Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Benjamin M. Riley April 15, 2021

Warmth-insensitive fields: a tool for the psychophysical study of innocuous and noxious thermal perception

by

Benjamin M. Riley

Daniel Harper
Adviser

Neuroscience and Behavioral Biology

Daniel Harper

Adviser

Shawn Hochman

Committee Member

Michael Crutcher

Committee Member

2021

Warmth-insensitive fields: a tool for the psychophysical study of innocuous and noxious thermal perception

By

Benjamin M. Riley

Daniel Harper

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Neuroscience and Behavioral Biology

2021

Abstract

Warmth-insensitive fields: a tool for the psychophysical study of innocuous and noxious thermal perception

By Benjamin M. Riley

Peripheral thermosensation and nociception are inextricably linked components of our somatosensory system. Despite over 120 years of vigorous debate on the matter, the mechanisms underpinning each term of this relationship remain nebulous. In recent years, the question at the forefront of research has been whether thermosensation is served by non-specific nociceptive afferents (i.e., C- and Aδ-fibers) or by integration of activity in many specific nociceptive and non-nociceptive fibers. Answers have naturally settled around a middle term that incorporates aspects of both ideas. Now, to determine where and how these mechanisms function, researchers require methods for isolating functional components and testing the response of the system. In the case of thermosensation and pain, this task can prove daunting. Issues of non-specific and incomplete isolation by traditional techniques are exacerbated by overlapping stimulus energies that excite both systems at once. In 1998, researchers proposed the exploitation of natural gaps in cutaneous warmth sensitivity as a research tool capable of circumventing these issues. Thermosensory properties of these *warmth-insensitive fields* (WIFs) suggested that within their boundaries it was possible to study perception of nociceptive heat and both innocuous and nociceptive cold in utter isolation from the innocuous warm sense. Despite this, WIFs have remained woefully understudied and underutilized in thermosensation research. This study aims to utilize quantitative sensory testing (QST) to better understand the thermosensory response properties of these fields and investigate how such gaps in perception may elucidate the relationships governing innocuous and noxious thermal sensations. Our findings that varying levels of warmth sensitivity may the perception of other aspects of thermosensation suggest that continued research in WIFs has the potential to elucidate mechanisms governing the relationship between innocuous and noxious thermal perception. Notably, we provide novel documentation of a relationship between warmth insensitivity and cold nociception that presents substantial theoretical implications for the field.

Warmth-insensitive fields: a tool for the psychophysical study of innocuous and noxious thermal perception

By

Benjamin M. Riley

Daniel Harper

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Neuroscience and Behavioral Biology

2021

Table of Contents

Tables and Figures

Introduction

Humans inhabit the widest range of environments of any animal on the planet. Our status as homeothermic mammals necessitates nearly constant regulation of core body temperature. Our special success in this task is owed at least in part to our talents for object manipulation and our ability to both harness natural as well as engineer artificial sources of heat. This unique combination of environmental pressures and evolutionary endowments manifests in a concert of autonomic processes (e.g., shivering, nociceptive flexion reflex, perspiration, vasodilation/constriction) and conscious behaviors (e.g., seeking shade, donning warm garments) that enable homeostasis, object perception, and protection from thermal injury (Filingeri, 2016).

A robust reliance exists between this dynamic range of responses and peripheral thermosensation, which enables us to detect the temperature of our immediate surroundings. Our skin serves as the primary interface with the ever-changing thermal environment. Innervation throughout this sensory organ commences causal sequences which culminate in both conscious and autonomic components of thermal homeostasis and generates awareness of our thermal milieu. Research on thermal innervation of the skin dates back over 120 years to the discovery of irregular warmth sensitivity of the skin and the proposition of morphologically distinct cutaneous receptor types for different sense modalities in the late 19th century (von Frey, 1895). Despite the long history of research, the understanding of thermosensation is far from comprehensive and many explanatory gaps persist in this field.

One such gap exists via the apparent paradox that despite our obvious reliance on peripheral thermosensation, the physiology dictating warmth detection in the skin seems to predict insensitivity. Strikingly, the fibers responsible for the transmission of innocuous warm signals occur at a density 3.5x lower than that of their cool-sensitive counterparts in the skin (Gerrett et al., 2015; Hensel, 1981). In one classic paper (Hallin et al., 1982) electrophysiological characterization of afferent C-fibers revealed that warm-specific fibers are a rarity even among this group of small-diameter, unmyelinated afferents. Of 125 C-fibers characterized, only five were found to be specific for detection of warming in the innocuous

range. The remainder of the C-fibers responded readily to moderately intense and noxious mechanical stimuli as well as heating in the noxious range (40-50°C). They are characteristically labeled *polymodal* C-nociceptors. In contrast to polymodal C-nociceptors which displayed large, complex receptive fields (RFs), the five warm-specific C-fibers (C-warm) studied were optimally activated by heat stimuli in the innocuous range (~32-40°C) and demonstrated small, dot-like RFs (*reviewed in* Torebjörk & Schmelz, 2005). The sparse distribution of warm-responsive afferents with small punctuate RFs ostensibly suggests that the skin would demonstrate properties of low warmth sensitivity in the innocuous range, an implication which is seemingly at odds with our reliance on peripheral thermosensation.

This may be partially explained by considering differences in energy costliness between homeothermic responses to inverse changes in temperature. Raising our core temperature has a high energy cost. To prevent excessive energy expenditure, rapid detection of cooling at the skin can serve to initiate preemptive autonomic and behavioral measures for thermal homeostasis. Human evolutionary descent from small, homeothermic mammals with high surface area to volume ratios that cause rapid radiative heat-loss (Louw, 1993) may also bolster our acuity for cool sensations. Conversely, reducing core body temperature is facilitated via evaporative cooling and the abatement of metabolic activity and thus requires comparatively little energy (Mitchell et al., 2018). Additionally, the most prominent environmental sources of heat, namely are solar radiation and ambient air temperature (Speakman & Król, 2010), typically persist over the span of whole days and stimulate the skin across a large spatial area. Thus, warming of the skin is most physiologically relevant when occurring across a wide area or persisting for a long period, as these types of stimuli are most likely to alter the body's core temperature in a significant way. Accordingly, homeostatic mechanisms for lowering core temperature likely experienced less selective pressure for early detection of spatially modest warming. Evidence of spatial and temporal summation of warmth sensation (Defrin et al., 2009; Marks & Stevens, 1971, 1973) suggests that naturally sparse innervation of the skin by warm fibers with small receptive fields may be the product of those selective pressures.

Despite evidence that increasing the size of a warm stimulus increases the salience of evoked warm sensations, conclusions are absent about altering stimulus configuration in the opposing direction. Even when examined independently of evidence for spatial summation, the seemingly obvious implication of sparse warmth fibers with small receptive fields would be the existence of naturally occurring gaps in the warmth sensitivity of the skin. However, publications investigating this possibility are sparser even than warm fibers themselves. Understanding how the history of theories regarding thermosensation and pain continue to drive modern research emphasizes not only the need but also the usefulness of research on punctuate warmth sensitivity.

Evolving theories of thermosensation and pain

Neural processes that mediate the relationship between stimulus and sensation have been pondered in one form or another since the days of Descartes. Among the range of sensory experiences humans encounter, pain is a particularly salient sensation and the clinical implications of its study made it an early target of empirical scientific inquiry. However, the scope of qualities and intensities attributed to pain and the difficulties parsing exactly what neural processes govern what aspects mean that the scientific understanding of pain is far from comprehensive. For the better half of the 20th century, scientists studying the mechanisms of pain largely fell into one of two opposing camps. Specificity theorists contended that pain is a specific sense (i.e., functionally and morphologically separate from other components of touch) and that differences in intensity and quality of pain could be attributed to distinct fibers. Pattern theorists, on the other hand, held that both painful and non-painful sensations could be transduced by the same fibers according to different patterns of activation.

Specificity theory has its roots in Johannes Muller's "law of specific nerve energies". In 1826, Muller contemplated the observation that stimulation of the optic nerve, either by light, pressure on the eye, or electrical stimulation, always elicited vision. From this, he posited that each of the five classical senses was served by distinct channels and that no matter the manner of stimulation, those channels would reliably produce a single type of sensation. Muller's idea was taken a step further in the late 19th

century when Blix, Goldscheider, Donaldson, and later von Frey published evidence of spot-like patterns of sensitivity of the skin (reviewed by Green, 2004 and Norrsell et al. 1999). These researchers identified spots on the skin where stimulation of various types would reliably result in a single sensation, either cool, warm, pain, touch, or itch. From this, it was proposed that each sub-modality of touch was transduced and perceived by distinct pathways (termed "labeled-lines") that span from the periphery to centers as high as the cortex. As with Muller's original idea, specificity theory stated that activation of a given pathway will reliably produce the same sensation, regardless of potential differences between activating stimuli.

Ironically, pattern theory can be traced back to Goldscheider, who was a part of the troupe that brought specificity theory to the forefront of pain research only years earlier. In his work studying tactile nerve fibers, Goldsheider concluded that three separate qualities (i.e., tickle, touch, and pain) could be encoded by a single fiber type using an intensity-based gradient (*reviewed in* Norrsell et al. 1999).

Specificity theory fared better against skepticism and persisted into the 20th century as the result of behavioral, electrophysiological, molecular, and genetic evidence demonstrating primary sensory afferents, specialized ion channels, and receptor subtypes that responded selectively to specific sensory stimuli (Ma, 2010; Perl, 2007). With time, however, studies also cast doubt on the degree to which either of the two prominent theories could account for the complexities of thermosensation, pain, and the relationship between them.

Between 1975 and 1990, multiple researchers reported that the use of ischemia or compression to block the activation of small-diameter, myelinated A-fibers resulted in activation of nociceptive C-fibers by innocuous cold and caused reports of heat or burning sensations (Mackenzie et al., 1975; Yarnitsky & Ochoa, 1990). This showed that A-fiber activation in response to innocuous cold is required for cold sensation and masks the simultaneous activation of C-nociceptors that would otherwise produce sensations typically associated with heat-evoked pain. Evidence of inter-afferent inhibition laid the

foundation for hypotheses that opposed the long assumed one-to-one relationship between receptor activation and sensation.

In 1994, Craig and Bushnell synthesized these ideas when they attributed a long-documented illusory pain phenomenon to central disinhibition between two spinothalamic tract neurons that receive input from distinct peripheral thermoreceptors. The thermal grill illusion of pain (TGI) is the sensation of burning pain produced by simultaneous and adjacent application of innocuous cool and warm stimuli, typically in the form of lateral bars (*first described by* Thunberg in 1894; *reviewed in* Green, 2004). Craig and Bushnell built on the earlier work of Mackenzie et al., Yarnitsky & Ochoa, and others by identifying these two separate spinothalamic tracts as the locus for Aδ inhibition of C-nociceptor activity during innocuous cooling. They hypothesized that in the TGI, C-warm fiber signaling in response to warm bars inhibits cool A-fibers, thereby disinhibiting C-nociceptor firing and unmasking the burning pain sensation they induce. Importantly, researchers followed up on this study by using positron emission tomography (PET) to perform functional imaging of the brain during the TGI. They found that the TGI, but neither of its innocuous components individually, correlated with activity in the same structures of the anterior cingulate cortex that are active during noxious heating or cold (Craig et al. 1996).

