can contribute to both depression and chronic pain. Cognitive-behavioral models highlight the role of catastrophizing and avoidance behaviors in maintaining these conditions (Romano & Turner, 1985).

However, in this study, the correlations between pain thresholds and psychological factors were not statistically significant (Table 2). This is most likely attributable to sample characteristics, as the participants were predominantly healthy, resulting in limited variability and restricted range in both pain perception and psychological distress. Furthermore, factors such as the relatively small sample size and the cross-sectional design may have limited the power to detect relationships. Psychological states like anxiety and depression can also fluctuate over time, and single-time assessments may fail to capture these dynamic changes (Linton & Shaw, 2011). Finally, our study may have been susceptible to retrospective bias, as participants may

inaccurately recall or report their symptoms and experiences (Romano & Turner, 1985). Future studies with larger, more diverse samples and longitudinal designs may provide a clearer understanding of these relationships.

Due to time constraints in the lab, the chronic pain data could not be fully analyzed within the scope of this project. However, further research will provide valuable insights into how psychological factors differentially influence pain perception in chronic pain patients compared to healthy controls. This understanding could guide the development of targeted interventions that integrate both pharmacological and nonpharmacological treatments, reducing the impact of anxiety and depression on pain management.

Fibromyalgia

Subject 26 and 28 displayed distinct pain profiles, with subject 26 exhibiting sensory threshold data comparable to healthy controls (Table 5) and moderate pain questionnaire scores (Table 3). In contrast, subject 28 reported severe, widespread pain with a high CSI score (Table 4) and a minimal 0.1˚C difference between warmth and heat pain thresholds in WIFs (Table 5). While the limited sample size prevents definitive conclusions, these results may indicate an emerging pattern for further investigation in future studies.

These preliminary findings align with current theories regarding nociplastic pain in fibromyalgia, which suggest altered central nervous system (CNS) processing as a primary contributor to heightened pain sensitivity (Clauw, 2014; Sarzi-Puttini et al., 2020; Häuser et al., 2015). Subject 28, who presented with severe symptoms of FM, exhibited a notably small gap between warmth and heat pain thresholds in both WIFs and normally-sensitive fields. This minimal distinction between regions indicates widespread sensitization, likely driven by central

mechanisms rather than peripheral nociceptive input. Extensive evidence supports this and suggests abnormal pain processing in the CNS of FM patients. Neuroimaging studies in FM patients have shown increased activation in brain regions associated with pain perception, including the insula, anterior cingulate cortex, and somatosensory cortex, while areas responsible for pain modulation and endogenous pain inhibition demonstrate reduced activity (Sarzi-Puttini, 2020). Additionally, increased connectivity between the default mode network (DMN) and insula is associated with spontaneous pain intensity, while reduced connectivity between pain-inhibitory regions further exacerbates pain perception (Häuser et al., 2015). The DMN is a system of interconnected brain regions that exhibit increased activity during rest and self-referential mental activities, such as daydreaming and introspection (Gillespie, Szabo, & Nemeroff, 2020). Meanwhile, the insula is a key brain region responsible for processing pain, interoception (awareness of internal body states), and emotional experiences (Uddin et al., 2017). The increased connectivity between these regions in FM may lead to a continuous awareness of pain, even without the presence of external stimuli, where the brain remains in a state of heightened pain perception. This is a relevant example of the complex interplay between psychological and physiological processes in pain perception.

Furthermore, cognitive influences, particularly pain catastrophizing and fear-avoidance behaviors, play a substantial role in amplifying pain experience. Since pain is not solely a physiological experience, it is heavily shaped by mental factors, particularly negative emotions like fear and anxiety (Linton & Shaw, 2011). Neuroimaging studies have demonstrated increased activity in limbic regions, including the amygdala and anterior insula, in individuals with high levels of catastrophizing (Häuser et al., 2015). Subject 28's significantly elevated FPQ-III score may have contributed to their enhanced pain sensitivity observed across both WIFs and control

fields. It is expected that a similar pattern between high FPQ-III scores and increased sensitivity is observed in future studies.

Finally, as this is an ongoing project, potential outcomes of the study are presented in the following tables, focusing on heat pain sensitivity in fibromyalgia patients and the clinical significance of these findings (Table 6). In future studies, FM patients will be tested in two regions: one experiencing clinical pain and one without pain. These potential results are compared to those of healthy controls as the overall aim is to compare sensory processing in healthy individuals versus those with chronic pain. The first potential outcome is that FM patients experience no change in sensitivity, with sensory testing data comparable to that of healthy controls (Table 6). This would suggest the absence of peripheral nociceptors, indicating that nociceptors in FM patients are not hyperactive and that central mechanisms may play a more dominant role in its pathophysiology. Prior research has shown that while some FM patients exhibit altered nociceptive processing, others demonstrate normal peripheral function, emphasizing the complex range of symptoms (Sluka & Clauw, 2016). The second potential outcome is heightened sensitivity only in clinically painful regions, suggesting localized inflammation and peripheral sensitization of nociceptors in these specific areas (Table 6). Peripheral sensitization occurs when nociceptive neurons in affected tissues become hyperexcitable due to inflammatory mediators such as cytokines, prostaglandins, and nerve growth factor (NGF), which lower nociception activation thresholds (Abeles et al., 2007). This mechanism aligns with findings that FM patients often have increased levels of inflammatory markers in muscle and skin biopsies, supporting the role of neurogenic inflammation in localized pain (Bazzichi et al., 2007). The third potential outcome is heightened sensitivity in both clinically painful and non-painful regions, indicating widespread sensitization and hyperalgesia

(Table 6). This would suggest central sensitization, a process in which repeated or prolonged nociceptive input leads to heightened responsiveness of the central nervous system (CNS). Central sensitization is characterized by increased levels of excitatory neurotransmitters such as glutamate and substance P, as well as impaired descending pain inhibition due to reduced serotonergic and noradrenergic activity (Sarzi-Puttini et al., 2020). This mechanism is widely supported as a key contributor to FM and is supported by neuroimaging studies showing altered pain processing in the CNS of FM patients (Gracely et al., 2002). By distinguishing between these potential outcomes, future studies will provide a more comprehensive understanding of sensitization mechanisms in chronic pain, ultimately guiding more targeted therapeutic interventions (Table 7).

Overall, while definitive conclusions cannot be drawn, the preliminary results align with current literature emphasizing the role of central nervous system dysfunction in nociplastic pain disorders like fibromyalgia. As this project continues, patterns between sensitivity and pain questionnaire scores, particularly the Catastrophizing Scale, CSI, CMSI, and FPQ-III, will continue to be evaluated. Understanding the role of CNS mechanisms may inform the development of targeted interventions designed to reduce pain sensitivity and improve overall pain regulation in FM patients.

