The Thermal Grill Illusion of Pain: Characterizing Differences in Response across Body Sites

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Abstract

The simultaneous application of interlaced innocuous warm and cool stimuli (a thermal grill stimulus, TGS) can elicit sensations of burning heat (the Thermal Grill Illusion, TGI). The TGS is thought to alter the central interactions between somatosensory sub-modalities (i.e. cold-inhibition of pain). Previous psychophysical findings point to body site differences in perceptual thermal thresholds. The primary objective of this study was to evaluate whether, using the same TGS, a TGI can be elicited at body sites other than the upper extremity. The present findings indicate that the TGI can be induced at the palm, back, calf, and foot. Pain and unpleasantness in response to the TGS were more frequent and intense following stimulation of the palm and back than the calf and foot. Lower cold pain thresholds were associated with lower pain intensity ratings in response to the TGS. These two findings may reflect differences in central integrative processes.
Acknowledgments

While writing this manuscript, I began to fully appreciate the astonishing knowledge pain researchers have accumulated in a relatively short period of time through meticulous and ingenious work. While it looks like my own (modest) contribution to the field ends here, I am excited by the ongoing work of my peers, and am confident of the clinical progress this body of knowledge will spearhead.

I feel immense gratitude towards my co-supervisors, Dr. Judith Hunter and Dr. Jonathan Dostrovsky. Their combined and complementary knowledge was a huge asset to me and provided me with great guidance at every stage of my Master’s degree. It speaks to their character that, despite knowing I was likely to move to other endeavors, they provided me with nothing short of enviable opportunities to partake in the field of pain research. I have also enjoyed their warm and positive attitudes. Judi, Jonathan: The pivotal role you played in this stage of my academic career will not be easily forgotten.

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1 Introduction

1.1 Description and Purpose

First characterized by Thunberg in 1896 (see Boring, 1942), the thermal grill illusion (TGI) describes a paradoxical burning heat sensation elicited by the simultaneous application of interlaced innocuous warm (40 °C) and cool (20 °C) stimuli on the skin (a thermal grill stimulus, TGS). Recent investigative efforts attempt to relate the central mechanism underlying the TGI to our current knowledge of pain pathways. However, its physiological underpinnings are still a matter of debate. The current prevailing theory suggests an interaction between thermoreceptive and nociceptive information (summarized in Craig, 2002). A compression block of peripheral cold thermoreceptive afferents results in a similar allodynic burning pain response (see, for example, Wahren et al, 1989). Researchers postulated that central cold thermoreceptive pathways modulate (inhibit) the perception of pain. During the TGS, activity at peripheral cold thermoreceptors is suppressed by the concurrent presence of warm temperature bars (Craig, 2002). This results in reduced activity at spinal Cold thermoreceptive neurons, which releases the inhibition typically held on the nociceptive pathway (Craig and Bushnell, 1994).

In this study, known body site differences in thermoreception were utilized to further investigate the physiological mechanisms underlying the TGI. Previous physiological and psychophysical findings point to body site differences in cutaneous somatosensory innervation density and perceptual thermal thresholds. Yet, to date, the TGS has only been tested on the palmar surface of the hand and the forearm. In particular, one of the purposes of this study was to assess whether the TGI demonstrated similar body site differences as those noted in response to uniform innocuous and noxious thermal stimuli. Additionally, because the TGI is not a ubiquitous phenomenon, this study also sought to explore the source of inter-individual variance in response to the TGS, and whether it was related to other physiological characteristics (e.g., thermal thresholds and sex differences).

1.2 Cutaneous Sensory System

The TGS is composed of interlaced bars set at warm and cool temperatures. Physiologically, it elicits activity at afferents sensitive to mechanical, thermal, and nociceptive
stimuli. The particular perceptual response it elicits suggests that this stimulus may be an interesting method by which to study the somatosensory system and, more specifically, the interactions between pain and temperature pathways.

The somatosensory system is comprised of peripheral afferents and central neurons which serve the physiological detection (sensation), and the subsequent awareness (perception), of various stimulus modalities, including touch, temperature, kinaesthesia and proprioception, itch, and pain. Distinct populations of peripheral afferents optimally respond to the specific modalities. Afferents innervating the muscles and joints transmit information on joint and limb positions, thus contributing to proprioception. Meanwhile, cutaneous afferents relay information regarding pressure, vibration, thermal, and/or chemical stimulation of the skin (for a review of the cutaneous sensory system, please refer to McGlone and Reilly, 2009).

The differentiation of sensory information is maintained in the central nervous system by neurons sensitive to particular modalities; other central neurons are multimodal and receive convergent information. Ascending somatosensory information projects to various brain regions; the forebrain is responsible for its perception. Pain, in particular, is a conscious sensory experience that both informs and motivates the individual. Thus, while nociception entails the detection of noxious stimuli by peripheral afferents, pain is conceptualized as a multi-faceted psychological phenomenon, having sensory-discriminative, cognitive-evaluative, and affective-motivational components (see for example, Treede et al, 1999).

1.2.1 Discriminative Touch

1.2.1.1 Peripheral Afferents

Cutaneous receptors show modality-specificity; therefore, they can be classified according to their functional and morphological characteristics (reviewed in Goodwin and Wheat, 2008). On glabrous skin, the tactile modality is principally subserved by four types of fast-conducting, large-diameter myelinated (A-β; conduction velocity: 35-75 m/s) afferents, sensitive to low-threshold mechanical forces. These afferents have specialized nerve endings. Of these four, two respond optimally to changes in pressure against the skin, firing vigorously at the beginning and end of the stimulus (“rapidly adapting”). Meissner corpuscles (abbreviated as RA) are mechanoreceptors with small, punctate receptive fields (RFs) and provide optimal information for tracking an object as it moves along the skin (maximum sensitivity to
frequencies below 30 Hz stimulus). Pacinian corpuscles (PC), also rapidly adapting receptors, have large and less defined RFs, and respond maximally to high frequency vibration (maximum sensitivity is to a 300 Hz stimulus).