Beyond corroborating the idea that low-threshold (i.e., innocuous) thermoreceptors and nociceptors combine in thermosensation, it became apparent that the interdependence between these components is fine-tuned to the extent that a simple deviation from normality such as concurrent warming and cooling can result in pain. The implications of researching the underlying mechanisms of thermosensation were more pervasive than ever before. As the 20th century drew to a close it was clear that novel research tools for exploiting aberrances in the relationship between innocuous and noxious thermosensation were needed to shed the dogmatic theories of the past 100 years.

In 1998, Barry Green and Alberto Cruz published a paper that presented just such a tool and appeared to open the enormous potential for studying thermosensation and pain. While running preliminary tests for a different study, Cruz noted that a 4.84 cm^2 Peltier thermode delivering a stimulus

of 40°C elicited no sensation of warmth at various locations on the body. The event was replicated in other laboratory members and led researchers to characterize these relatively large *warmth-insensitive fields* (WIFs) using rigorous psychophysical methods. While their published findings presented substantial implications for the study of thermosensation, particularly for better understanding the relation to nociception, these gaps in warmth sensitivity have remained almost entirely unstudied for over 20 years.

The WIF: discretizing innocuous and noxious thermosensation

One question of particular interest for better understanding how thermosensation and pain interact is whether conduction via specialized nociceptive channels (namely, C and A-δ fibers) is sufficient to encode the various characteristics of thermal pain or if the integrated activity of both nociceptive and non-nociceptive systems is required (Defrin et al., 2002). While psychophysical methods for studying each term of this relationship are well-developed, the field currently lacks sufficient means of studying their connection. However, WIFs may provide researchers with a unique opportunity.

To investigate the influence of one sensory system on another, experimenters must identify and develop paradigms that allow them to selectively isolate the activation of each system across a range of stimulus energies. In the case of innocuous thermosensation and nociception, this task is made difficult by the overlap of psychophysical thresholds for activation. By stimulating the skin with a heat stimulus that elicits pain, fibers that detect changes in temperature below the nociceptive range are also recruited. Even if firing in these innocuous thermoreceptors trails off or ceases at higher temperatures, their initial activation may have important effects on nociception. Thus, isolating activation of thermally responsive nociceptors requires intricate methods of inhibiting or tuning out concurrent warm fiber activation. In mice, genetic knockout of thermally responsive subtypes of transient receptor potential ion channels (TRP channels) in primary sensory afferents provides the option of creating different “thermosensory phenotypes” and adequately reduces warm fiber firing. However, the understanding of exactly which TRP channels are activated in innocuous and noxious thermosensation is incomplete (Wang & Siemens, 2015).

In humans, ischemic nerve block and selective attenuation of fiber types are used to non-invasively and reversibly induce similar thermosensory phenotypes (Glencross & Odenfield, 1975; Harper & Hollins, 2014). However, these methods are prone to incomplete isolation of the perceptual category of interest (Frustorfer, 1984). When selective activation/inactivation of a given system is unreliable, the gold standard in neuroscience is to examine behavioral changes in cases where a lesion to the system of interest has altered normal processing. Psychophysics studies done in patients with spinal cord injury resulting in either partial or complete loss of thermosensation have provided unique insight on the relationship between thermal pain and innocuous thermosensation (Defrin et al. 2002). Researchers found that the quality and threshold of both hot and cold thermal pain was dependent on the sensitivity to non-noxious warm and cool stimuli. Lesions to the spinal cord undeniably result in specific thermosensory phenotypes that are useful for isolating different components of thermosensation and nociception. Conclusions from such studies also run the risk of overlooking the complex dependence of thermosensation and nociception on ascending and descending spinal tracts. Damage to these tracts could have cascading effects on both excitatory and inhibitory signaling in central and peripheral components of the pathways for pain and thermal sensation. Between-subject reproducibility regarding the location and extent of the lesion as well as potential up/downstream effects on the thermosensory and nociceptive networks are important methodological concerns that are very difficult to control for. Additional practical concerns include the limits placed on data collection when working with clinical populations.

Vital for human-subjects research on mechanisms of interaction between thermosensation and nociception is a non-invasive method of reproducibly isolating these components. Identifying and characterizing such a method in healthy populations would have the added benefit of increasing data collection. Exploiting WIFs, pronounced sites of naturally occurring warmth insensitivity on the skin, is a promising method that may meet these requirements.

Upon discovering WIFs in 1998, Green and Cruz attributed localized inability to detect warming below the noxious range (i.e., temperatures as high as 42°C) to a regional lack of cutaneous C-warm

fibers. This was consistent with previous work documenting the sparsity of these fibers (Hensel, 1981; Stevens & Marks, 1971) and their small RFs (Hallin et al., 1982). They set out to test this theory by identifying and characterizing other thermosensory attributes of WIFs in healthy individuals. Their findings (summarized in Table 1 below) substantiate the sparsity of low-threshold warm fibers with small RFs. Most crucially, they point to WIFs as potential areas where innocuous warm and nociceptive heat responses can be functionally discretized to research the mechanisms governing their relationship.

TABLE 1. Summary of main findings, Green & Cruz, 1998.

Psychophysical finding	**Conclusion**
At least one WIF was located on the forearms of 65% of young, healthy subjects screened. WIFs ranged in size from $5cm^2$ to as large as $15cm^2$.	Cutaneous innervation by warmth sense is remarkably punctuated and sparse, corroborating electrophysiological reports of low occurrence of C-warm fibers with small RFs in human skin.
Detection thresholds for heating were significantly higher in WIFs than in adjacent fields, by a margin of 7.6 °C.	Thresholds for heating in WIFs reflect the activity of C-nociceptors rather than low-threshold C-warm fibers.
Thresholds for heat pain were significantly higher in WIFs than in adjacent WSFs, by a margin of 2.0°C.	Activity of innocuous warm fibers modulates nociceptive thresholds for heat.
Ratings of perceived intensity of heating for temperatures 37-44°C were lower in WIFs than in adjacent WSFs. In WIFs, temperature spanning into the noxious range (42-44°C) evoked innocuous sensations, no stronger than sensations evoked by a mere 37°C in adjacent WSFs.	Activity of innocuous warm fibers contributes to the perceived intensity of heating. The continuation of this trend into the noxious range suggests that supraliminal stimulation of C-nociceptors can yield weak and innocuous sensations.
Sensitivity to cooling was not different in WIFs.	WIFs are not anomalous areas of compromised sensory function.

TABLE 2. A summary of Green and Cruz's findings and the conclusions drawn from them.

WIF = *warmth-insensitive field*
WSF = *warmth-sensitive field*

In the 23 years since these findings were published, research on thermosensation and pain has continued to provide insight. Mechanisms that endow these afferent fibers with their sensory profiles have been predominantly attributed to the relative expression of TRP channel subtypes at receptor terminals. Fibers may possess the ability to discriminate between several stimuli and respond accordingly based on which subpopulations of TRP channels are activated (Voets et al., 2004; Zheng, 2013). Attempts to correlate TRP subtype activation to specific sensations typically investigate polymodal C-fiber subtypes, which are capable of transmitting combinations of thermal and mechanical information in the innocuous and noxious range (Campero et al., 2009; Dubin & Patapoutian, 2010). While this may seem to favor a reversion towards pattern theory and the discrimination of several stimulus characteristics by single fiber types, contemporaneous studies highlight the importance of integration and inhibition between sensory neurons in generating and conditioning peripheral sensation (Bokiniec et al., 2018; Ma, 2010, 2012; Romanovsky, 2007).

Despite this, WIFs have been incredibly understudied and consequently underutilized as a research method for studying nociception in the absence of low-threshold warm signaling. In only two of 13 publications that cite Green and Cruz's 1998 paper do researchers actively seek to identify WIFs in human subjects (Defrin et al., 2009; Wager et al., 2006). Even still, Wager et al. only remark that WIFs were identified to actively avoid thermosensory testing in these sites (citation metrics obtained from *NCBI's National Library of Medicine*). Further investigation of WIFs is needed to understand their thermosensory response profile and provide insight into how innocuous and noxious components of thermosensation inform perception.

The aim of the present study is, therefore: (1) to assess factors that may influence the occurrence of warmth-insensitive fields (WIFs) in healthy subjects, (2) to measure and compare the thermosensory response profile of WIFs and warmth-sensitive fields (WSFs), (3) to utilize the WIF to better understand how the innocuous warm sense informs sensation of noxious thermal stimuli.

Methods

Participants

Subjects were recruited from the Emory University Atlanta campus and surrounding areas. The Emory University Institutional Review Board (IRB) approved all testing procedures. Subjects provided written informed consent before the start of the experiment. The study included a total of 12 healthy participants, 9 of whom were women. All subjects were between the ages of 18 and 21, with a mean age of 19.25. Potential study participants were excluded if they had skin lesions, histories of nerve damage, impaired nervous function, or other illness (i.e., diabetes) or injury that could affect sensory function. All subjects completed one test session. Sessions had a maximum length of two hours, per the Emory University IRB's guidelines for human-subjects research during the COVID-19 pandemic.

Equipment

Measurements of skin temperature were recorded using a TPI 368 Close-Focus infrared thermometer (Test Products International, Beaverton, Oregon, USA). All psychophysical test paradigms and visual interfaces were designed and run in LabVIEW 2019 (National Instruments, Austin, Texas, USA). Thermal stimulation defined by LabView programs was administered via direct connection with a Medoc TSA-II Neurosensory Analyzer (Medoc Ltd., Ramat Yishay, Israel). The thermal contactor had a surface area of 2.56 cm^2 (1.6 x 1.6 cm).

WIF Identification

A grid of 2.56 cm^2 (1.6 x 1.6 cm) squares was marked on the volar surface of the participant's left forearm to reproducibly apply the thermode to delineated areas of skin (fields). The center reference line of the grid was aligned along the vector between the participant's middle finger and elbow. Based on evidence that low-threshold mechanoreceptors structurally associated with hair follicles/erector pili (Glatte et al., 2019) demonstrate temperature-dependent changes in firing (Ackerley et al., 2014), a high-resolution photo was taken of the volar forearm with the grid applied. This was later used to identify and assess potential effects of the presence/absence of hair on thermosensory phenotype. Three measurements

were taken of the arm. Circumference of the wrist and upper forearm were taken at the distal and proximal boundaries of the grid. This was done to test the *a priori* hypothesis that distension of the epidermis resulting either from normal growth or other factors such as weight gain may have some effect on the absolute spatial density of thermosensory afferents. The subject was then asked to stand with their left arm outstretched, parallel to the floor to measure the distance from the distal edge of the grid to the midline of the cervical spine (i.e., the "gridspan") to assess how thermal sensitivity may vary as a function of distance from the midsagittal plane (Gerrett et al., 2015).