Limitations

While this study provides insights about nociceptive processing, several limitations should be acknowledged. The most major limitation, as mentioned throughout the paper, is the small sample size, which potentially reduced the statistical power to detect significant relationships. Additionally, due to time constraints and laboratory issues, the study could not

include much data from fibromyalgia patients. Another potential limitation involves the thermode size used in sensory testing. Warmth detection is not highly specific and is greatly influenced by spatial summation (Green, 2004). The thermode size used in this study, 16x16mm, may have activated nearby warm fibers, leading to an underreporting of warmth-insensitive fields. Green and Cruz demonstrated that smaller thermodes (6.4mm^2) identified WIFs more effectively (Green & Cruz, 1998).

Additionally, the presence of bias must be considered which can significantly affect the validity of research findings. As mentioned, retrospective bias, also known as recall bias, occurs when participants inaccurately remember past events, leading to skewed results. This type of bias is particularly relevant in clinical studies such as this one where variables are evaluated based on self-reporting measures, such as the many self-administered questionnaires present in this study (Althubaiti, 2016). Recall bias has been associated with many factors, including the length of the recall period and the nature of the disease being studied, whether acute or chronic (Althubaiti, 2016). Studies have found that chronic pain patients tend to recall higher pain levels in clinical studies than those recorded in daily diaries, particularly when assessing their greatest and average pain. However, the inclusion of current pain ratings in retrospective assessments improved accuracy (Haase, 2023). In our study, our questionnaires ask participants, both healthy controls and FM patients, to report their current clinical pain as well as their pain levels over the past week and the past six months. This comprehensive approach allows for a comparison of short-term and long-term recall, providing a broader understanding of each participant's experience. Future studies could further minimize retrospective bias by asking participants to record daily fluctuations in pain in a diary.

Finally, a software inconsistency was recently identified within the HADS and CMSI pain questionnaires. Specifically, two items were missing from the HADS, while one item in the CMSI was duplicated. Although data collected prior to this discovery was retained and included as a variable, these scores should be interpreted with some caution. The software has since been corrected for all future participants.

Conclusion

This study provides insights into thermal perception and nociception, revealing significant differences in heat and heat pain perception between WIFs and normally sensitive fields. These findings contribute to the growing evidence supporting the role of low-threshold warm fibers in pain detection. A deeper understanding of how these fibers mediate the transition from innocuous warmth to pain could lead to the development of more targeted pain management strategies by focusing on specific sensory pathways. Furthermore, the potential observed patterns linking central sensitization to a reduced gap between nociceptor activation and pain perception suggest the involvement of central sensitization in enhancing peripheral signals. Future research exploring these mechanisms could advance therapeutic approaches aimed at restoring the balance between nociceptive activation and pain experience. Ultimately, this study lays the groundwork for a more comprehensive understanding of pain processing, with the potential to advance our knowledge of the neurobiological mechanisms underlying nociplastic pain.

Figures and Tables

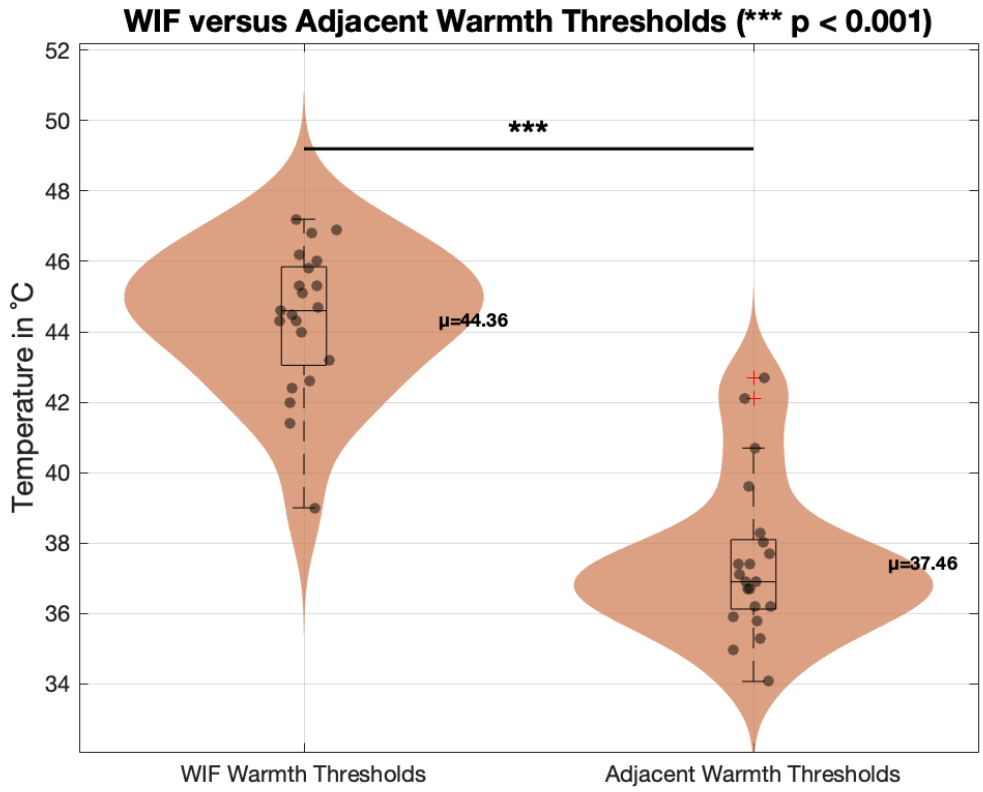

Figure 1. Thresholds testing protocol demonstrated a 6.90˚C increase in warmth detection thresholds in WIFs. ***, $p < 0.001$