Merkel disks have well-defined, spot-like RFs that are sensitive to the indentation of skin and aid in the detection of texture and form. Merkel disks are categorized as slowly adapting receptors (SAI) since their activity persists throughout the presentation of a stimulus. Lastly, Ruffini’s endings (SAII) have diffuse RFs, which are sensitive to the stretching of skin; however, their contribution to the conscious perception of touch is not well understood.

Analogous slowly adapting receptors, as well as Pacinian units, also innervate hairy skin. In addition, hairy skin contains two other classes of mechanoreceptors that do not have glabrous-skin counterparts. Hair units innervate single or several follicles and are activated by the deflection of hairs. Hair units can have either large-diameter (A-β) or small-diameter (A-δ afferents; conduction velocity: 3-30 m/s) myelinated axons. A less well-understood type of receptor in the hairy skin is the unmyelinated (C afferents; conduction velocity 0.5-2 m/s) low-threshold mechanoreceptors responsive to light brushing, which may be associated with pleasurable touch (refer to Olausson et al., 2002). The central projection pattern of the unmyelinated afferents is unlike that of A-β mechanoreceptors (discussed below); instead, they follow a similar pattern to that of thermoreceptive and nociceptive units (discussed in section 1.2.2.3).

1.2.1.2 Central Projections

Tactile and proprioceptive peripheral afferents innervating the body below the neck enter the central nervous system at the dorsal aspect of the spinal cord (Goodwin and Wheat, 2008). Most first-order large-diameter myelinated afferents from the body ascend to the somatosensory cortex through the dorsal column-medial lemniscus pathway (A-β afferents also branch into the spinal dorsal horn and synapse onto spinal neurons; discussed in section 1.2.2.3). As the name of the pathway implies, the afferents travel up the spinal cord through the dorsal columns, a dorsomedial axonal tract. The fibres maintain somatotopic organization, with axons from afferents innervating the lower body forming a bundle termed the gracile fasciculus, and those from the upper body forming the cuneate fasciculus. The axons of these ascending tracts synapse with second-order neurons in the gracile nucleus and cuneate nucleus of the brainstem, respectively. In turn, secondary neurons decussate and project through the medial lemniscus to
the ventral posterolateral nucleus (VPL) of the thalamus. From there, third-order neurons travel to the primary somatosensory cortex (SI), which mediates conscious perception of touch. The cortical areas of SI representing certain body sites (for example, the hands) are disproportionately large with relation to their actual size. This reflects greater peripheral innervation at the sites, and a resultant higher perceptual sensitivity.

1.2.2 Innocuous Thermoreception and Pain

1.2.2.1 Peripheral Thermoreceptors

Unlike afferents encoding discriminative touch information, thermal receptors (or, thermoreceptors) have simple unencapsulated or morphologically distinct “free” nerve endings with either thinly myelinated or unmyelinated axons. Electrophysiological recordings from these afferents show background discharge to ambient skin temperature (Bessou and Perl, 1969; LaMotte and Campbell, 1978). Thermoreceptors are better able to encode relative changes in, rather than absolute, temperature. They also respond to thermal stimuli applied to discrete areas of the skin (e.g., detecting the temperature of objects being held). Thermoreceptors are insensitive to mechanical deformation of their RF (Bessou and Perl, 1969; Iggo, 1969; Hensel and Iggo, 1971; Dubner et al, 1975; Kenshalo and Duclaux, 1977; LaMotte and Campbell, 1978).

Two functional groups of afferents are responsible for innocuous thermal sensation: receptors sensitive to temperatures perceived as cold, and those sensitive to warmth.

1.2.2.1.1 Peripheral Afferents Responsive to Cooling

Peripheral thermoreceptive afferents responsive to innocuous cold temperatures have been identified in various mammalian species, including rats (Iggo, 1969), cats (LaMotte and Thalhammer, 1982), dogs (Iggo, 1969), monkeys (Bessou and Perl, 1969; Iggo, 1969; Hensel and Iggo, 1971; Darian-Smith et al, 1973; Long, 1973; Dubner et al, 1975; Kenshalo and Duclaux, 1977; Long, 1977; LaMotte and Thalhammer, 1982), and humans (Konietzny and Hensel, 1983; Campero et al, 2001).

Cold afferents are characterized by: a dynamic response to cooling, a tonic discharge to sustained cold temperatures, and a suppression of activity in response to warming of their RF. Cold afferents code temperature in a complex manner: They develop a steady-state response to
adapting temperatures between approximately 20-40 °C (Bessou and Perl, 1969; Iggo, 1969; Darian-Smith et al, 1973). On average, the maximal steady-state activity of these afferents is obtained with temperatures between 29-33 ºC; however, this value varies between individual afferents and can be as low as 19 ºC (Darian-Smith et al, 1973; Dubner et al, 1975). Amongst four cold afferents innervating human hairy skin, the maximal firing frequency in response to a static cold stimulus occurred between the temperatures 22-28 ºC (Konietzny and Hensel, 1983). In monkeys, it was found that the response of cold afferents to the static maxima is often characterized by periodic bursting (Iggo, 1969; Dubner et al, 1975). As the adapting temperature was lowered, this bursting behaviour persisted; however, the time interval between bursts increased (Iggo, 1969; Darian-Smith et al, 1973; Dubner et al, 1975; Kenshalo and Duclaux, 1977). It has been suggested that this bursting behaviour may be less prominent in human cold afferents (Konietzny and Hensel, 1983; Campero et al, 2001). Another population of mechanically-insensitive, high-threshold cold afferents has been identified in monkeys and cats (LaMotte and Thalhammer, 1982). These high-threshold cold afferents respond to temperatures between 0-27 ºC. It is unclear whether their function is thermoreceptive or nociceptive. The investigators suggested that they may underlie the perception of sustained cold temperatures below 20 ºC.