Following measurements, participants placed their arm on a memory foam pad with the volar surface and the grid facing upwards. This was done to minimize the subject's exposure to external temperature stimuli (e.g., a table surface that may be perceived as cold). Subjects were then familiarized with thermoneutral sensations and sensations of warmth/heat by applying the thermode to a randomly selected field of the grid. The thermode was set to either skin temperature (measured in the corresponding field using the IR thermometer) or to increase from skin temperature to 40.5°C at a rate of 5°C/s. Upon reaching the 40.5°C target temperature, the thermode maintained that temperature for 3 seconds before returning to skin temperature at a rate of -5 °C/s. For both stimulus types, subjects were asked to provide a verbal rating of the stimulus intensity from 0-100, with 0 indicating "no thermal sensation at all" and 100 indicating "the hottest sensation you can imagine from this stimulus". All subjects reported a rating of 0 for the skin temperature stimulus.

Participants were asked to use the same scale to provide a rating of thermal intensity for the 40.5°C ramp-and-hold stimulus at each field within the grid. Measurements of skin temperature were taken at points along the grid's center reference line for each set of three rows. The thermode was presented first at the participant's leftmost field in the row closest to the hand. Testing progressed row-wise in this way from the wrist up towards the elbow. The experimenter allowed a minimum of 2 seconds to pass between the end of one stimulus and the start of the next. Skin temperatures and intensity ratings were recorded in a spreadsheet designed to emulate the shape of the grid.

Skin areas in which subjects provided a thermal intensity rating of 0 in response to the 40.5°C stimulus were classified as WIFs. In subjects where a WIF was not located within the bounds of the grid, the area with the lowest thermal intensity rating was cataloged as the minimally warm-sensitive field (WSF_{min}). In all subjects, thermal testing was conducted first in the WIF or WSF_{min} and subsequently repeated in the field of the grid where the subject provided the highest thermal intensity rating, i.e., the maximally warmth-sensitive field (WSF_{max}). Throughout testing, subjects completed a demography survey, the Pain and Negative Aspect Schedule (PANAS) (Watson, Clark, & Tellegen, 1988), and the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983). These tests were administered based on links between depression and somatic symptoms (Simon et al., 1999) as well as the co-occurrence of chronic pain with anxiety and mood disorders (McWilliams, Cox, & Enns, 2003).

A. WIF 2

Elbow			
20	20	10	5
10	20	15	20
10	5	25	25
0	20	20	20
5	5	20	20
0	5	20	20
5	0	20	10
5	30	30	25
20	0	10	20
10	20	5	20
Wrist			

B. Non-WIF 7

Elbow			
60	63	60	60
40	55	52	50
15	38	30	25
12	45	30	22
33	25	42	40
16	37	20	23
20	38	33	25
39	33	30	37
28	35	30	27
25	9	28	24
22	27	12	25
18	26	20	22
Wrist			

C. WIF 3

Elbow			
30	30	25	20
15	35	30	20
35	20	20	0
25	35	15	10
30	20	20	5
30	35	25	10
0	25	10	0
45	30	35	15
25	40	45	45
30	45	35	40
Wrist			

Figure 1. Mapping the warmth sensitivity of the left volar forearm.

Two subjects with WIFs (A&C) and one subject where a WIF was not identified (B). In subjects who had more than one WIF or more than one field with the same minimum/maximum intensity rating, the field used as a test site was selected at random.

Test sites are indicated by bolded borders. *Grey = WIF; Pink = WSF_{min}; Dark Red = WSF_{max}.*

Threshold measurements

Thresholds for cool, warm, cold pain, and heat pain were assessed sequentially using either ascending or descending method of limits (MOL) (Gescheider, 1997). Subsequently, thresholds for cool and warm were assessed again, this time using a temporal forced-choice variation on the *staircase* MOL in which stimuli vary up and down by small increments to narrow in on the subject's threshold for detection. To reduce the potential effects of repeated stimulation on response profiles of thermosensory receptors and afferents (Vriens, Nilius, & Voets, 2014), the next threshold measurement task in the sequence was not initiated until skin temperature fell within 0.5°C of the temperature recorded at the outset of testing for that field. All absolute threshold values obtained using MOL were calculated by averaging the temperature of detection across three successive trials. Based on evidence that ambient temperature has effects on pain and temperature perception (Strigo, Carli & Bushnell, 2000), all thresholds are reported here as the difference between the absolute threshold and the subject's skin temperature (ΔT) recorded immediately before thermal testing.

To determine the thresholds for cold and warm detection, three successive ramps of steadily increasing or decreasing temperature were administered. The Peltier thermode was applied to the field manually by the experimenter such that contact with the skin was as consistent as possible in location and pressure. Beginning at skin temperature, the thermode increased or decreased temperature at a rate of ±0.5 °C/s. This rate of change was chosen based on evidence that using slow rates of temperature change in MOL threshold reduced intra-individual differences in threshold and minimized measurement artifact (Palmer et al., 2000). Subjects were given the task of pressing a button at the moment they first perceived a cool or warm sensation. Pressing the button caused the computer program to record the thermode temperature and return to the baseline temperature at the maximum rate of temperature change which the Medoc system was capable of (approximately ±8-10°C/s). Successive stimuli were separated by inter-stimulus intervals (ISIs) between 10 and 15 seconds. The exact length of the ISI within this range was randomized to prevent response errors caused by habituation and anticipation of sensation onset.

A similar protocol was employed to determine thresholds for cold pain and heat pain. Three successive increasing or decreasing thermal stimuli were again administered with a rate of change of ±0.5 °C/s. Subjects were instructed to press the button at the moment that the sensation first became painful. Randomized ISIs between 10 and 15 seconds were used to minimize habituation and anticipation but also to avert potential tissue damage. Limits of 0 and 50°C were set for cold and hot stimuli, respectively. If the subject did not signal the onset of pain sensation at temperatures between these bounds, the minimum or maximum temperature was recorded and the thermode was returned to baseline temperature as rapidly as possible.

To examine the possibility that the thermal response profiles of WIFs are exacerbated by response biases that are inherent in MOL threshold measurement, cool and warm thresholds were measured once more using a temporal two-alternative forced-choice (2AFC) variation of the staircase MOL. Thresholds determined with this method are not subject to the same effects of reaction time that are seen in MOL (i.e., the latency of the button press). It was initially proposed that the difference in thresholds determined using the two methods could be used to assess reaction time in response to cooling and warming in WIFs and WSFs to provide a measure of peripheral conduction velocity (Susser, Sprecher, & Yarnitsky, 1999). However, the ability to conduct these tests was limited by the length of the study session.

Subjects were instructed to pay careful attention and identify which of two, 5-second intervals (A or B) contained a 3-second temperature pulse. The duration of each interval was signified on a computer screen and accompanied by an auditory queue. In cases where the subject was unsure or could not discriminate the stimulus, they were asked to make their best guess. This repeated for a total of 20 trials each for warm and cool.

The test utilized a 3-down, 1-up decision rule. If the subject correctly identified the stimulus-interval on 3 successive trials, then the stimulus temperature for the next trial would decrease in salience (make a downward step). For every incorrect response, the temperature for the next trial would increase in salience (an upward step). The step value was 0.5°C for the first 10 trials and 0.2°C for the last 10 trials. For all cool tasks and the warm task in WSFs, the baseline temperature between pulses was set to skin

temperature. For warm tasks in WIFs, a baseline of 39°C was used to ensure that subjects could not discriminate the trial containing the heat pulse by a sensation of coolness upon the thermode returning to baseline. For all trials, the target temperature for the first pulse was 1°C closer to baseline than the subject's warm or cool threshold determined using MOL. Thresholds were calculated as the average temperature for the last 4 trials.

Measures of Pain Intensity

To determine the perceived intensity and quality evoked by stimuli in the nociceptive range, the method of magnitude estimation (ME) was employed for 45°C and 48°C stimuli (Stevens, 1956). A temperature of 39°C was used as the baseline for these tests in all field types. This was done to maximize the activation of any C-warm fibers that may have been present in the field immediately before each of three successive stimuli. Thermode temperature increased from baseline temperature to 45 or 48°C at a rate of 5°C/s. The target temperature was maintained for 5 seconds before returning to baseline. Successive trials were separated by ISIs of 20 seconds. Subjects rated the intensity of the pain evoked on a 0-100 scale, where 0 represented "no pain at all" and 100 represented "the most intense pain imaginable from this stimulus".

Measures of Pain Quality

Subjective reports of the quality of heat-evoked pain were recorded to determine the extent to which the low-threshold system for detecting warm may inform concurrent or subsequent C-nociceptor activity to discriminate the "hot" quality of pain from the other pain types which these polymodal fibers transduce (i.e., mechanical, cold). For all tests where stimulus intensity extended into the nociceptive range (MOL pain thresholds and ME) subjects were tasked with describing the quality of each stimulus that they deemed to be painful. For each nociceptive trial, subjects were instructed to select one or more adjectives from the following list to describe their paint: cold, cool, aching, sharp, stinging, neutral, warm, hot, burning. List items were presented on a sheet of paper and were positioned in no particular sequence or rank. Trials in which a subject did not report pain (e.g., in MOL for pain thresholds, if the upper or

lower temperature limit were insufficient for pain detection; in ME, if subjects provided a pain rating of 0) were recorded as *"neutral"*. For heat-evoked pain, the proportions of trials in which a subject described the pain as *"hot"* were compared between field types.

The same task and list of descriptors were given in trials of cold pain MOL to assess whether the low-threshold warm system had any bearing on the quality of cold-evoked pain. Green and Cruz reported that thresholds for cooling were normal in WIF and adjacent WSF areas, but did not investigate any metrics about cold pain. Based on their incidence in WIF and WSF areas, the proportions of trials where subjects described the pain as "cold," "burning," and "hot" were compared. Reports of the latter two terms are commonly referred to as *paradoxical heat* (PH) (Susser, Sprecher, & Yarnitsky, 1999).

Data Analysis

Despite Green & Cruz's documentation of WIFs in healthy, young individuals and due to the lack of literature regarding variables that may influence the occurrence of WIFs, an abundance of caution was used when assessing differences in thermosensory phenotype between WIF and Non-WIF subject groups. Differences between numerical variables concerning demography, arm measurements, and field parameters (Table 2) as well as detection and pain thresholds (Figure 2), and intensity ratings (Figure 3), were assessed by analyzing the difference of means. Between-group tests (WIF v. Non-WIF subjects, see Table 3) treated the data as independent, even for data collected in the WSF_{max} of both groups Within each group, between-field tests (WIF or WSF_{min} v. WSF_{max}, see Figures 2-5) treated the data as paired or related (dependent).