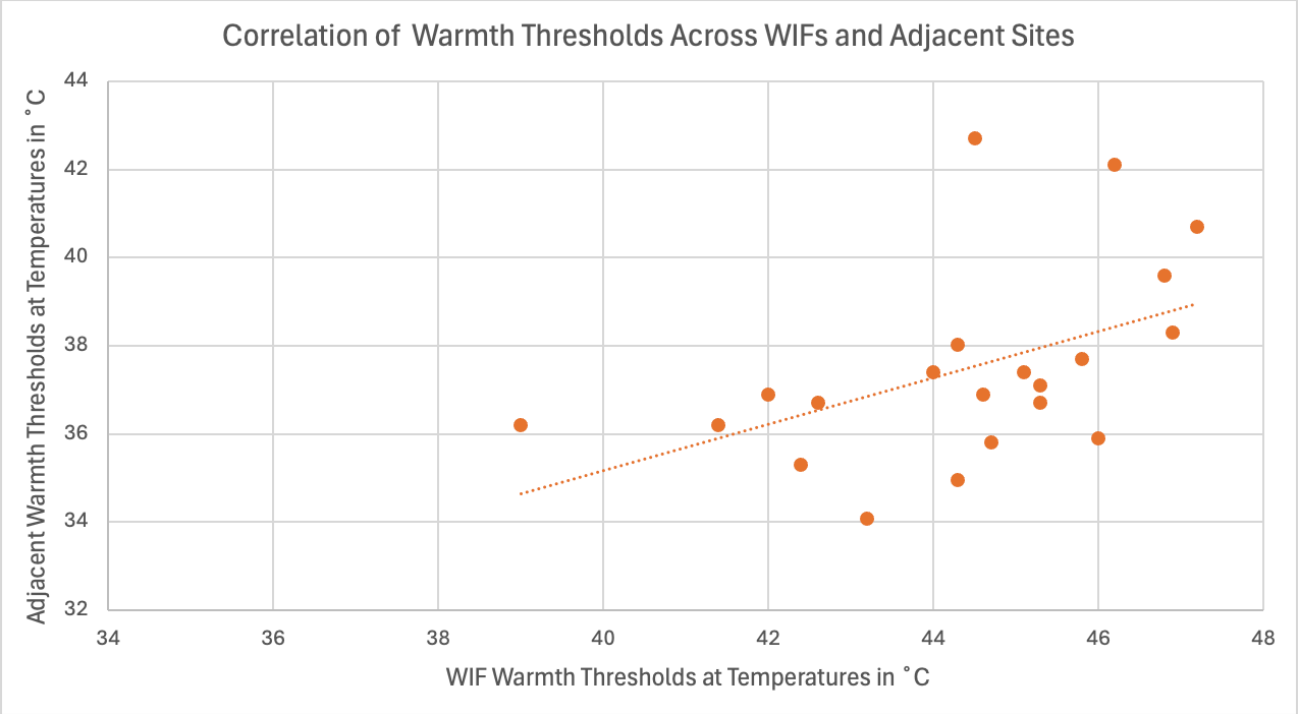

Figure 2. A moderately positive correlation found between WIF and adjacent warmth thresholds. $r = 0.481$, $p = 0.027$

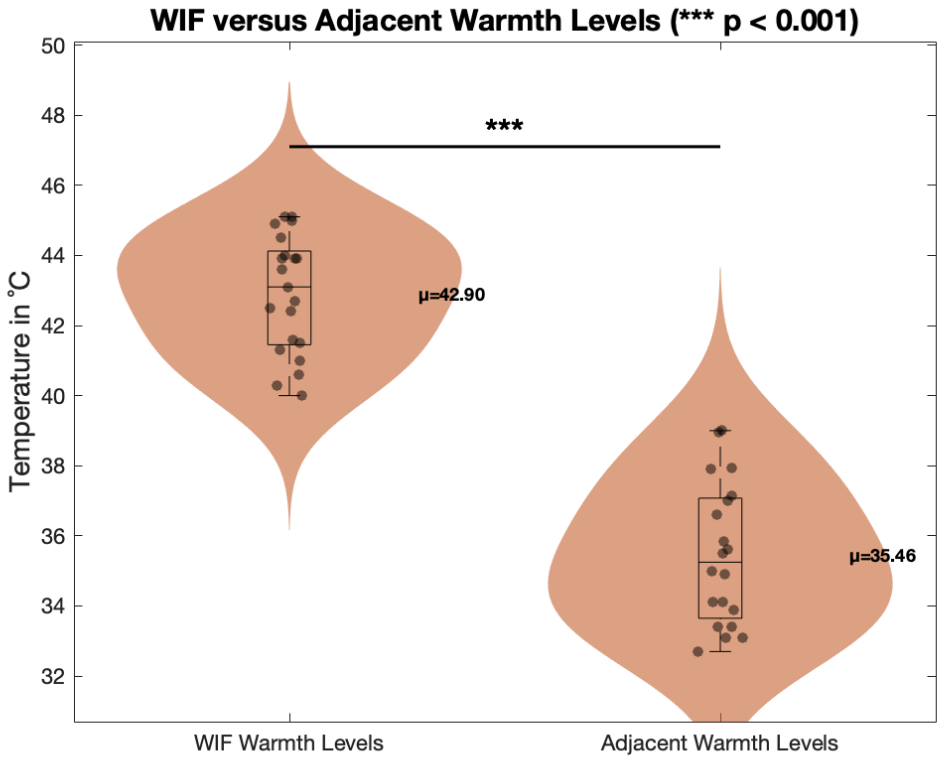

Figure 3. Levels testing protocol demonstrated a 7.28˚C increase in warmth detection in WIFs. ***, $p < 0.001$

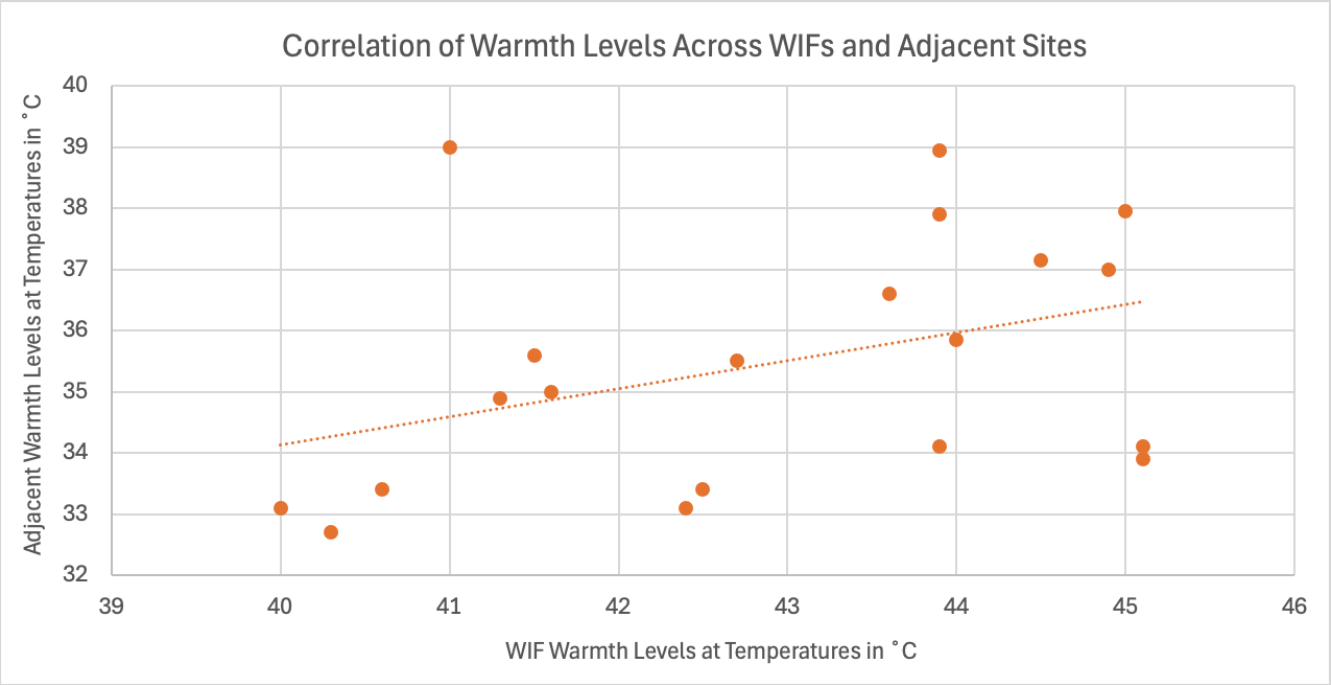

Figure 4. Moderate positive correlation found between WIF and adjacent warmth levels. $r = 0.452$, $p = 0.045$