Cold afferents display a graded, dynamic response to cooling of the skin by 1-10 ºC, even when the initial or final temperatures fall outside of their range of sensitivity to static thermal stimuli (Iggo, 1969; Darian-Smith et al, 1973; Dubner et al, 1975). Conversely, cold afferent activity is suppressed by dynamic warming (Iggo, 1969; Darian-Smith et al, 1973; Dubner et al, 1975), even when the final temperature of the warming step is closer to the unit’s static maximum (Iggo, 1969). Rapid cooling elicits a large transient response in cold afferents, where the peak phasic firing frequency overshoots the cell’s adapted firing rate for the final temperature. The transient response decays over 2-15 s (Darian-Smith et al, 1973; Dubner et al, 1975), and within 30 s of the implementation of a new adapting temperature, the corresponding steady-state activity is established (Iggo, 1969; Kenshalo and Duclaux, 1977). The dynamic response can be suppressed by frequent cooling steps. This temporal suppression persists for at least 10 s (Darian-Smith et al, 1973; Dubner et al, 1975), and the strength of this phenomenon is dependent on the intensity of the previous stimuli (Dubner et al, 1975).
Some cold afferents exhibit “paradoxical” discharges to noxious heating (Bessou and Perl, 1969; Long, 1973; Dubner et al, 1975; Kenshalo and Duclaux, 1977; Long, 1977; Campero et al, 2001). Bessou and Perl (1969) described a transient response to sudden increases in skin temperatures up to noxious levels. Other researchers reported the threshold for this paradoxical firing to be on average 55 °C, although previous exposure to a noxious heat stimulus lowered the threshold below 50 °C (Dubner et al, 1975). Higher core temperatures also reduced the heat threshold of cold afferents (Long, 1973; Dubner et al, 1975; Long, 1977). Kenshalo and Duclaux (1997) found that certain cold afferents developed tonic activity to an adapting temperature of 45 °C, activity which was then suppressed by rapid cooling.

Electrophysiological recordings of peripheral nerves in monkeys revealed that cold afferents were located on both hairy and glabrous skin (Iggo, 1969). The conduction speed of some of these afferents fell within the range of thinly myelinated fibres, while others were unmyelinated; similar findings were reported from studies conducted on monkeys (Iggo, 1969; Hensel and Iggo, 1971; Dubner et al, 1975). Iggo found that the afferents innervating glabrous areas were all thinly myelinated, while those whose RFs were on hairy skin could be either myelinated or unmyelinated (Iggo, 1969). This author did not find functional differences between A-δ and C afferents. The conduction velocities of the cold afferents recorded from human hairy skin ranged between 0.8-3.0 m/s² (Campero et al, 2001).

Cold afferents have spot-like RFs, ranging in diameter between 0.25 to 5 mm (Iggo, 1969; Darian-Smith et al, 1973; Dubner et al, 1975; Kenshalo and Duclaux, 1977; Campero et al, 2001). A single cold afferents can also innervate multiple discrete RFs (Bessou and Perl, 1969; Dubner et al, 1975; Kenshalo and Duclaux, 1977; Campero et al, 2001). When recording from 28 cold afferents from the fore- and hindlimbs of rhesus monkeys, Kenshalo & Duclaux did not find a difference in size or number of RFs across hairy and glabrous skin (Kenshalo and Duclaux, 1977).

It has been noted that certain A-β mechanoreceptors (specifically, SAII afferents) are also sensitive to cooling. Lowering the temperature of a probe kept at a constant pressure increases the discharge of SA afferents (Iggo, 1969; Johnson et al, 1973; Georgopoulos, 1976; Simone and Kajander, 1997). However, these large-diameter myelinated afferents are not thought to play a prominent role in thermoreception, since their sensitivity to different graded cooling steps is less than the human ability to perceive these differences (Johnson et al, 1973).
What is more, during nerve blocks, the detection of cold temperatures delivered to hairy skin is impaired only when A-δ fibre-mediated potentials are abolished (Mackenzie et al., 1975). Thus, the perception of cold is principally subserved at the periphery by thinly myelinated thermoreceptors. The functional significance of the cold sensitivity of SA afferents remains unknown, although it has been hypothesized that they may serve a modulatory role in the transmission of cold thermoreceptive information through the central nervous system (Simone and Kajander, 1997).

1.2.2.1.2 Peripheral Neurons that Respond to Warming

Peripheral thermoreceptive afferents responsive to innocuous warm temperatures have been described in the monkey (Hensel and Iggo, 1971; LaMotte and Campbell, 1978; Darian-Smith et al., 1979a; Darian-Smith et al., 1979b; Duclaux and Kenshalo, 1980). Warm afferents have also been recorded from in humans (Hallin et al., 1981; n = 5).

Warm afferents develop graded, steady-state firing frequencies to maintained stimulus temperatures between 30-40 °C (Hensel and Iggo, 1971; Duclaux and Kenshalo, 1980). In effect, the static range of warm and cold thermoreceptors overlaps. At temperatures above 40 °C, warm afferents display one of two response profiles (Hensel and Iggo, 1971; Duclaux and Kenshalo, 1980): The adapted firing frequency of some warm afferents continues to increase linearly as temperatures approach noxious levels. Amongst other warm afferents, the static firing frequency reaches a maximum between 40-43 °C, and then levels-off with higher temperatures (Hensel and Iggo, 1971; Duclaux and Kenshalo, 1980). The steady-state activity of warm afferents is suppressed by dynamic cooling (Darian-Smith et al., 1979b; Duclaux and Kenshalo, 1980).

Warm afferents display a strong response to rapid warming of their RF (Duclaux and Kenshalo, 1980), where the transient firing frequency is significantly greater than the tonic firing frequency coding the new adapting temperature (Hensel and Iggo, 1971). They respond to more intense warm pulses (up to 8 °C above the adapting temperature) with a higher dynamic firing frequency and a shorter latency in activation (Darian-Smith et al., 1979b). However, when the final temperature of a warming pulse is above 45 °C, the dynamic response of warm afferents plateaus, or changes into short bursts followed by complete cessation of activity (LaMotte and Campbell, 1978; Darian-Smith et al., 1979b). The repeated presentation of warm
stimuli at inter-stimulus intervals shorter than 60 s depresses the activity of these afferents (Darian-Smith et al, 1979b).