Given the small sample sizes for both groups (WIF, $n = 5$; Non-WIF, $n=7$), the normality of distribution for all numerical variables was assessed using z-scores for skewness and excess kurtosis (Kim, 2013). All variables were normally distributed with the exception of WSF_{max} warm thresholds in the WIF group. For normally distributed variables, the Independent-samples T-test was used between-group (Tables 2 &3) and the paired-sample T-test was used between-field (Table 2, *Field Parameters*; Figure 2). For the non-normally distributed variables, non-parametric alternatives for independent and paired samples T-tests were used. The Wilcoxon-Mann-Whitney test was used between-groups (Table 3)

and the related-samples Wilcoxon signed rank test was used between-fields (Figure 2A, *Warm*). The latter test was also used for between-field comparison of pain intensity ratings provided during ME (Figure 3), as the distribution of the between-field differences in these data were not normally distributed.

Associations between all categorical variables (Tables 2 &3, Figures 4 & 5) were assessed using Fisher's exact test. All statistical tests were run in SPSS Statistics v.27 (International Business Machines Corporation, Armonk, New York, USA). All plots were generated using MATLAB v.R2020b (MathWorks, Natick, Massachusetts, USA).

Results

At least one WIF was identified on the left volar forearm of 5 out of 12 subjects screened (41.67%). Among these subjects, the number of WIFs identified ranged from 1 to 17. It is worth noting that the occurrence of 17 WIFs seems to constitute an outlier, even when accounting for the relatively large size of the subject's forearm. The next-highest number identified was four. The subject with 17 recorded WIFs appeared to show abnormally low warmth-sensitivity across the entire forearm, providing a maximum thermal intensity rating of only four on the scale from 0-100. Nonetheless, the field type (WIF vs. WSF_{max}) reliably predicted the difference in warmth detection thresholds measured in the two sites and so the subject's data were included in the study.

The identification of WIFs in the present study occurred less frequently than expected based on preliminary pilot testing among lab members as well as Green and Cruz's reports of WIFs occurring in 65% of subjects screened. This difference can likely be attributed to confining our search to the volar surface of a single forearm.

TABLE 2. Between-group comparisons of demography and arm measurements; bidirectional comparisons of field parameters.

	Variable	WIF (*n*=5)	Non-WIF (*n*=7)	Significance
Demography	**Age**	20.00 (± 0.45)	18.71 (± 0.29)	*p = 0.029*
	Sex			
	Female	60.0%	85.7%	*p = 0.523*
	Male	40.0%	14.3%	
	Dominant Hand			
	Right	100.0%	71.4%	*p = 0.470*
	Left	0.0%	28.6%	
	PANAS Score			
	Positive Affect (0-50)	30.80 (± 2.94)	34.00 (± 1.73)	*p = 0.341*
	Negative Affect (0-50)	17.60 (± 4.03)	22.29 (± 9.86)	*p = 0.421*
	HADS Score			
	Anxiety (0-21)	7.20 (± 3.51)	7.29 (± 1.57)	*p = 0.983*
	Depression (0-21)	4.80 (± 1.99)	2.57 (± 1.17)	*p = 0.327*
Arm Measurements	**Gridspan** (*cm*)	71.50 (± 3.78)	66.93 (± 0.61)	*p = 0.296*
	Wrist circumference (*cm*)	16.07 (± 0.92)	15.18 (± 0.32)	*p = 0.323*
	Forearm circumference (*cm*)	25.82 (± 1.82)	23.79 (± 1.10)	*p = 0.335*
Field Parameters	**Skin Temperature** (*°C*)			
	WIF or WSF_{min}	32.38 (± 0.37)	31.66 (± 0.61)	*p = 0.381*
	WSF_{max}	32.54(± 0.68)	31.97 (± 0.20)	*p = 0.459*
	Significance	*p = 0.741*	*p= 0.559*	
	Hair Presence			
	WIF or WSF_{min}	40.0%	0.0%	*p = 0.152*
	WSF_{max}	20.0%	0.0%	*p = 0.417*
	Significance	*p = 1.000*	*p = 1.000*	

TABLE 2: All continuous variables are represented as μ (± SEM). All categorical variables are listed as a percent value, representing frequency of occurrence within their group (column). Refer to *Methods: Data Analysis* for information regarding statistical tests.

WIF = *warmth-insensitive field*
WSF_{min} = *minimally warmth-sensitive field*
WSF_{max} = *maximally warmth-sensitive field*

One aim of the present study was to examine variables that could potentially influence the occurrence of WIFs. The result of these investigations is summarized in Table 2, above. Among demographic variables tested, only age was shown to be significantly different between WIF and Non-WIF subjects. This result was unexpected, as all participants were between 18 and 22 years of age, a

range across which significant differences in warmth sensitivity are not thought to occur (Stevens & Choo, 1998). Potential confounding factors in this determination are considered in the *Discussion* section.

An analysis of forearm and wrist circumference, as well as the distance of the grid from the midsagittal plane, revealed that these were not significant factors influencing the occurrence of WIFs among our study sample. However, the subject who had 17 documented WIFs also had the largest value of these 3 measurements across the entire study cohort. Further research regarding the effects of skin surface area on warmth sensitivity and the occurrence of WIFs is needed.

The presence of hair and the skin temperature of test sites were assessed following their identification to investigate the possibility that these factors, which were observed to vary across the forearm during pilot testing, affect regional warmth sensitivity. Green and Cruz did not specify which region of the forearm where WIFs were most commonly identified. Consequently, the hairiness and skin temperature of these fields were also unreported. In the present study, neither hair presence nor skin temperature was shown to have significant differences between fields (WIF or WSF_{min} v. WSF_{max}) within groups nor between groups (WIF v. Non-WIF) (Table 2). Thus we ruled out the possibility that warmth sensitivity or the occurrence of WIFs were influenced by hair follicle-associated low-threshold mechanoreceptors which may encode small amounts of thermal information (Ackerley et al., 2014; Glatte et al., 2018). We similarly ruled out that variations in skin temperature between fields or between groups had a significant effect on warmth sensitivity or later determinations of thresholds, pain intensity, and pain quality (Wu et al., 2001).

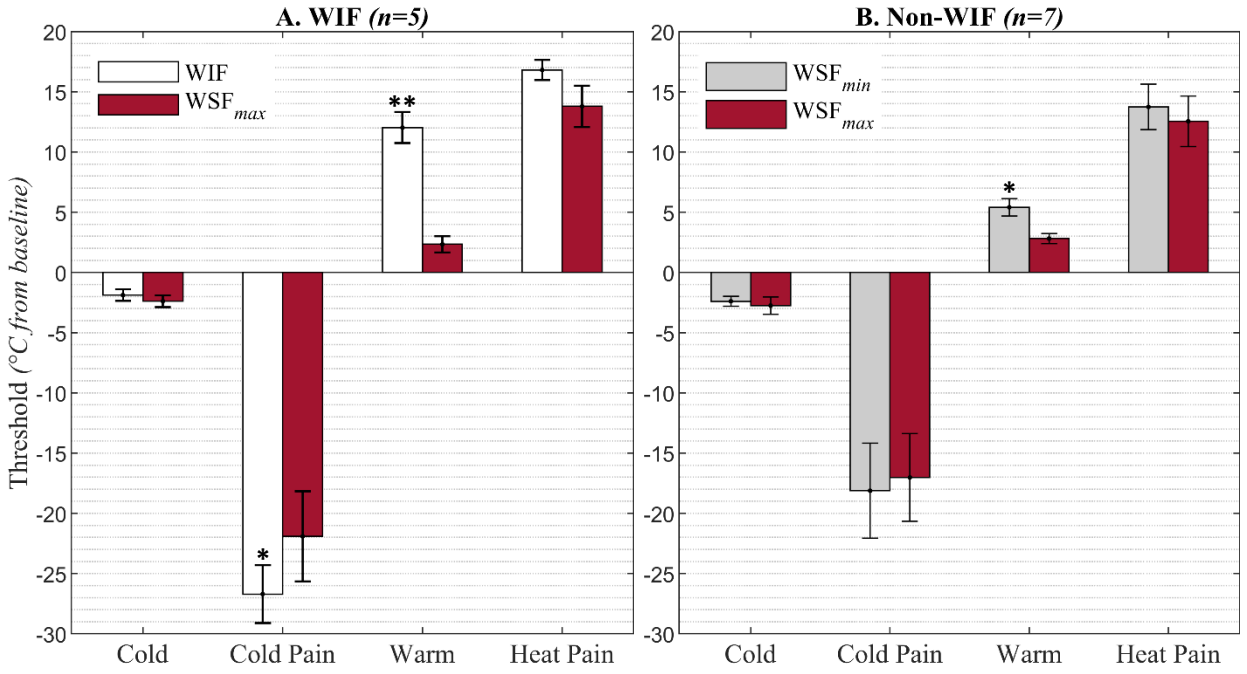

FIGURE 2: Average thresholds for the detection of cold, cold pain, warming/heating, and heat pain in the WIF (A) and Non-WIF group (B). Bars represent mean threshold for each thermosensory modality (determined using MOL) as the change in temperature (°C) from baseline; error bars represent standard error of the mean (μ ± SEM).

Note that thresholds are reported as the difference from skin temperature. In 2A, a significant difference is indicated (*) between cold thresholds in WIF and WSF_{max} (Paired samples T-test, *p = 0.45*) and also indicated (**) between warm thresholds in WIF and WSF_{max} (Paired samples T-test, *p= 0.003*). In 2B, significant difference is indicated (*) between warm thresholds in WSF_{min} and WSF_{max} (Related-Samples Wilcoxon signed rank test, *p = 0.043).*

Refer to *Methods: Data Analysis* for information regarding statistical tests. For between-group significance values, see *Table 3.*

WIF = *warmth-insensitive field*
WSF_{min} = *minimally warmth-sensitive field*
WSF_{max} = *maximally warmth-sensitive field*

Within both WIF and Non-WIF groups, field type had a significant effect on warm detection thresholds. In WIF subjects (Figure 2A), the average warm threshold was 12.02 (± 2.87)°C above baseline temperature. When compared with the average heat pain threshold in WSF_{max} of the same subjects (13.79 ± 3.84°C), the difference was not significant (Independent samples T-test, *p = 0.538*). This finding replicates that of Green and Cruz (1998) and reinforces their hypothesis that detection of heating in WIFs is facilitated by C- nociceptors due to the lack of low-threshold C-warm fibers.