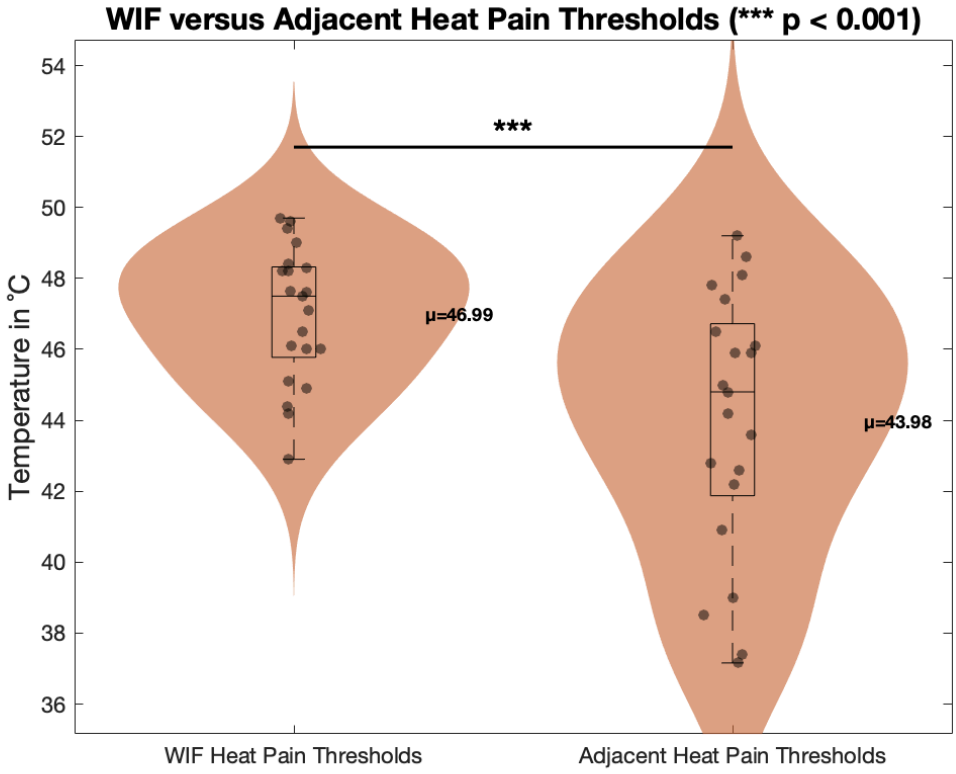

Figure 5. Thresholds testing protocol demonstrated a 3.00˚C increase in heat pain detection thresholds in WIFs. ***, $p < 0.001$

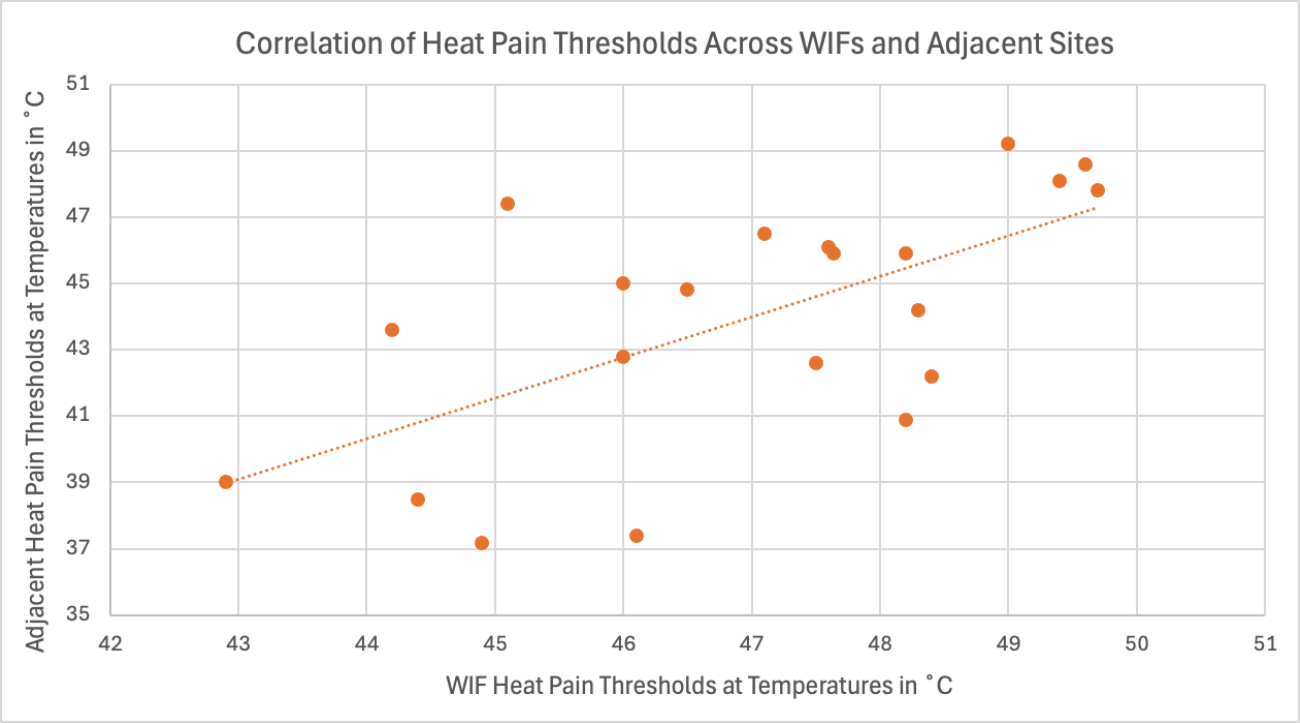

Figure 6. Positive correlation found between WIF and adjacent heat pain thresholds. r = 0.637, p = 0.002