Warm afferents are unmyelinated (Iggo, 1969; Hensel and Iggo, 1971; LaMotte and Campbell, 1978; Darian-Smith et al, 1979b; Duclaux and Kenshalo, 1980; Hallin et al, 1981). The RFs of warm afferents are characterized as single spot-like areas (< 1 mm in diameter) (Hensel and Iggo, 1971; LaMotte and Campbell, 1978; Darian-Smith et al, 1979b; Hallin et al, 1981), although warm afferents with several receptive spots have been described (Duclaux and Kenshalo, 1980). Warm afferents are more difficult to isolate than cold units (Bessou and Perl, 1969; Hensel and Iggo, 1971), and are relatively sparse (Iggo, 1969; Hallin et al, 1981; Moss-Salentijn, 1992).

1.2.2.2 Primary Afferent Nociceptors


Nociceptive afferents can be either sensitive to a single modality, or are polymodal. Thermally insensitive, high-threshold mechanoreceptors represent a large portion of peripheral nociceptors (approximately 50% of A-δ and 10-30% of C nociceptive fibres, based on data from the hairy skin of cats and the glabrous skin of monkeys; however, this proportion may vary across body sites, skin type, and species, and may be biased by the experimental technique used to locate the RF of the afferent) (Bessou and Perl, 1969; Georgopoulos, 1976; Adriansen et al, 1983). A small group of unimodal nociceptive afferents exclusively sensitive to either noxious heat or cold stimuli have also been identified in the glabrous and hairy skin of monkeys (Georgopoulos, 1976; Meyer et al, 1991; Treede et al, 1998). However, the majority of
nociceptors respond to a combination of mechanical, thermal, and/or chemical stimuli (Bessou and Perl, 1969; Georgopoulos, 1976; LaMotte and Thalhammer, 1982).

Nociceptive afferents can be grouped according to their response to mechanical stimuli. Mechanically sensitive nociceptive afferents (MSAs) have a higher mechanical threshold than A-β mechanoreceptors (36 g/mm² for mechanically sensitive nociceptors, compared to 0.1-1 g for low-threshold mechanoreceptors) (Bessou and Perl, 1969; Georgopoulos, 1976; Järvilehto et al, 1976; Davis et al, 1993; Schmidt et al, 1997). In contrast, mechanically insensitive nociceptive afferents (MIAs) can have mechanical thresholds as high as 150.0 g/mm², or are mechanically unresponsive (Meyer et al, 1991; Davis et al, 1993).

The threshold of nociceptive afferents to thermal stimuli falls within the range of 39-53 °C (Georgopoulos, 1976; Georgopoulos, 1977; Van Hees and Gybels, 1981; LaMotte and Thalhammer, 1982; Treede et al, 1995; Campero et al, 1996; Schmelz et al, 1996; Schmidt et al, 1997; Treede et al, 1998). It has been suggested that A-δ polymodal nociceptors exposed to a prolonged noxious heat stimulus exhibit one of two response patterns (Treede et al, 1995; Treede et al, 1998). Type I mechano-thermal nociceptive afferents, which have high heat thresholds (53 °C), slowly increase their firing rate as the heat stimulus progresses. Type II mechano-thermal nociceptive afferents have lower heat thresholds (median temperature of 46 °C), and respond more quickly to noxious heat stimuli. The response of Type II afferents rapidly decays. In comparison to Type I A-δ mechano-thermal nociceptive afferents, Type II afferents have a higher mechanical threshold (Treede et al, 1998). In one study, type II nociceptive afferents were not found in glabrous skin (Treede et al, 1995).

Myelinated and unmyelinated nociceptive afferents in cats, monkeys, and humans sensitized by exposure to a supra-threshold noxious heat stimulus exhibit lower thermal and mechanical thresholds (Bessou and Perl, 1969; LaMotte and Thalhammer, 1982; 1479 LaMotte, R.H. 1982). This sensitization can persist for as long as two hours (Bessou and Perl, 1969). Conversely, the pulsed presentation of a noxious heat stimulus at a short inter-stimulus interval has been shown to suppress the excitation of nociceptors (Price et al, 1977; Adriaensen et al, 1984).

Nociceptive afferents sensitive to noxious heat are thought to be more common than those responsive to noxious cold temperatures (Bessou and Perl, 1969; Georgopoulos, 1976;
Georgopoulos, 1977; Campero et al, 1996). However, a study investigating A-δ polymodal nociceptive afferents in rats found that they all responded to temperatures below 0 °C (Simone and Kajander, 1997). Approximately 20% of cold-responsive nociceptive afferents begin firing at temperatures between 25-31 °C; the remaining cold-responsive nociceptors have cold thresholds below 20 °C (Georgopoulos, 1976; Simone and Kajander, 1997).

Activity at A-δ and C nociceptive afferents can also be induced by chemical agents such as mustard oil, capsaicin, bradykinin, and histamine (Adriaensen et al, 1983; Meyer et al, 1991; Davis et al, 1993; Schmelz et al, 1996; Serra et al, 2004). The firing rates of polymodal nociceptive afferents elicited by noxious cold and chemical irritants are relatively weak in comparison to the response of these nociceptors to heat and mechanical stimuli (Bessou and Perl, 1969; Georgopoulos, 1976; Van Hees and Gybels, 1981; LaMotte and Thalhammer, 1982; Campero et al, 1996). However, the response of MIAs to an intradermal injection of a mixture of chemical inflammatory mediators is robust (Meyer et al, 1991; Davis et al, 1993). Exposure to chemical irritants can lower the mechanical and/or thermal thresholds of nociceptive afferents (Davis et al, 1993; Schmelz et al, 1996; Yeomans and Proudfit, 1996; Yeomans et al, 1996; Serra et al, 2004).