In contrast to the findings of Green and Cruz, between-field differences in heat pain thresholds were not determined to be significant within either group. However, mean values in both WIF (Figure 2A) and Non-WIF (Figure 2B) subjects may suggest an emergent trend that could become significant as more data is collected. Notably, cold pain thresholds were significantly lower in WIFs than in WSF_{max} (Figure 2A, Paired sample T-Test, $p = 0.045$). This finding is notable as it is the first known report of altered perception of cold pain in WIFs.

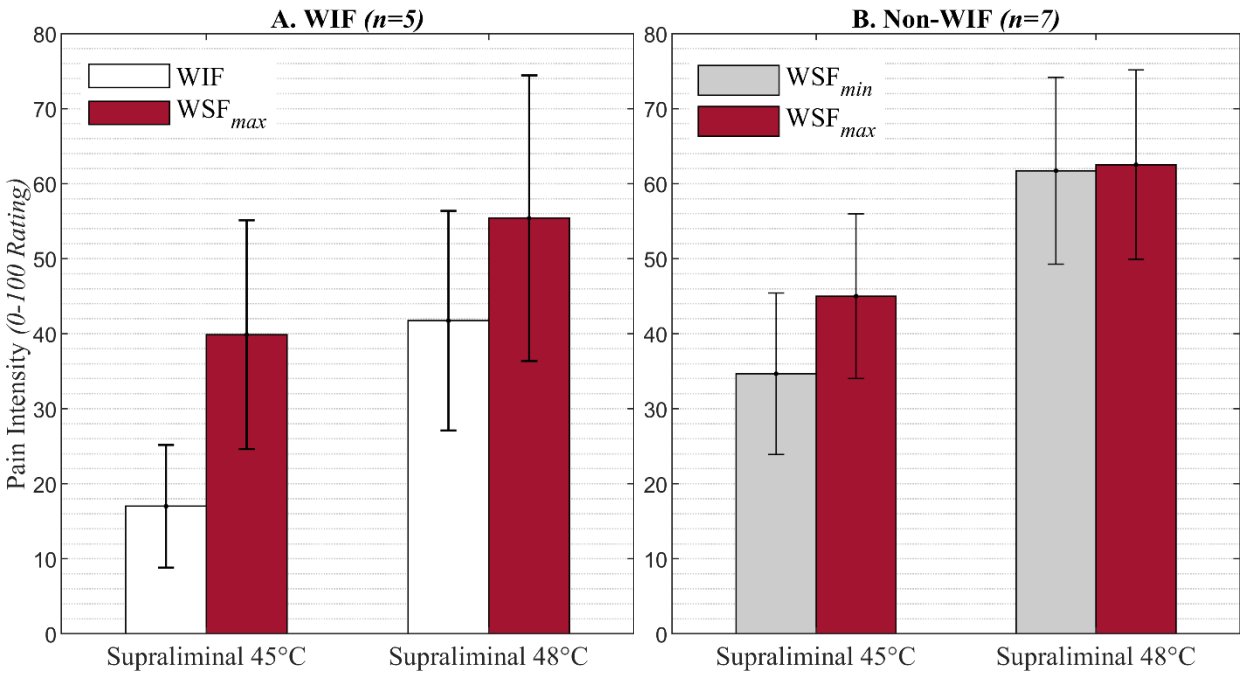

FIGURE 3: Average intensity ratings of heat-evoked pain in the WIF (A) and Non-WIF group (B). Bars represent mean pain intensity rating for magnitude estimation using supraliminal temperatures of 45°C and 48°C; error bars represent standard error of the mean ($\mu \pm SEM$).

In both groups, differences between fields did not demonstrate significance at threshold (Heat Pain) or in either magnitude estimation task (Supraliminal 45°C and 48°C) (Related-samples Wilcoxon signed rank test, $p > 0.05$).

Refer to *Methods: Data Analysis* for information regarding statistical tests For between-group significance values, see *Table 3*.

WIF = *warmth-insensitive field*
WSF_{min} = *minimally warmth-sensitive field*
WSF_{max} = *maximally warmth-sensitive field*

Pain intensity ratings of supraliminal nociceptive heat stimuli were not significantly different across between-fields (Figure 3) or between-groups (Table 3). A lack of statistical significance can be attributed to the large variation around the mean for these data. However, as is the case with heat pain thresholds, a non-significant trend suggests that lower warmth sensitivity may reduce the perceived intensity of nociceptive heat stimuli. More data is required to determine if this emergent trend would become significant across a larger sample. Additionally, implementation of a visual analog scale (VAS) for rating painfulness in these tasks may help subjects develop a more uniform concept of the rating scale and could reduce between-subject variations in reporting (Haefeli & Elfering, 2005).

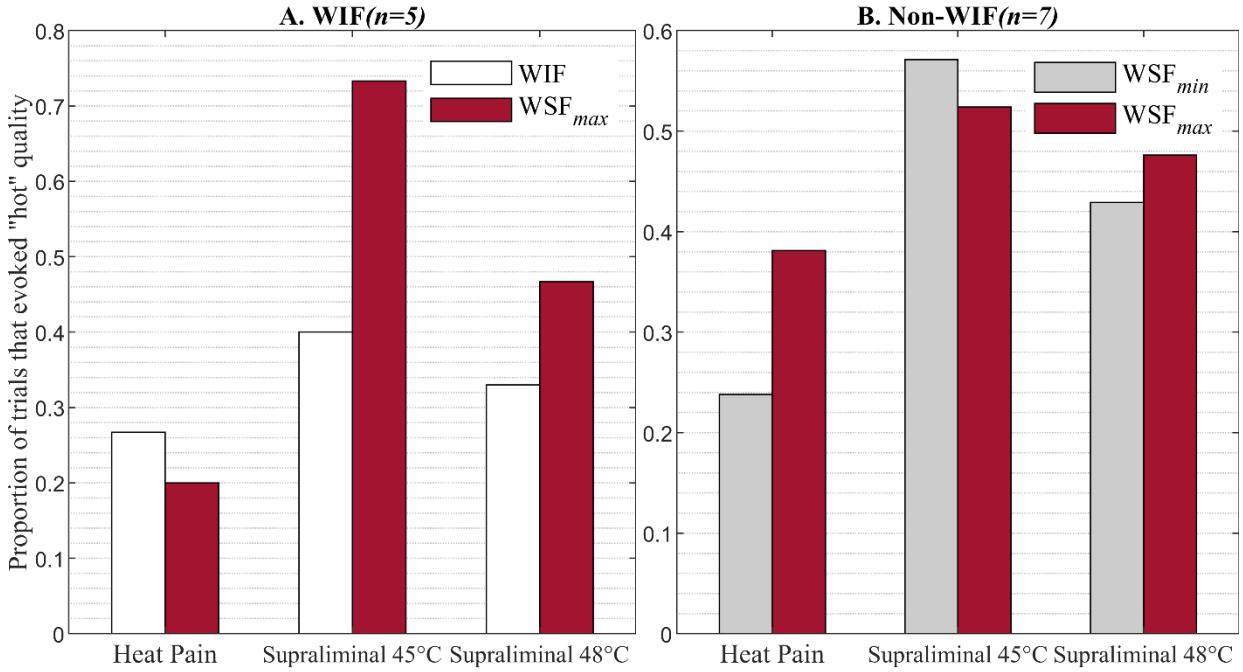

FIGURE 4: Thermal quality of heat-evoked pain in the WIF (A) and Non-WIF groups (B). Bars represent the proportion of trials where subjects described the pain as *"hot"*.

Within both groups, differences between fields failed to demonstrate significance at threshold (Heat Pain) or in either magnitude estimation task (Supraliminal 45°C and 48°C) (Fisher's Exact Test, $p > 0.05$).

Refer to *Methods: Data Analysis* for information regarding statistical tests. For between-group significance values, see *Table 3*.

WIF = *warmth-insensitive field*
WSF_{min} = *minimally warmth-sensitive field*
WSF_{max} = *maximally warmth-sensitive field*

Warmth sensitivity of a field did not have a significant effect on the proportion of trials in which heat-evoked pain was described by the subject as "hot" in either WIF or Non-WIF groups (Figure 4). This suggests that integration of activity from the low-threshold warm system may not be required for polymodal C-nociceptors to distinguish between types of pain.

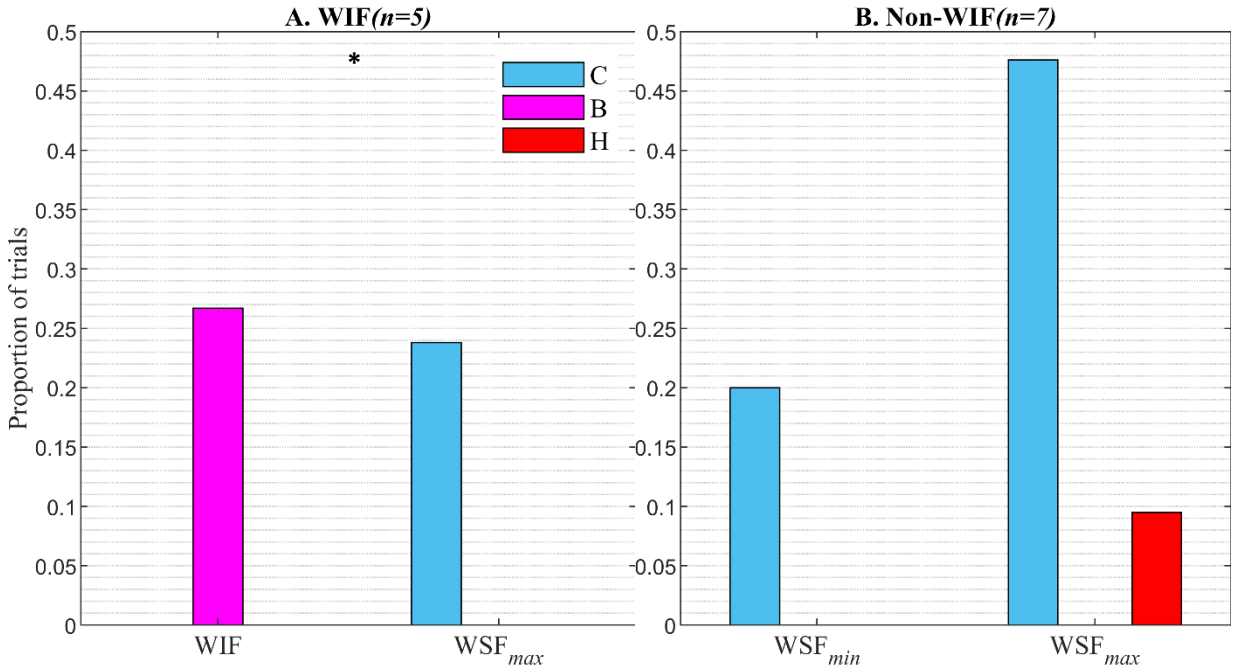

FIGURE 5. Thermal quality of cold-evoked pain in the WIF (A) and Non-WIF groups (B). Bars represent the proportion of cold pain threshold trials (using MOL) for which the subjects described the pain as either "cold", "burning", or "hot".