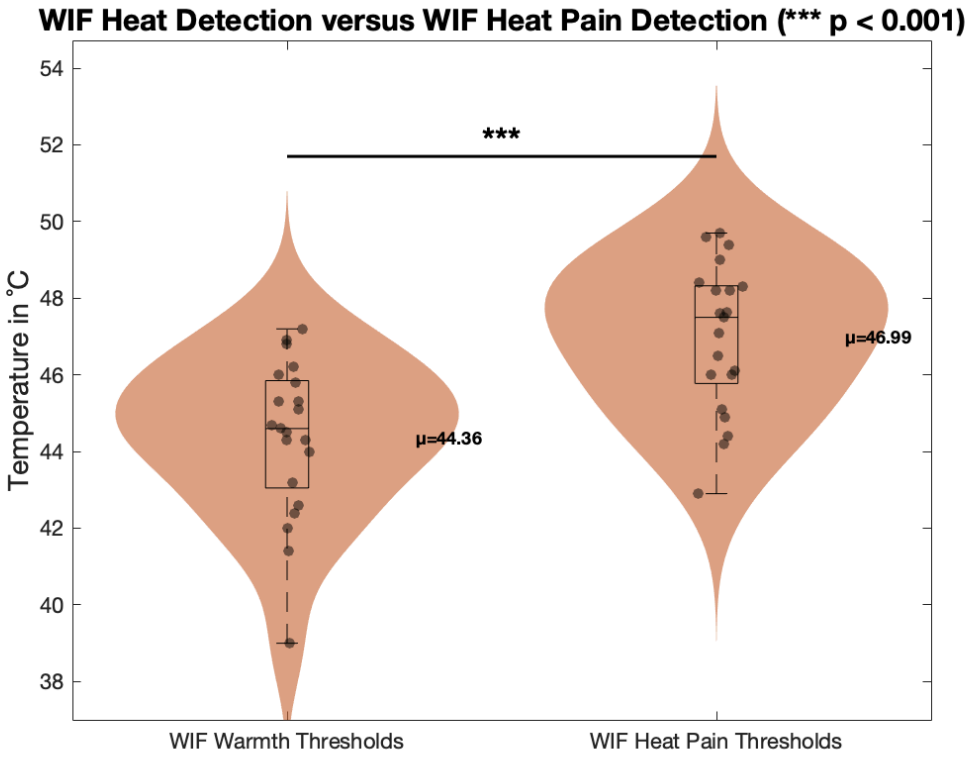

Figure 7. Thresholds testing protocol demonstrated a 2.63˚C increase in heat pain detection thresholds of WIFs. ***, $p < 0.001$

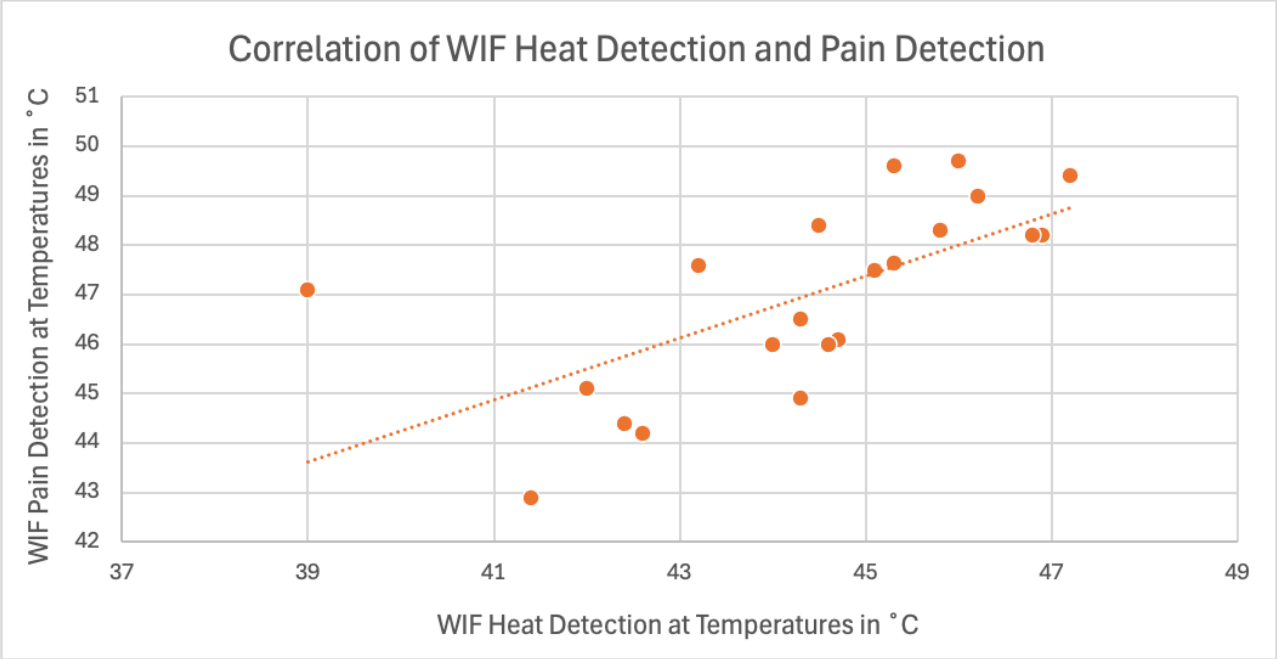

Figure 8. Positive correlation between heat detection and pain detection thresholds in WIFs. $r = 0.663$, $p < 0.001$

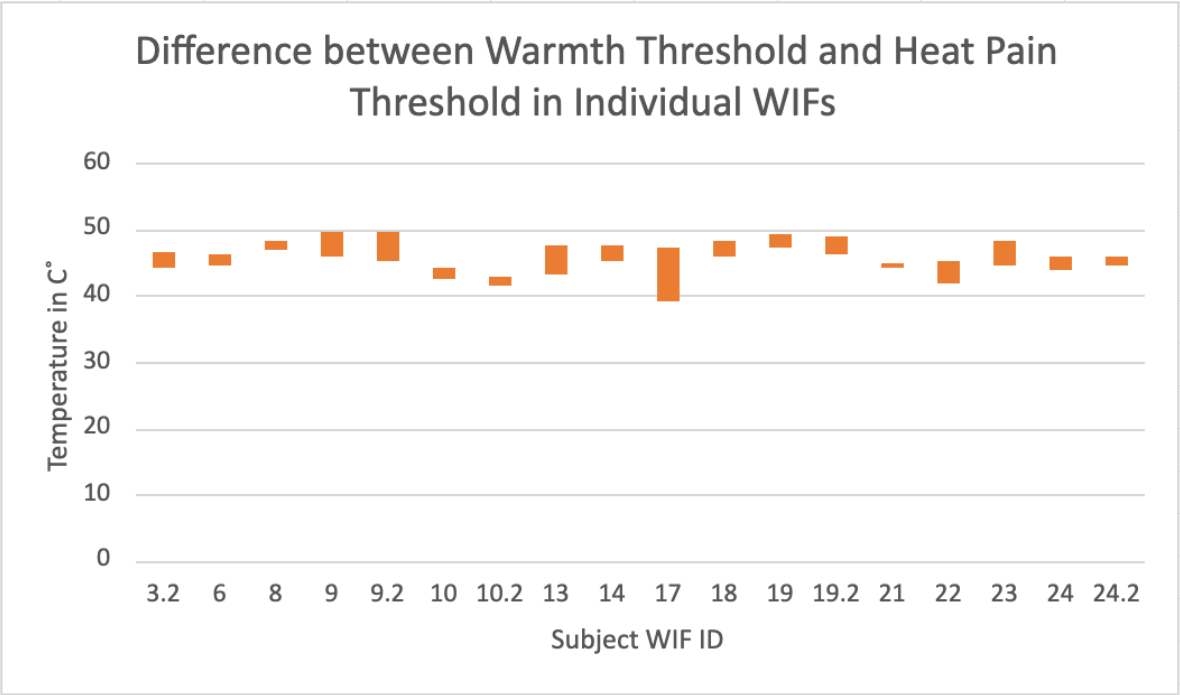

Figure 9. Variability in individual threshold differences in WIFs by displaying the difference values between WIF warmth and WIF heat pain thresholds for each subject.