The RFs of A-δ and C nociceptive fibres are of varied shape. Some nociceptors have punctate RFs approximately 1 mm in diameter; others innervate areas of 5-22 mm in diameter (Bessou and Perl, 1969; Georgopoulos, 1976; Hallin et al, 1981; Van Hees and Gybels, 1981; Bromm et al, 1984; Meyer et al, 1991; Campero et al, 1996; Schmelz et al, 1996; Schmidt et al, 1997; Schmidt et al, 1997). Nociceptive afferents can also have non-continuous fields, innervating various spots (Georgopoulos, 1976; Hallin et al, 1981; Schmidt et al, 1997). In humans, the RF size of unmyelinated nociceptive afferents was found to vary significantly by location (Hallin et al, 1981; Schmidt et al, 1997). The RFs of nociceptive afferents innervating the toe were described as being smaller than those of the foot, which in turn were smaller than those localised on the leg (Schmidt et al, 1997). Correspondingly, human participants were better able to localize noxious stimuli on the foot than on the leg (Schmidt et al, 1997).

1.2.2.3 Spinal Termination Patterns

The following findings are from studies conducted on rats (Hellon and Misra, 1973; Hellon and Mitchell, 1975; Dickenson et al, 1979; Bouhassira et al, 1995), guinea pigs (Sugiura et al, 1986), mice (Dhaka et al, 2008), rabbits (Dickenson et al, 1979), cats (Wall, 1967;
Christensen and Perl, 1969; Light and Perl, 1977; Dostrovsky and Hellon, 1978; Brown et al., 1978; Light and Perl, 1979a; Light and Perl, 1979b; Craig and Hunsley, 1991; Han et al., 1998; Andrew and Craig, 2001), and monkeys (Willis et al., 1973; LaMotte, 1977; Light and Perl, 1977; Kumazawa and Perl, 1978; Price et al., 1978; Light and Perl, 1979a; Light and Perl, 1979b; Willis et al., 1979; Kenshalo et al., 1982; Maixner et al., 1986; Ferrington et al., 1987; Dostrovsky and Craig, 1996).

The axons of thermal and nociceptive afferents innervating the body (except the orofacial region) join a posterolateral (Lissauer’s) tract and send collaterals into the dorsal horn at various levels of the spinal cord to terminate on second-order neurons in the dorsal horn of the spinal cord (LaMotte, 1977; Light and Perl, 1977; Sugiura et al., 1986). Important projection tracts – conveying information regarding temperature, pain, and crude touch – originate from dorsal horn neurons. Most second-order neuronal axons immediately decussate, and then project to the brain through the spinothalamic tract (STT; the main ascending projection located on the anterolateral quadrant of the spinal cord), or through other ascending tracts, including the spinoreticular and spinomesencephalic tracts. Approximately 15% of the ascending axons remain on the ipsilateral side (Apkarian and Hodge, 1989).

The spinal cord grey matter consists of ten cytoarchitectonically distinct layers referred to as laminae I-XI (Rexed, 1952): The dorsal horn extends from lamina I through VI; the intermediate zone includes laminae VII and X; the ventral horn contains laminae VIII, IX, and IX. First-order afferents follow termination patterns in these laminae according to their morphological and functional characteristics. However, no lamina is the exclusive site of termination for any one population of afferents. Differences in organization at the various levels of the spinal cord have also been noted (Sugiura et al., 1986). Dorsal horn neurons can receive specific or convergent input from innocuous and noxious peripheral units. A proportion of spinal neurons feature large and complex RFs with sensitive central areas, less effective edges (Wall, 1967; Christensen and Perl, 1970; Price et al., 1978; Ferrington et al., 1987; Dostrovsky and Craig, 1996), and inhibitory regions (Willis et al., 1973; Ferrington et al., 1987).

Several studies have been undertaken to describe the responses of dorsal horn cells (see for example: Wall, 1967; Hellon and Misra, 1973; Dostrovsky and Hellon, 1978; Kumazawa and Perl, 1978), in particular those that comprise the spinothalamic tract (Christensen and Perl, 1970; Willis et al., 1973; Price et al., 1978; Kenshalo et al., 1982; Ferrington et al., 1987;
Cells in lamina I (the marginal zone) receive monosynaptic input from thinly myelinated and unmyelinated nociceptors (Christensen and Perl, 1970; Light and Perl, 1979b; Sugiura et al., 1986). Thus, nociceptive neurons are often found in the marginal zone (Christensen and Perl, 1970). These neurons can signal graded increases of noxious thermal and mechanical intensity (Ferrington et al., 1987). The RFs of nociceptive lamina I neurons are relatively small (e.g., spanning a few digits of the paw) (Christensen and Perl, 1970; Willis et al., 1973; Price et al., 1978; Ferrington et al., 1987). Distal RFs are smaller than those located more proximal to the spinal cord (Willis et al., 1973). Lamina I cells may play a role in pain localization, due to the small size of their RFs (Ferrington et al., 1987; Craig, 2003).

Distinct populations of lamina I neurons have been characterized: One group responds exclusively to noxious mechanical and/or noxious heat stimulation of their RF (referred to as nociceptive-specific, NS) (Christensen and Perl, 1970; Willis et al., 1973; Kumazawa and Perl, 1978; Price et al., 1978; Ferrington et al., 1987; Dostrovsky and Craig, 1996; Zhang et al., 2006). Polymodal nociceptive neurons have been identified that are transiently sensitive to innocuous cooling, as well as exhibiting sustained firing to noxious cold, heat, and mechanical stimuli (Christensen and Perl, 1970; Ferrington et al., 1987; Craig and Bushnell, 1994; Zhang et al., 2006). These units, termed heat, pinch, and cold (HPC) cells, respond to temperatures below 25 °C and above 45 °C (Craig and Bushnell, 1994; Dostrovsky and Craig, 1996; Han et al., 1998; Craig, 2003, Davidson et al, 2008). NS and HPC cells are thought to receive mostly A-δ and C nociceptive input, respectively (Craig, 2003). A group of polymodal cells coding a wider dynamic range of mechanical intensities, including a weak response to innocuous pressure and brushing, have also been recorded from the marginal zone (wide-dynamic-range cells, WDR) (Ferrington et al., 1987; Dostrovsky and Craig, 1996).