Nociceptive cold (using MOL) evoked a relatively high rate of reports of "burning" and no reports of "cold" in WIFs. This represented a significant difference (*) from ratings provided in the WSF_{max} within the same group (Fisher's exact test, $p = 0.024$). The same difference was not shown between fields in Non-WIF subjects (Fisher's exact test, $p = 0.07$), though reports of cold sensation were notably less frequent in the WSF_{min} than in the $WSF_{max.}$ Subjects reported two incidences of a hot sensation in the WSF_{max} of Non-WIF subjects.

Refer to *Methods: Data Analysis* for information regarding statistical tests. For between-group significance values, see *Table 3*.

WIF = *warmth-insensitive field*	C = *"cold"*
WSF_{min} = *minimally warmth-sensitive field*	B= *"burning"*
WSF_{max} = *maximally warmth-sensitive field*	H= *"hot"*

In stark contrast to the findings shown in Figure 4., robust differences in the quality of cold-evoked pain descriptors were present between the WIF and WSF_{max} of subjects in the WIF group (Figure 5A.) as well as between-groups for the WIF and WSF_{min} (Table 3). Cold-evoked pain in the WIF was unique, as reports of *"cold"* were not provided in any of the cold pain MOL trials. Rather, cold pain in WIFs was highly likely to evoke reports of *"burning"* compared to other skin areas. This is the first known evidence that the lack of low-threshold C-warm fiber signaling found in WIFs may contribute in some way to an altered perception of cold pain. This revelation is supported by the difference in cold pain thresholds observed between WIF and WSF_{max}, shown in Figure 2A.

While these data are from small samples, the indication of warmth sensitivity exerting an effect on cold but not heat-evoked pain presents exciting new research questions (visited at length in *Discussion*).

At the other end of Figure 5 (B), reports of *"cold"* were most common in the WSF_{max} of Non-WIF subjects. Interestingly, two reports of *"hot"* also were given. More data is needed to determine if these reports of paradoxical heat represent an emerging trend or an outlier.

TABLE 3. Between-group comparisons of thermosensory response profiles.

	Thermosensory attribute	WIF (*n*=5)	Non-WIF (*n*=7)	Significance
Threshold (ΔT from baseline, °C)	**Cold** (*MOL*)			
	WIF or WSF_{min}	-1.86 (± 0.47)	-2.39 (± 0.42)	*p = 0.428*
	WSF_{max}	-2.39 (± 0.49)	-2.75 (± 0.71)	*p = 0.710*
	Cold (*2AFC*)			
	WIF or WSF_{min}	-1.36 (± 0.36)	-1.20 (± 0.34)	*p = 0.751*
	WSF_{max}	-1.48(± 0.54)	-1.97 (± 0.56)	*p = 0.557*
	Cold Pain			
	WIF or WSF_{min}	-26.71 (± 2.40)	-18.12 (± 3.95)	*p = 0.096*
	WSF_{max}	-21.91 (± 3.73)	-17.02 (± 3.63)	*p = 0.382*
	Warm (*MOL*)			
	WIF or WSF_{min}	+12.02 (± 1.28)	+5.41 (± 0.72)	*p = 0.001*
	WSF_{max}	+2.33 (± 0.68)	+2.81 (± 0.42)	*p =0.268*
	Warm (*2AFC*)			
	WIF or WSF_{min}	+11.25 (± 0.84)	+3.84 (± 0.92)	*p = 0.0002*
	WSF_{max}	+2.42 (± 0.51)	+2.10 (± 0.55)	*p =0.698*
	Heat Pain			
	WIF or WSF_{min}	+16.80 (± 0.83)	+13.74 (± 1.88)	*p = 0.225*
	WSF_{max}	+13.79 (± 1.72)	+12.54 (± 2.09)	*p =0.677*
Intensity (0-100)	**Search Stimulus** (*40.5°C*)			
	WIF or WSF_{min}	0.0 (± 0.0)	19.86 (± 4.55)	
	WSF_{max}	35.00 (± 13.67)	61.14 (± 10.98)	*p =0.161*
	Supraliminal Heat (*45°C*)			
	WIF or WSF_{min}	17.00 (± 8.17)	34.67 (± 10.78)	*p = 0.254*
	WSF_{max}	39.87 (± 15.23)	45.00 (± 10.98)	*p =0.784*
	Supraliminal Heat (*48°C*)			
	WIF or WSF_{min}	41.73 (± 14.61)	61.71 (± 14.62)	*p = 0.323*
	WSF_{max}	55.40 (± 19.04)	62.52 (± 12.61)	*p =0.751*
Pain Quality	**Cold Pain** (*Threshold*)			
	WIF or WSF_{min}	B, 26.7%	C, 23.8%	*p =0.005*
	WSF_{max}	C, 20.0%	C, 47.6% H, 9.5%	*p =0.053*
	Heat Pain (*Threshold*)			
	WIF or WSF_{min}	H, 26.7%	H, 23.8%	*p = 1.000*
	WSF_{max}	H, 20.0%	H, 38.1%	*p =0.295*
	Supraliminal Heat (*45°C*)			
	WIF or WSF_{min}	H, 40.0%	H, 57.1%	*p = 0.499*
	WSF_{max}	H, 73.3%	H, 52.4%	*p =0.355*
	Supraliminal Heat (*48°C*)			
	WIF or WSF_{min}	H, 33.3%	H, 42.9%	*p =0.817*
	WSF_{max}	H, 46.7%	H, 47.6%	*p = 1.000*

TABLE 3. All continuous variables are represented as μ (± SEM). All categorical variables are listed as a percent value, representing frequency of occurrence within their group (column). Refer to *Methods: Data Analysis* for information regarding statistical tests.

WIF = *warmth-insensitive field*
WSF_{min} = *minimally warmth-sensitive field*
WSF_{max} = *maximally warmth-sensitive field*

C = *"cold"*
B= *"burning"*
H= *"hot"*

Discussion

Factors affecting the occurrence of WIFs

The significant effect of age on the identification of WIFs is perplexing. While it is well-established that thermal sensitivity declines with age (Stevens & Choo, 1998), it is unlikely that the gap between mean ages of WIF and Non-WIF subjects in this study, a mere 1.29 years, would result in the complete loss of thermal sensitivity at specific sites. Furthermore, the mean age of subjects with WIFs reported by Green and Cruz was higher than for either of the groups in the present study. Recruitment for this study was mainly conducted by posting flyers on Emory's main campus. The narrow age range of subjects who responded to recruitment materials may be because many individuals on campus during the COVID-19 pandemic were undergraduate residents. The tightly distributed variations around the mean age for both groups may represent a resultant sampling bias. Further investigation of WIFs encompassing a wider age range is needed to better understand the potential effects of aging on the occurrence of localized warm insensitivity. If these studies show that larger age differences are required for significant differences in WIF occurrence to arise, then the small age range would present an advantage for our study by indicating that variability in warmth sensitivity shown here is age-independent.

The lack of significant effects for all other variables reported in Table 2 contraindicates the possibility that WIFs co-occur with certain demographics and/or physical attributes. Furthermore, the coincidence of several key thermosensory differences between test sites in WIF and Non-WIF subjects advocates the study of disparate warmth sensitivity more widely as it pertains to nociception. In support of this, two subjects in our study who were found to have WIFs were asked to return for a brief follow-up session. The second session was required to remeasure 2AFC warm and cool thresholds that were not collected in the first session due to a technological malfunction. In both subjects, the WIF used for testing in the first session was located at the same spot on the forearm. Green and Cruz were also notably able to identify WIFs in the same areas across as many as five sessions. This indicates that WIFs are

physiologically stable and not merely artifacts of shifting detection thresholds based on environmental or other factors.

Practical considerations for identification of WIFs

In all but one of the 5 subjects where a 2.56 cm^2 WIF was located, warmth-insensitivity was remarkably spatially isolated, i.e., surrounding areas did not exhibit warmth insensitivity or characteristically low ratings of warmth intensity. WIFs identified by Green and Cruz reportedly ranged in size from 5cm^2 to as large as 15cm^2. However, WIF boundaries beyond the site where the 4.84 cm^2 thermode failed to elicit warmth sensation were distinguished using a thermode size of only 0.64 cm^2. It is possible that the first reports of WIFs as large as 15 cm^2 were biased by differences in spatial summation that arise when using a small contactor.

The only other study to adopt Green and Cruz's technique for identifying and subsequently testing WIFs (Defrin et al., 2009) showed that significant spatial summation of warm detection occurs within these sites. In their study, a medium-sized (2.25 cm^2) thermode was used to identify WIFs and WSFs and test detection thresholds for heating in these areas. Subsequent testing was conducted using a small (0.5 cm^2) thermode placed within the bounds of the originally identified field. Thresholds were significantly higher when measured with the small thermode in both WIFs and WSFs and extended into the nociceptive range within WSFs. This suggests that even within a site that is sensitive to warming across a modest stimulus area, a sufficiently small stimulus may still exploit gaps in C-warm fiber innervation. Therefore, measuring the spatial extent of an insensitive site by summing the areas of smaller, individually measured insensitive sites may predispose researchers to over-report the size of WIFs.

Consistent with this idea, but at odds with their reports of WIF sizes, Green and Cruz specify that placing the 4.84 cm^2 thermode over areas where the smaller thermode had detected as few as one or two warmth-sensitive spots resulted in warmth detection thresholds similar to those measured in WSFs. Accordingly, researchers seeking to employ the WIF as a tool for divorcing low-threshold warm fiber

activity from other sensory mechanisms should limit thermosensory testing to the largest area which can be identified as warmth-insensitive under a single, continuous stimulus.

Trends in processing heat pain accordant with disparate warmth sensitivity

Thermosensory testing in both WIF and Non-WIF subjects confirmed that ratings of thermal sensation in response to a stimulus of 40.5°C reliably predict differences in detection threshold for heating. This difference was especially pronounced between the two sites tested in the WIF group, as thresholds in WIFs extended into the nociceptive range. The similar predictive effect of warm intensity ratings on warm threshold differences between WSF_{min} and WSF_{max} in Non-WIF subjects indicates that perceived intensity and threshold are closely linked in a more complex manner than would be revealed by treating WIFs and WSFs as diametrical terms. Principles of spatial summation of thermal sensation tell us that the salience of a stimulus increases as a function of how many relevant sensory fibers are recruited (Marks & Stevens, 1971, 1973). By applying the same concept to a fixed rather than changing stimulus area, we show that mapping the warmth intensity evoked by identical stimuli across the forearm may serve as a marker for warm-fiber innervation density. This extends beyond determinations of mere presence or absence of these fibers when binarily distinguishing WSFs from WIFs. Future neurophysiological studies aiming to record the activity of rarely occurring C-warm fibers in waking human subjects could employ these methods to narrow in on areas of high warmth sensitivity where these fibers may be particularly concentrated. This would not only cut down on time and resource expenditure but would also spare subjects potentially extraneous invasive probes.