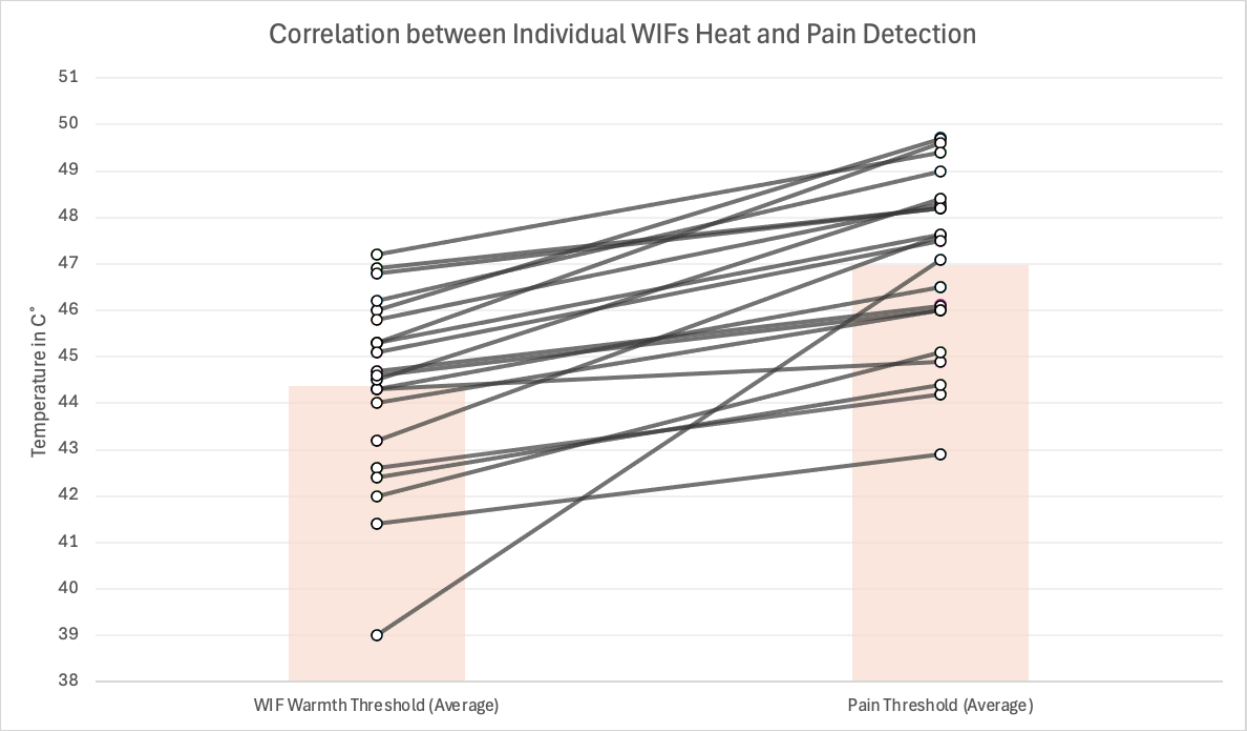

Figure 10. Another representation of the variability in individual WIF warmth and heat pain thresholds. Each line is one subject and connects that subject's WIF warmth threshold to their corresponding WIF heat pain threshold.

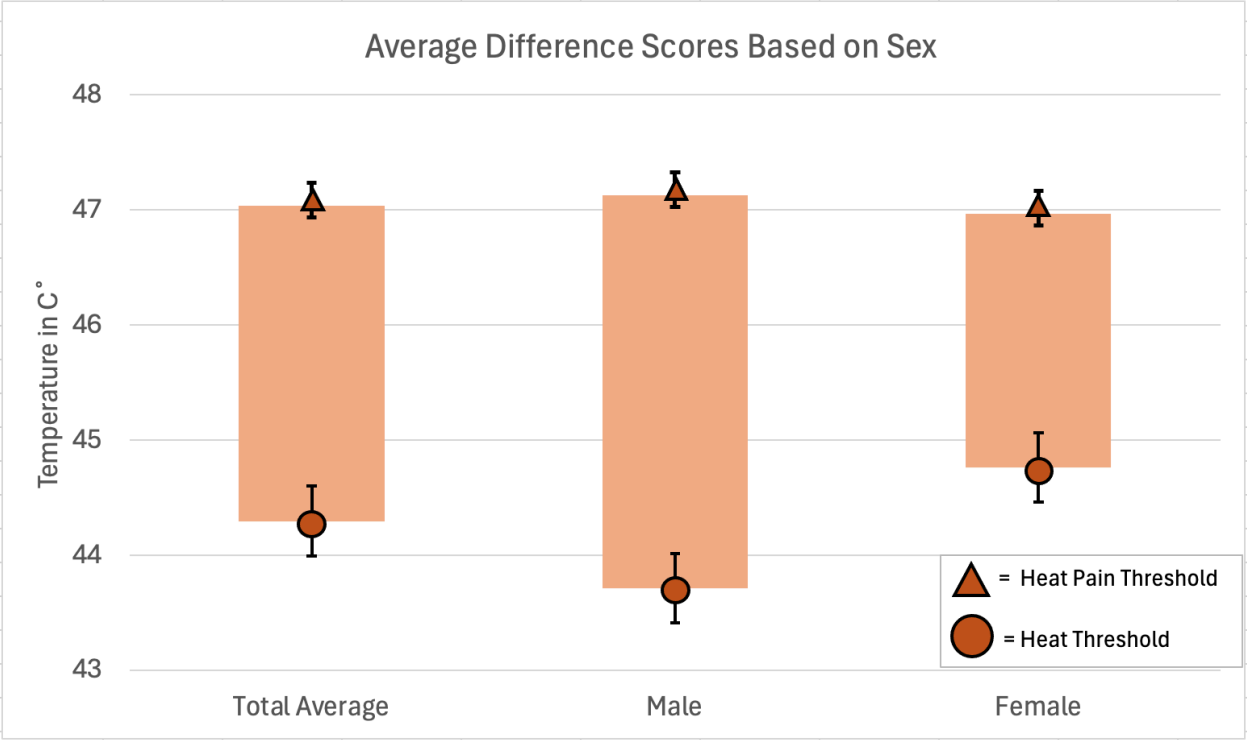

Figure 11. Average difference scores between WIF heat and heat pain thresholds for the entire sample of healthy controls, as well as separately for males and females.