In lamina I there are also pure thermoreceptive units activated only by innocuous cool (Cold cells) or warm temperatures (Warm cells) (Hollon and Misra, 1973; Dostrovsky and Hollon, 1978; Kumazawa and Perl, 1978; Dickenson et al., 1979; Dostrovsky and Craig, 1996; Zhang et al, 2006). In fact, afferents expressing the innocuous cold-sensitive transient receptor potential channel TRPM8 project primarily to lamina I (Dhaka et al., 2008). Like their peripheral counterparts, Cold cells show a graded phasic and tonic response to cooling. This response
plateaus at temperatures around 15 °C (Craig and Bushnell, 1994; Dostrovsky and Craig, 1996; Zhang et al, 2006). Central Cold cells also exhibit paradoxical excitation to noxious heat above 44 °C (Dostrovsky and Craig, 1996; Zhang et al, 2006). Warm cells receive monosynaptic input from C fibre thermoreceptors, and show a graded response to innocuous warming, developing a plateaued response to noxious heat temperatures (Andrew and Craig, 2001). As in the periphery, the relative abundance of second-order dorsal horn neurons sensitive to warming is low in comparison to the number of cool-specific neurons (relative ratio of 1 : 10-20) (Dostrovsky and Hellon, 1978; Dickenson et al, 1979; Andrew and Craig, 2001). The RF size of thermoreceptive cells is generally larger than those of nociceptive cells in this lamina, and can span the entire hindlimb of an animal (Andrew and Craig, 2001; Zhang et al, 2006). The RFs of spinal thermoreceptors are also much larger when compared to the small, punctuate RFs of peripheral thermoreceptive cells; this finding has led to the suggestion that a single spinal thermoreceptive neuron receives convergent input from many peripheral afferents (Hellon and Mitchell, 1975; Dickenson et al, 1979; Dostrovsky and Craig, 1996; Andrew and Craig, 2001; Zhang et al, 2006).

Unmyelinated and thinly myelinated afferents have arborizations into lamina II (the substantia gelatinosa) (Light and Perl, 1979a; Sugiura et al, 1986). The afferents that have been characterized responded to low- and high-threshold mechanical stimuli (Sugiura et al, 1986). Another study found evidence indicating that nociceptors, but not cold thermoreceptors, terminate in lamina II (Dhaka et al, 2008). Few of the ascending fibres comprising the STT originate from this dorsal horn layer (Apkarian and Hodge, 1989). Instead, this lamina mainly consists of interneurons, with connections to lamina I cells, a few to lamina V cells, as well as interneurons terminating a segment rostrally or caudally.

The nucleus proprius of the dorsal horn includes laminae III-V. Laminae III-V neurons receive innocuous touch information from large- and small-diameter mechanoreceptors sensitive to hair brush and innocuous pressure (including SAI receptors) (Willis et al, 1973; Brown et al, 1978; Kumazawa and Perl, 1978; Price et al, 1978; Light and Perl, 1979b). Lamina V, as well as deeper laminae VI-VII, is the site of arborization of collaterals from muscle afferent fibres (Brown and Fyffe, 1979). Lamina V neurons also receives nociceptive input from thinly myelinated afferents (Light and Perl, 1979b).
The cell bodies of spinothalamic tract axons activated by light brushing localize to laminae IV-V of the dorsal horn (Willis et al., 1973). These neurons show a graded response to increasing intensities of mechanical stimulation (Wall, 1967; Willis et al., 1973; Price et al., 1978), and some are also likely to display a strong response to noxious heating (WDR cells) (Willis et al., 1973; Price et al., 1978). Their response is optimal for intensity coding (Maixner et al., 1986; Craig, 2003). High-threshold afferents (NS neurons) similar to those identified in lamina I have also been characterized in lamina V (Willis et al., 1973).

The RFs of laminae IV and V cells vary in size, and can include a single digit, span the dorsal surface of the foot, or extend across dermatomes (Willis et al., 1973; Price et al., 1978). Lamina V neurons in particular have larger RFs than similar lamina I or IV cells (Wall, 1967; Willis et al., 1973). Unlike the cells in laminae I and IV, cells in lamina V do not follow a somatotopic organization (Wall, 1967; Willis et al., 1973).

Laminae IV-VIII contain neurons that fire to joint or muscle manipulation (Willis et al., 1973).

1.2.2.4 Thalamic and Cortical Projections

Studies investigating the termination patterns of STT axons and thalamic sensory neurons have been conducted on rats (Dostrovsky and Guilbaud, 1988; Zhang et al., 2006), cats (Craig and Burton, 1981; Smith et al., 1991; Craig and Dostrovsky, 1991; Craig and Dostrovsky, 2001), monkeys (Giesler et al., 1981; Apkarian and Hodge, 1989; Bushnell and Duncan, 1989; Craig et al., 1994; Blomqvist et al., 2000; Davidson et al., 2008), and humans (Lenz et al., 1993; Davis et al., 1999).

STT afferents ascend to the midbrain through the contralateral, anterolateral quadrant of the spinal cord. In monkeys, a considerable proportion of these neurons originate from the upper three segments of the spinal cord (Apkarian and Hodge, 1989). Approximately half of STT afferents have their cell bodies in lamina I, and the remainder have their cell bodies in laminae IV-V, and deeper laminae VII-VIII (Apkarian and Hodge, 1989). In lower segments, the proportion of lamina I projections drops to 34% (lower cervical) and 18% (lower lumbar) (Apkarian and Hodge, 1989). STT neurons terminate onto cells of the lateral and/or medial thalamus (Giesler et al., 1981). Neurons that project to the lateral, or both the lateral and medial thalamus are often from the dorsal horn of the spinal cord and respond to A-δ and C fibre
volleys. Neurons that only project to the medial thalamus are mostly from the intermediate zone or ventral horn (Giesler et al., 1981). In turn, thalamic neurons ascend to various loci in the cerebral cortex.