The continued use of psychophysics for investigating the thermosensory characteristics of sites exhibiting warmth sensitivity across a broad scale could shed light on the non-significant trends presented in this paper. Namely, areas of little or no warm sensitivity expressed higher average heat pain thresholds than their highly warm-sensitive counterparts. While this finding is consistent with Green and Cruz's reports, the significance of the effects was not demonstrated in either WIF (Figure 2 A.) or Non-WIF subjects (B.). Perceived intensity of heat-evoked pain (Figure 3) indicates a similar non-significant trend that links warmth sensitivity and salience of heat pain. Alternatively, it has been suggested that

descending inhibition of the structures that integrate innocuous thermal and nociceptive inputs may result in the high irregularity of these effects (Green et al., 2008). Conditioned pain modulation (CPM), a method that attenuates ongoing nociception via the distal application of a competing pain stimulus, could be used in conjunction with WIFs to experimentally separate the two terms of this relationship. CPM is thought to work by activating diffuse noxious inhibitory controls (DNIC) which project to dorsal horn convergent neurons of the spinal cord (Le Bars et al., 1979). CPM has been used experimentally to attenuate the intensity and quality of noxious heat stimuli and pain resulting from the TGI (Harper & Hollins, 2014). These findings together suggest that dorsal horn convergent neurons are likely sites for the integration of innocuous and nociceptive signals involved in the TGI. Future studies could use a similar approach by examining the ability of CPM to attenuate the intensity and quality of heat pain administered in WIF and WSF skin of varying warmth sensitivity. If such studies were to demonstrate differences in attenuation of pain that scaled with warmth sensitivity, then the dorsal horn convergent neurons would be a likely site of integration of low-threshold warm and nociceptive heat signals.

Altered processing of cold pain: an unexpected feature of the WIF

During protocol design, measurements of cold-threshold were mainly included as a means of providing a comparison point for warm-sensitivity mediated differences in threshold, intensity, and quality of *heat* evoked pain. And while trends from our data still point to the possibility of better understanding and characterizing these relationships, factors surrounding cold-evoked pain constitute the most striking finding of this study.

Differences in cold pain threshold and quality, especially reports of *"burning"* rather than *"cold"* (Figure 5A), were unexpected features of the WIF thermosensory profile. Green and Cruz reported that cold detection thresholds were highly similar between WIFs and adjacent sites, and implying that the value of WIFs laid exclusively in the opportunities they present for studying the role of low-threshold warm fiber activity in heat pain processing. However, to the current author's best knowledge, the findings presented herein are the first reports of altered cold pain sensitivity and notable occurrence of burning

sensation occurring in WIFs. This presents exciting possibilities for the expansion of WIFs as tools for studying the complex connections between warm, cold, and nociceptive components of thermosensation.

In 1999, Susser, Sprecher, & Yarnitsky used a psychophysical reaction-time-based measure of peripheral fiber conduction velocities for cold, heat, and paradoxical heat on the legs of healthy individuals. They concluded that experience of paradoxical heat was mediated by C-fibers, which have a conduction velocity nearly tenfold slower than thin, myelinated A-fibers. This was corroborated in the following years by the discovery of cool-responsive C-fibre activation at temperatures as low as 0°C (Campero & Bostock, 2010). Crucially, the transmission of PH by C-fibers suggests potential overlap with theories generated by studies on the disinhibition of cool-driven C-nociceptor burning sensations by A-fiber block and the TGI (Mackenzie et al., 1975; Yarnitsky & Ochoa, 1990; Craig et al., 1996).

The account of the TGI put forth by Craig et al. (1994) (*refer to Introduction: Evolving theories of thermosensation and pain*) proposed that the TGI is the result of the disinhibition of cool-driven C-nociceptor activity by C-warm thermoreceptors. Based on this finding and the body of work published both before and after, it is the opinion of the author that high instances of PH in WIFs may be attributed to limited C-warm fiber density. In the absence of inhibitory input from C-warm fibers, the aberrant activity of A-δ fibers, the central components which they innervate, or some combination of these two occurs and results in a failure to inhibit cool-driven C-fiber activity that encodes for hot/burning sensations.

To test this theory, two foundational concepts must be demonstrated in this system. First, the described effect of a lack of warm-fiber input must be shown to alter a component of the A-delta system. Various types of changes in peripheral sensitization have been shown to cause central sensitization implicated in disorders of pain processing (Koltzenburg et al., 1994; Robowtham &Fields, 1996; Woolf, 1983). Additionally, the effects of disinhibition on post-synaptic cells are widely studied phenomena shown to have profound effects in many systems. A parsimonious placeholder may not exist within this theory for the significant between-group difference in pain quality shown for WSF_{max} and the occurrence of PH ratings in WSF_{max} in Non-WIF subjects. If this effect persists as data collection continues and N

increases, it may be worth investigating the hypothesis that A-δ inhibition of C-nociceptor fibers is finely tuned such that shifting C-warm activity in either direction may result in paradoxical heat.

Second, it must be demonstrated that A-delta fibers remain intact for the sensation of innocuous cool, as this thermosensory component is not significantly altered in WIFs. Mechanisms governing the experience of cold-evoked pain remain elusive and ambiguous (Foulkes & Wood, 2007) making this an exciting avenue of study.

Conceptual backing for this theory is provided by Susser and colleagues' discovery that PH was optimally induced under a small probe and high rates of temperature change. The increased efficacy of a small probe is consistent with poor spatial summation of warm fibers. A rapid decrease in temperature provides optimal conditions for early activation of A-fibers primarily during the innocuous range of cold stimuli (Campero et al., 2009).

Research on this topic may be difficult without a method that provides sufficient means of comparison with naturally occurring WIFs. Selectively blocking C-warm fibers without outright affecting the function of C-nociceptive fibers is not well documented in the literature. Novel methods of targeting TRP channels may serve as a promising method for achieving this, but evidence of low fiber-specificity of TRP subtype expression may present limits on the specificity of this method as well (Belmonte & Viana, 2008).

One alternative explanation for the high incidence of PH in WIFs may result from the arrangement of stimulus presentation in the current study. PH was most reliably induced by Susser et al. when the cold stimulus was administered following completion of MOL heat pain threshold determination. In the present study, cold pain MOL trials were administered following MOL for warmth detection. In WIF subjects, where these thresholds extended into the nociceptive range, the stimulus paradigm may have emulated this method of inducing PH. However, the heat pain thresholds reported in these subjects were approximately 6 °C higher than warm detection thresholds in WIFs. Additionally, as spelled out in the methods, sufficient time was given between threshold measurements for the skin to return to its approximate baseline temperature. As cold-evoked PH in WIFs had not been reported before

our study, future work done investigating this phenomenon will employ rigorous psychophysical examination. At this point, more data is required to assess the worth of pursuing this line of inquiry, though early signs show promise.

Conclusions

This study was undertaken to replicate but also build upon Green and Cruz's original work. Factors of potential influence on the prevalence of WIFs were shown to be largely non-significant, with the one exception being subject age. While this was likely not a confounding factor in our study cohort, deficits in thermal sensation that progress with age (Stevens & Choo, 1998) emphasize the importance of studying WIFs in a more age-diverse dataset to determine if WIFs may increase in number or spatial extent. A finding of this nature would potentially contraindicate the use of thermal QST for the diagnosis of peripheral neuropathy, as naturally occurring WIFs may be misconstrued as artifacts of pathological issues.

Quantifying thermosensory profiles in WIFs and non-WIFs confirmed and expanded on Green and Cruz's discovery by indicating that differences between warmth-sensitive sites can also be used as a method for studying the effects of innocuous thermosensation on nociception. When used in conjunction with WIFs, the study of multiple WSFs in this manner can better quantify the dynamics of this relationship via quantifying the strength and upper bounds of effects.

Thermosensory testing in WIFs and WSFs most importantly indicated a significant effect of warmth sensitivity on the intensity and quality of cold-evoked pain. While exciting theoretical implications discussed here are highly contingent on the production of several key findings, the broader and more widely applicable suggestion is that warm-fibers may communicate with various nociceptive and non-nociceptive somatosensory components in nonobvious ways.

References

Ackerley, R., Backlund Wasling, H., Liljencrantz, J., Olausson, H., Johnson, R. D., & Wessberg, J. (2014). Human C-tactile afferents are tuned to the temperature of a skin-stroking caress. *Journal of Neuroscience, 34*(8), 2879-2883. doi:10.1523/jneurosci.2847-13.2014

Belmonte, C., & Viana, F. (2008). Molecular and cellular limits to somatosensory specificity. *Molecular Pain, 4*. doi:10.1186/1744-8069-4-14

Bokiniec, P., Zampieri, N., Lewin, G. R., & Poulet, J. F. (2018). The neural circuits of thermal perception. *Current Opinion in Neurobiology, 52*, 98-106. doi:10.1016/j.conb.2018.04.006

Campero, M., & Bostock, H. (2010). Unmyelinated afferents in human skin and their responsiveness to low temperature. *Neuroscience Letters, 470*(3), 188-192. doi:10.1016/j.neulet.2009.06.089

Campero, M., Baumann, T. K., Bostock, H., & Ochoa, J. L. (2009). Human cutaneous C fibres activated by cooling, heating and menthol. *The Journal of Physiology, 587*(23), 5633-5652. doi:10.1113/jphysiol.2009.176040

Craig, A. (2003). Pain mechanisms: Labeled lines versus convergence in central processing. *Annual Review of Neuroscience, 26*(1), 1-30. doi:10.1146/annurev.neuro.26.041002.131022

Craig, A. D., Reiman, E. M., Evans, A., & Bushnell, M. C. (1996). Functional imaging of an illusion of pain. *Nature, 384*(6606), 258-260. doi:10.1038/384258a0

Craig, A., & Bushnell, M. (1994). The thermal grill illusion: Unmasking the burn of cold pain. *Science, 265*(5169), 252-255. doi:10.1126/science.8023144

Defrin, R., Ohry, A., Blumen, N., & Urca, G. (2002). Sensory determinants of thermal pain. *Brain, 125*(3), 501-510. doi:10.1093/brain/awf055

Defrin, R., Petrini, L., & Arendt-Nielsen, L. (2009). Spatial summation of thermal sensations depends on skin type and skin sensitivity. *Experimental Brain Research, 198*(1), 29-36. doi:10.1007/s00221-009-1934-y

Dubin, A. E., & Patapoutian, A. (2010). Nociceptors: The sensors of the pain pathway. *Journal of Clinical Investigation, 120*(11), 3760-3772. doi:10.1172/jci42843

Filingeri, D. (2016). Neurophysiology of skin thermal sensations. *Comprehensive Physiology*, 1429-1491. doi:10.1002/cphy.c150040

Foulkes, T., & Wood, J. (2007). Mechanisms of cold pain. *Channels, 1*(3), 154-160. doi:10.4161/chan.4692

Frey, M. V. (1895). Beiträge zur Sinnesphysiologie der Haut. *Saechsischen Akademie Der Wissenschaften Zu Leipzig. Math-Phys CI, 47*, 166-184.