Measure	*t*-value	Two-tailed Significance
WIF Warmth Thresholds	0.399	0.700
WIF Heat Pain Thresholds	-0.510	0.624
WIF Levels	1.404	0.184
WIF Difference Score	0.668	0.516

Table 1: Results for Welch's t-tests conducted in WIFs between female and male groups

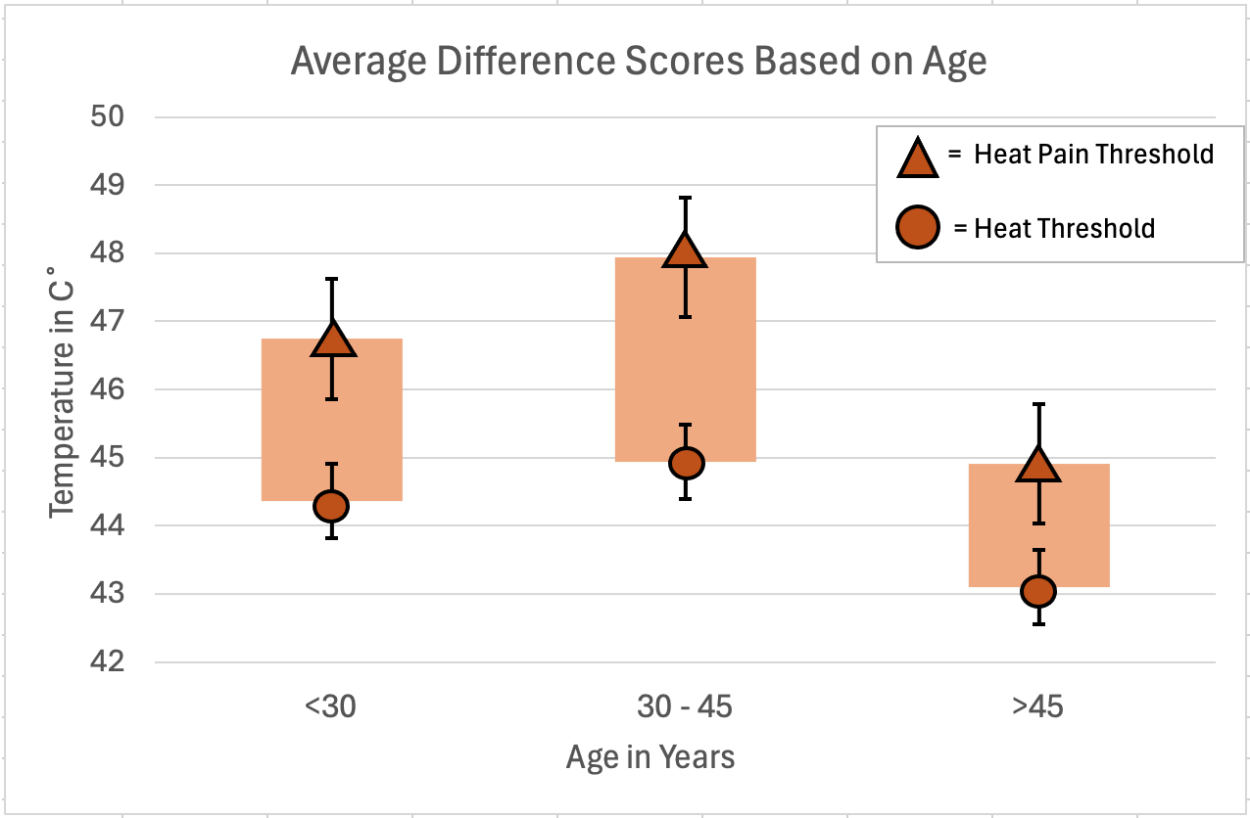

Figure 12. Average WIF difference scores across three age groups present in the sample of healthy controls.

Questionnaire	**Pearson's correlation (*r*)**	**Two-tailed significance**
Pain Catastrophizing Scale	-0.156	0.550
Positive and Negative Affect Scale	-0.159	0.542
Fear of Pain Questionnaire-III	0.172	0.509
PainDETECT	0.135	0.606
Hospital Anxiety and Depression Scale	0.282	0.272
Complex Medical Symptoms Inventory	0.290	0.259
Central Sensitization Inventory	0.225	0.386
Brief Pain Inventory	-0.011	0.996

Table 2. Pearson's correlation analysis between individual WIF difference thresholds and the total scores of the questionnaires.

Questionnaire	Subject (FM 26) Score		Max Possible Score	
Pain Catastrophizing Scale	13		36	
PANAS	37	21	50	50
FPQ-III	45		150	
painDETECT	7		38	
HADS	9	7	21	21
CMSI	6*	8*	12	19
CSI	45*		100	
BPI	4	2.43	10	10

Table 3. Questionnaire scores of the fibromyalgia patient (FM 26). Cells that are split represent subscale scores of the questionnaire. Scores marked with an asterisk are generally considered clinically significant.

Questionnaire	Subject (FM 28) Score		Max Possible Score	
Pain Catastrophizing Scale	23		36	
PANAS	24	21	50	50
FPQ-III	102		150	
painDETECT	26*		38	
HADS	4	13	21	21
CMSI	11*	14*	12	19
CSI	56*		100	
BPI	7.25*	8.29*	10	10

Table 4. Questionnaire scores of the fibromyalgia patient (FM 28). Cells that are split represent subscale scores of the questionnaire. Scores marked with an asterisk are generally considered clinically significant.

Subject ID	Warmth Thresholds (˚C)		Warmth Levels (˚C)		Heat Pain Thresholds (˚C)	
	WIF	**Adjacent**	**WIF**	**Adjacent**	**WIF**	**Adjacent**
26 (FM)	45.03	37.8	44.3	36.1	47.96	46.37
28 (FM)	43.4	35.67	40.9	32.3	43.5	35.7
Average of Healthy Controls	44.36	37.46	42.9	35.46	46.99	43.98

Table 5. Quantitative sensory testing results of the fibromyalgia patients.

Potential Outcomes	Sensitivity in Clinically Painful Region	Sensitivity in Non-Painful Region	Meaning of Finding
No Change in Sensitivity	Similar to Pain-Free Healthy Controls	Similar to Pain-Free Healthy Controls	Nociceptors are not sensitized
Changes in Sensitivity only in Painful Region	⬆ than Pain-Free Healthy Controls	Similar to Pain-Free Healthy Controls	Localized inflammation and nociceptive sensitization
Widespread Changes in Sensitivity	⬆ than Pain-Free Healthy Controls	⬆ than Pain-Free Healthy Controls	Widespread sensitization of nociceptors

Table 6: Potential Outcomes of Future Fibromyalgia Studies

Potential Mechanism	Potential Treatment
Central Sensitization (Potential Outcomes 1 and 3)	Treatment should primarily target central mechanisms such as enhancing descending pain modulation through SNRIs (serotonin-norepinephrine reuptake inhibitors) or tricyclic antidepressants, both of which increase inhibitory neurotransmitter levels (Arnold et al., 2012)
Peripheral Inflammation and Sensitization (Potential Outcome 2)	Treatment should focus on localized inflammation through topical analgesics which desensitize nociceptors or anti-inflammatory drugs such as NSAIDs (Bazzichi et al., 2007)

Table 7: Potential Mechanisms of Fibromyalgia and Corresponding Treatment

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Appendix A

WIF Mapping Scripts

Preparation Tasks

Applying the Grid

Apply the 16x16 mm grid to the participant's volar forearm using the grid template from the crease at the junction between the palm and the inner wrist, to the bend of the elbow. Apply the grid such that the grid's center line aligns with the subject's middle finger, up to the elbow.