Information relayed through the lateral and medial thalamus are thought to have differential roles in pain perception (for a topical review, see Treede et al., 1999). The somatosensory information projecting through the lateral thalamus is understood to mediate the sensory-discriminative dimension of pain (Treede et al., 1999). Generally, STT neurons terminating at lateral thalamic nuclei originate from laminae I and V (Apkarian and Hodge, 1989). Anterior lateral thalamic nuclei, including the ventroposterior lateral and inferior nuclei (VPL and VPI) are important targets of laminae IV-V STT neurons (Apkarian and Hodge, 1989; Craig, 2006), although some marginal zone thermoreceptive and nociceptive neurons are also known to terminate here (Ferrington et al., 1987; Andrew and Craig, 2001; Zhang et al., 2006; Davidson et al., 2008). Accordingly, STT neurons terminating on this thalamic region have complex RFs responsive to mechanical and/or thermal stimuli that span a single digit, or include an entire limb (Giesler et al., 1981; Davidson et al., 2008). The RFs of high-threshold STT neurons terminating at the lateral thalamus are smaller than those of WDR neurons (Giesler et al., 1981; Davidson et al., 2008).

Cells in the VPL project to the primary and secondary somatosensory cortex (SI & SII, respectively) (Treede et al., 1999). The somatosensory areas are thought to play a role in coding the intensity, location, and duration of nociceptive input, and are often activated during noxious thermal tasks (reviewed in Bushnell et al., 1999). The SII may have a more specific role in sensory integration across modalities (Treede et al., 1999). Cortical nociceptive cells in the postcentral gyrus are organized somatotopically, have small RFs, and show a graded response to increasing thermal heat stimulus intensity (Kenshalo, 1988; Kenshalo et al., 2000). Cells in SI can also be sensitized by previous exposure to noxious stimuli, paralleling the phenomena of allodynia and hyperalgesia (Whitsel et al., 2009). There is also a response in the SI to innocuous warming and cooling of the skin (Duclaux et al., 1974; Chatt and Kenshalo, 1977; Tsuboi et al., 1993; Craig et al., 1996).

A high proportion of marginal zone projections terminate at the posterior thalamus (Apkarian and Hodge, 1989; Craig, 2006; Davidson et al., 2008). This input is mostly nociceptive, sensitive to both thermal and chemical irritants, although some units also respond
to innocuous temperatures (Zhang et al., 2006; Davidson et al., 2008). The posterior aspect of the ventral medial nucleus (VMpo) receives dense lamina I input from nociceptive (NS and HPC) and thermoreceptive (Warm and Cold) cells (Craig and Hunsley, 1991; Craig et al., 1994; Dostrovsky and Craig, 1996; Blomqvist et al., 2000). Microstimulation of this thalamic area in human patients can elicit reports of cool, warmth, and pain (Lenz et al., 1993; Davis et al., 1999). Cells from the VMpo project to the posterior region of the insular cortex (see Blomqvist et al., 2000). The insula is implicated in the cognitive control of various homeostatic functions (Craig, 2009). Activity at the insular cortex is also seen in response to both non-painful and painful thermal stimuli (Casey et al., 1996; Craig et al., 1996; Ostrowsky et al., 2002). Thus, the involvement of the insula in thermal perception may reflect the relevance, and motivational valence, of these external stimuli to physiological function (e.g., thermoregulation) (Craig, 2002; Craig, 2009).

Nuclei of the medial thalamus relay STT input that is thought to be mainly relevant to the affective evaluative components of pain perception (Apkarian and Hodge, 1989; Treede et al., 1999). Neurons in these nuclei respond to noxious stimulation over large, often bilateral, and complex RFs, which can span the entire body (Giesler et al., 1981; Dostrovsky and Guilbaud, 1988; Dostrovsky and Guilbaud, 1990). It is unlikely that nociceptive neurons in the medial thalamus transmit information regarding stimulus location; however, these neurons have been shown to code stimulus intensity with high fidelity (Bushnell and Duncan, 1989). The response of medial thalamic neurons in monkeys to noxious stimuli is modulated by attention, and the administration of analgesics (Bushnell and Duncan, 1989).

In cats and rats, the medial thalamic submedial nucleus (Sm) receives both thermoreceptive (mostly cold) and nociceptive lamina I input. Yet, neurons in the Sm nucleus fire only in response to nociceptive input; they are not excited by innocuous thermal stimuli (Dostrovsky and Guilbaud, 1988; Dostrovsky and Guilbaud, 1990; Craig and Dostrovsky, 1991). It has been hypothesized that, in humans, a STT projection to the ventral caudal area of medial dorsal nucleus (MDvc) is phylogenetically similar to the input into the Sm seen in other species (Craig and Burton, 1981; Craig and Dostrovsky, 1991; Craig et al., 1994). It has been further postulated that medial thalamic nuclei, and the MDvc in particular, project to the anterior cingulate (ACC) (Craig et al., 1996; Vogt, 2005), as well as the prefrontal areas of the cortex (PFC) (Craig et al., 1996). Alongside the SI, SII, and insula, activity at the ACC and the PFC is
often elicited during painful tasks (Craig et al., 1996; Hutchison et al., 1999; Treede et al., 1999). The ACC has been implicated in the affective evaluation of pain (Rainville et al., 1997; Hutchison et al., 1999; Treede et al., 1999). The function of the cingulate cortex in pain has also been construed as an evaluation of the personal salience of the stimulus, thus encompassing factors such as attention, anticipation, and behavioural output (Vogt, 2005).

Medial thalamic centre lateral (CL), centre median (CM), and parafascicular (Pf) nuclei are also known to receive STT input (Giesler et al., 1981; Apkarian and Hodge, 1989). These areas connect with areas involved in motor control, including the basal ganglia and the premotor cortex (Craig and Burton, 1981). Their role in pain perception is unclear.