Fruhstorfer, H. (1984). Thermal sensibility changes during ischemic nerve block. *Pain, 20*(4), 355-361. doi:10.1016/0304-3959(84)90112-x

Gerrett, N., Ouzzahra, Y., & Havenith, G. (2015). Distribution of Skin Thermal Sensitivity. In 1282297767 945848131 P. G. Agache, 1282297768 945848131 P. Humbert, 1282297772 945848131 F. Fanian, & 1282297773 945848131 H. Maibach (Eds.), *Measuring the skin: Non-invasive investigations, physiology, normal constants*. Berlin: Springer. doi:DOI 10.1007/978-3-319-26594-0_72-1

Gescheider, G. A. (1997). *Psychophysics: The fundamentals* (3rd ed.). Lawrence Erlbaum Associates.

Glatte, P., Buchmann, S. J., Hijazi, M. M., Illigens, B. M., & Siepmann, T. (2019). Architecture of the cutaneous autonomic nervous system. *Frontiers in Neurology, 10*. doi:10.3389/fneur.2019.00970

Glencross, D., & Oldfield, S. (1975). The use of ischemic nerve block procedures in the investigation of the sensory control of movements. *Biological Psychology, 2*(3), 227-236. doi:10.1016/0301-0511(75)90022-8

Gray, H., Gray, H., & Lewis, W. H. (1918). Anatomy of the human body. doi:10.5962/bhl.title.20311

Green, B. G. (2004). Temperature perception and nociception. *Journal of Neurobiology, 61*(1). doi:10.1002/neu.20081

Green, B. G., & Akirav, C. (2010). Threshold and rate sensitivity of low-threshold thermal nociception. *European Journal of Neuroscience*. doi:10.1111/j.1460-9568.2010.07201.x

Green, B. G., & Cruz, A. (1998). "Warmth-insensitive fields": Evidence of sparse and irregular innervation of human skin by the warmth sense. *Somatosensory & Motor Research, 15*(4), 269-275. doi:10.1080/08990229870682

Haefeli, M., & Elfering, A. (2005). Pain assessment. *European Spine Journal, 15*(S1). doi:10.1007/s00586-005-1044-x

Hallin, R. G., Torebjork, H. E., & Wiesenfeld, Z. (1982). Nociceptors and warm receptors innervated by C fibres in human skin. *Journal of Neurology, Neurosurgery & Psychiatry, 45*(4), 313-319. doi:10.1136/jnnp.45.4.313

Handwerker, H. O., & Kobal, G. (1993). Psychophysiology of experimentally induced pain. *Physiological Reviews, 73*(3), 639-671. doi:10.1152/physrev.1993.73.3.639

Harper, D. E., & Hollins, M. (2014). Coolness both underlies and protects against the painfulness of the thermal grill illusion. *Pain, 155*(4), 801-807. doi:10.1016/j.pain.2014.01.017

Hensel, H. (1981). *Thermoreception and Temperature Regulation*. London: Academic Press.

Kim, H. (2013). Statistical notes for clinical researchers: Assessing normal distribution (2) using skewness and kurtosis. *Restorative Dentistry & Endodontics, 38*(1), 52. doi:10.5395/rde.2013.38.1.52

Koltzenburg, M., Torebjörk, H. E., & Wahren, L. K. (1994). Nociceptor modulated central sensitization causes mechanical hyperalgesia in acute chemogenic and chronic neuropathic pain. *Brain, 117*(3), 579-591. doi:10.1093/brain/117.3.579

Le Bars, D., Dickenson, A. H., & Besson, J. (1979). Diffuse noxious inhibitory controls (DNIC). I. effects on dorsal horn convergent neurones in the rat. *Pain, 6*(3), 283-304. doi:10.1016/0304-3959(79)90049-6

Louw, G. N. (1993). *Physiological animal ecology*. Essex: Longman Scientific & Technical.

Lue, Y., Wang, H., Cheng, K., Chen, C., & Lu, Y. (2018). Thermal pain tolerance and pain rating in normal subjects: Gender and age effects. *European Journal of Pain, 22*(6), 1035-1042. doi:10.1002/ejp.1188

Ma, Q. (2010). Labeled lines meet and talk: Population coding of somatic sensations. *Journal of Clinical Investigation, 120*(11), 3773-3778. doi:10.1172/jci43426

Ma, Q. (2012). Population coding of somatic sensations. *Neuroscience Bulletin, 28*(2), 91-99. doi:10.1007/s12264-012-1201-2

Mackenzie, R. A., Burke, D., Skuse, N. F., & Lethlean, A. K. (1975). Fibre function and perception during cutaneous nerve block. *Journal of Neurology, Neurosurgery & Psychiatry, 38*(9), 865-873. doi:10.1136/jnnp.38.9.865

Marks, L. E., & Stevens, J. C. (1971). Spatial summation and the dynamics of warmth sensation. *Perception & Psychophysics, 9*(5), 391-398. doi:10.3758/bf03210236

Marks, L. E., & Stevens, J. C. (1973). Spatial summation of warmth: Influence of duration and configuration of the stimulus. *The American Journal of Psychology, 86*(2), 251. doi:10.2307/1421436

McKemy, D. D. (2012). The molecular and cellular basis of cold sensation. *ACS Chemical Neuroscience, 4*(2), 238-247. doi:10.1021/cn300193h

McWilliams, L. A., Cox, B. J., & Enns, M. W. (2003). Mood and anxiety disorders associated with chronic pain: An examination in a nationally representative sample. *Pain, 106*(1), 127-133. doi:10.1016/s0304-3959(03)00301-4

Mitchell, D., Snelling, E. P., Hetem, R. S., Maloney, S. K., Strauss, W. M., & Fuller, A. (2018). Revisiting concepts of thermal physiology: Predicting responses of mammals to climate change. *Journal of Animal Ecology, 87*(4), 956-973. doi:10.1111/1365-2656.12818

Norrsell, U., Finger, S., & Lajonchere, C. (1999). Cutaneous sensory spots and the "law of specific nerve energies": History and development of ideas. *Brain Research Bulletin, 48*(5), 457-465. doi:10.1016/s0361-9230(98)00067-7

Palmer, Denis J. Martin, Wilma M, S. T., Ravey, J., Steedman, W., & Martin, D. J. (2000). C- and aδ-fibre mediated thermal perception: Response to rate of temperature change using method of limits. *Somatosensory & Motor Research, 17*(4), 325-333. doi:10.1080/08990220020002033

Perl, E. R. (2007). Ideas about pain, a historical view. *Nature Reviews Neuroscience, 8*, 71-80. doi:10.1038/nrn2042

Romanovsky, A. A. (2007). Thermoregulation: Some concepts have changed. functional architecture of the thermoregulatory system. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 292*(1). doi:10.1152/ajpregu.00668.2006

Simon, G. E., VonKorff, M., Piccinelli, M., Fullerton, C., & Ormel, J. (1999). An international study of the relation between somatic symptoms and depression. *New England Journal of Medicine, 341*(18), 1329-1335. doi:10.1056/nejm199910283411801

Speakman, J. R., & Król, E. (2010). Maximal heat dissipation capacity and hyperthermia risk: Neglected key factors in the ecology of endotherms. *Journal of Animal Ecology*. doi:10.1111/j.1365-2656.2010.01689.x

Stevens, J. C., & Choo, K. K. (1998). Temperature sensitivity of the body surface over the life span. *Somatosensory & Motor Research, 15*(1), 13-28. doi:10.1080/08990229870925

Stevens, S. S. (1956). The direct estimation of sensory magnitudes: Loudness. *The American Journal of Psychology, 69*(1), 1. doi:10.2307/1418112

Strigo, I., Carli, F., & Bushnell, M. (2000). Effect of ambient temperature on human pain and temperature perception. *Anesthesiology, 92*(3), 699-707. doi:10.1097/00000542-200003000-00014

Susser, E., Sprecher, E., & Yarnitsky, D. (1999). Paradoxical heat sensation in Healthy subjects: Peripherally conducted BY Aδ or C fibres? *Brain, 122*(2), 239-246. doi:10.1093/brain/122.2.239

Torebjörk, E., & Schmelz, M. (2005). Single-Unit Recordings of Afferent Human Peripheral Nerves by Microneurography. In 1282229338 945805099 P. K. Thomas & 1282229341 945805099 P. J. Dyck (Eds.), *Peripheral Neuropathy* (4th ed., Vol. 1, pp. 1003-1014). Philadelphia, PA: W.B. Saunders.

Voets, T., Droogmans, G., Wissenbach, U., Janssens, A., Flockerzi, V., & Nilius, B. (2004). The principle of temperature-dependent gating In Cold- and heat-sensitive TRP channels. *Nature, 430*(7001), 748-754. doi:10.1038/nature02732

Vriens, J., Nilius, B., & Voets, T. (2014). Peripheral thermosensation in mammals. *Nature Reviews Neuroscience, 15*(9), 573-589. doi:10.1038/nrn3784

Wager, T. D., Matre, D., & Casey, K. L. (2006). Placebo effects in laser-evoked pain potentials. *Brain, Behavior, and Immunity, 20*(3), 219-230. doi:10.1016/j.bbi.2006.01.007

Wang, H., & Siemens, J. (2015). TRP ion channels in thermosensation, thermoregulation and metabolism. *Temperature, 2*(2), 178-187. doi:10.1080/23328940.2015.1040604

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: The PANAS scales. *Journal of Personality and Social Psychology, 54*(6), 1063-1070. doi:10.1037/0022-3514.54.6.1063

Woolf, C. J. (1983). Evidence for a central component of post-injury pain hypersensitivity. *Nature, 306*(5944), 686-688. doi:10.1038/306686a0

Wu, G., Campbell, J. N., & Meyer, R. A. (2001). Effects of baseline skin temperature on pain ratings to suprathreshold temperature-controlled stimuli. *Pain, 90*(1), 151-156. doi:10.1016/s0304-3959(00)00399-7

Yarnitsky, D., & Ochoa, J. L. (1990). Release of cold-induced burning pain by block of cold-specific afferent input. *Brain, 113*(4), 893-902. doi:10.1093/brain/113.4.893

Zheng, J. (2013). Molecular mechanism of TRP channels. *Comprehensive Physiology*. doi:10.1002/cphy.c120001

Zigmond, A. S., & Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica, 67*(6), 361-370. doi:10.1111/j.1600-0447.1983.tb09716.x