We will now apply the grid on "location" for our testing.

Preparing the Medoc and LabVIEW Programs

Ensure that the Medoc TSA 2 is on, the laptop is working, and the monitor is working. Participants should be able to view the computer monitor, but they should not be able to see the laptop screen. The computer mouse and Medoc remote control should be near the subject so they can reach it for later tasks

This is the Medoc TSA II device, and we'll be using it for our thermal testing today. It cannot cause any damage to your skin with the temperatures it applies, but if at any point you would like to stop the testing please let me know.

Pretest

Conduct a pretest to help the participant learn to use the rating scale. For this test you will open the table titled "NoSH WIF MAP 38". Apply the stimulus for 2 seconds using the 16x16 mm probe. Have the participant rate the intensity of a warm and neutral (skin temperature) sensation using the rating scale. If participant requests, you may do this a second time. There is no need to record this data.

"The device placed on your skin is able to either warm or cool the skin. In addition, for some of the tests, you will be given a stop button that enables you to immediately stop the ongoing test stimulus at any time. For every test, I will explain to you when to use the stop button.
Before we begin, we're going to quickly practice rating the intensity and quality of the sensation. I'm going to apply a thermal stimulus to your palm. Please tell me whether the device on your skin feels warm, cool or neutral. ***(Wait for participant to respond)*** *Please rate the intensity of the sensation from 0-100 with 0 being no sensation at all, and 100 being the most intense sensation you can imagine from this stimulus. There will be times when you may not feel anything at all, and that's completely okay— Do you have any questions?*

Thermal Mapping

Use "NoSH WIF MAP 38" with baseline temperature set to 35 degrees Celsius. Align the cell with the bottom left cell on the arm, moving up along columns, and systematically moving across the width of the arm. Prompt the participant to rate the intensity of the warmth sensation

from 0-100 on the VAS scale. Allow for at least 2 seconds from the prior stimulus before stimulating the next cell. Record ratings in the appropriate spreadsheet.
I will now begin mapping the sensitivity of your arm. Please rate the intensity of the sensation from 0-100 (how warm the stimulus feels to you) with 0 being not warm at all and 100 being the warmest sensation that you can imagine from this stimulus. Do you have any questions?

Thermal Mapping at 40˚C
Use "NoSH WIF MAP 40" with baseline temperature set 40˚C. Use this in the lowest 10 ratings from the WIF MAP 38 test. Prompt the participant to rate the intensity of the warmth sensation from 0-100 on the VAS scale. Allow for at least 2 seconds from the prior stimulus before stimulating the next cell. Record ratings in the appropriate spreadsheet.
I will now go back and retest some of the areas. Please rate the intensity of your sensation from 0-100 (how warm the stimulus feels to you) with 0 being not warm at all and 100 being the warmest sensation that you can imagine from this stimulus. Also give us a sensation quality from the list in front of you, or any other sensation that you might be feeling if none of the sensations provided describe what you are perceiving. You may pick as many or as few as you feel. There will be times when no stimulus is introduced, so please only provide a rating when you feel a sensation or when we ask you if you felt anything. Do you have any questions?

WIF Diagonal Configurations
Use "NoSH WIF MAP 40" with baseline temperature set 40˚C. Use data figures from data sheets to know the order of configuration.
I will now change the location of the thermode in various placements. Please rate (the feeling) or the intensity of your sensation from 0-100 with 0 being no sensation at all and 100 being the most intense sensation you can imagine from this stimulus. Also give us a sensation quality from the list in front of you or any other sensation that you might be feeling. Pick as many or as few as you feel. Do you have any questions?

WIF Testing
If the subject gives a low rating, we need to confirm that it is a potential WIF. Warm limits, Heat pain thresholds and Warm thresholds tasks will be performed to confirm this. We will also conduct cool limits, Cold pain thresholds and Cool threshold tasks to further characterize the location. These four tests will also be done on another normally sensitive area of the skin to provide a point of comparison. Right after the test is performed on one of the two areas, the same test will be performed on the second spot. The order will be counterbalanced among participants.

Warm Thresholds (Limits)

Select the program "NoSH WIF map Warm Limits". Enter 3-digit subject ID. Change the Baseline Temp to the skin temperature.

In this set, we will test your ability to perceive a change in temperature. Please press the Y button on the remote immediately once you perceive a change in temperature to warm/warmer for the first time. Please also provide a sensation quality from the list provided. You can pick as many or as few as you feel. Subsequently, the thermode will cool down again to baseline temperature and the task will repeat several times. We will repeat this test several times with short breaks in between. We will begin in a few seconds.

Heat Pain Thresholds (Warm Limits)

Select the program "NoSH WIF map Warm Limits". Enter 3-digit subject ID.

Next, we will test your thresholds for perceiving heat pain. Please press the Y button on this remote the moment you feel the sensation become painful, not warm. Please also provide a sensation quality from the list provided. You can pick as many or as few as you feel. This task will repeat 3 times and will begin in a few seconds.

Warm levels (Warm Levels)

Select the program "NoSH Warm levels".

Next, we will test your ability to perceive different temperatures. Please say "YES" the moment you feel a warm sensation. The temperature will be changing; just keep your response according to your sensation. We will do multiple trials and sometimes there will be some breaks between trials, so do not worry if you are not feeling a sensation for a time.

Cool Thresholds (Cool Limits)

In this set, we will test your threshold for perceiving cool sensations. Please press the Y button on the remote immediately once you perceive a change in temperature to cool/cooler for the first time. Please also provide a sensation quality from the list provided. You can pick as many or as few as you feel. Subsequently, the thermode will warm up again, until it reaches baseline temperature, and the task will repeat several times. Press the button every time you feel a shift to cooler temperature. We will begin in a few seconds.

Cold Pain Thresholds (Cool Levels)

Next, we will test as to when you perceive the cooling of the thermode as painful. Please press the Y button on this remote the moment you feel the sensation become painful, not cold.

Please also provide a sensation quality from the list provided. You can pick as many or as few as you feel. This task will repeat 3 times and will begin in a few seconds.
Cool Levels (Levels) *Next, we will test your ability to perceive different temperatures. Please say "YES" the moment you feel a cool sensation. The temperature will be changing; just keep your response according to your sensation. We will do multiple trials and sometimes there will be some breaks between trials, so do not worry if you are not feeling a sensation for a time.*

Prolonged Noxious Stimulation

Now we are going to test how your skin reacts to heat. We will apply a warm feeling to your skin that might hurt a little, but it won't burn you or cause any permanent damage. Your skin might turn red or feel sensitive, but it won't be harmful.
At the beginning, we will test two different heat levels to find out what feels comfortable for you. When you feel any kind of warmth, press the button right away. After that, we will apply heat for 10 seconds and ask you to rate how much it hurts and describe the feeling. Then, we will test two more heat levels the same way. We will repeat this process a total of 8 times.
If at any point it becomes too painful or you want to stop, just let us know! Do you have any questions before we begin?