### 1.3 Psychophysical Data of Thermoreceptive and Nociceptive Function

While the sensory modalities are differentially encoded at the periphery by specialized populations of afferents, activity of the specific peripheral neurons is insufficient to predict the perception of warmth, cold, or pain in individuals (Hardy and Oppel, 1937; Van Hees and Gybels, 1981; Bromm et al., 1984). For example, in the case of noxious heat, nociceptors begin responding to temperatures below the heat pain threshold. Only as the intensity of the stimulus is raised, and the firing frequency of these units increases, does a reliable relationship between firing rate and pain ratings emerge (Van Hees and Gybels, 1981).

Complementing electrophysiological data, psychophysical studies measure and characterize the perceptual experience of normal and pathological pain. These studies have elucidated various phenomena, which inform on underlying somatosensory mechanisms. In fact, a number of variables affect the transmission of somatosensory information through the central nervous system, including the intensity, location, temporal pattern, and spatial area of stimulation. Second-order neurons in the nociceptive pathway are also subject to top-down modulation. The perception of pain itself varies according to cognitive and psychosocial factors. Effectively, the experience of pain varies between individuals in terms of: the intensity of pain reported; the motivational and emotional valence attributed to the experience; as well as cognitive and behavioural responses that result from it.
1.3.1 Detection of Noxious and Innocuous Thermal Stimuli

Detection thresholds are a common measurement of thermoreceptive and nociceptive functioning. In human studies, from a base skin temperature between 32-34 °C, the temperature of a contact thermode placed on the skin is increased or decreased until individuals first feel innocuous warmth (warmth detection threshold, WDT), cold (cold detection threshold, CDT), heat pain (heat pain threshold, HPT), or cold pain (cold pain threshold, CPT). These thresholds amongst healthy volunteers are approximated to be: 34 °C; 32 °C; 45 °C; 15 °C, respectively. However, different studies have reported these threshold values to range between 33-36 °C; 28-32 °C; 39-46 °C; and 10-25 °C (for the WDT, CDT, HPT, and CPT, respectively; Wahren et al, 1989; Yarnitsky and Ochoa, 1990; Greenspan et al, 1993; Taylor et al, 1993; Meh and Denislic, 1994; Hagander et al, 2000; Harju, 2002; Rolke et al, 2006; Wasner and Brock, 2008).

Threshold values are variable between individuals (Meh and Denislic, 1994; Hagander et al, 2000; Rolke et al, 2006); The CDT and CPT show the least and most variance, respectively (Rolke et al, 2006). Threshold measurements also fluctuate according to measurement parameters, including: the size of the stimulation area; testing paradigm; body site tested; and individual characteristics, such as sex and age. When sex differences are found, females typically show lower thermal pain thresholds (Feine et al, 1991). Skin temperature has not been shown to be a significant factor in threshold measurements (Meh and Denislic, 1994; Pertovaara et al, 1996; Hagander et al, 2000).

1.3.2 Differential Contribution of A-δ and C Fibre Nociceptors to the Perception of Pain

Because of the difference in conduction velocities between A-δ and C afferents, nociceptive input enters the central nervous system in two waves. In certain paradigms of noxious stimulation, the differential contribution of these two afferent types is perceptually discernable. The faster conducting A-δ afferents are associated with the initial perception of “sharp” pain, which is well localized to the site of injury or harm (“first pain”; Price et al, 1977). This is followed by a dull, more diffuse “burning” sensation mediated by unmyelinated input that often outlasts the noxious stimulus itself (“second pain”; Price et al, 1977; Ochoa and Torebjörk, 1989). In the spinal system, blocking A-δ input stops the perception of first pain without affecting second pain sensations (Price et al, 1977). The two temporal components of pain are oppositely affected by the presentation of repetitive noxious heat stimuli: As the heat pulses progress, the perceived intensity of the sharp first pain decreases while that of second
pain increases (Price and Dubner, 1977). Since the activity of both peripheral A-δ and C nociceptive afferents decreases during a train of heat pulses, the temporal summation of second pain reflects a central process by which dorsal horn nociceptive neurons become sensitized to peripheral C fibre input (Price and Dubner, 1977). This effect has been selectively inhibited by the administration of an NMDA receptor antagonist, which blocks the spinal neurotransmitter system that is thought to be responsible for the facilitative process (Price et al, 1994).

Craig (2003) proposed that the aversive perceptual qualities of sharp and burning pain reflect the differential input received by lamina I NS and HPC cells, respectively. Recordings from STT neurons in laminae I and V of monkeys revealed that the activity of HPC cells (and not that of WDR neurons) faithfully paralleled the perceptual increase in second pain intensity following repeated contact with noxious heat (Craig, 2004). Thus, this research insinuated the possibility that nociceptive inputs perceived as sharp and burning pain are independently transmitted, and modulated, through the central nervous system.

1.3.3 Spatial Summation and Discrimination

An interaction between the intensity of the stimulus and the area of cutaneous stimulation affects the ability to detect both innocuous warmth (Hardy and Oppel, 1937; Kenshalo et al, 1967; Cain, 1973; Marks and Stevens, 1973; Nadel et al, 1973; Stevens et al, 1974; Greenspan and Kenshalo, 1985; Green and Zaharchuk, 2001), and cold (Rózsa and Kenshalo, 1977; Stevens and Marks, 1979; Greenspan and Kenshalo, 1985; Green and Zaharchuk, 2001). The intensity of the stimulus required for the detection of warmth, cold, and pain decreases as the area of stimulation increases (Hardy and Oppel, 1937; Kenshalo et al, 1967), even as stimulation spans two dermatomes (Marks and Stevens, 1973) or includes two bilateral sites (Cain, 1973; Rózsa and Kenshalo, 1977). However, as the intensity of the stimulus increases, the relative importance of stimulus area lessens (Stevens and Marks, 1979). Similarly, for the WDT, increasing the area of stimulation above approximately 10 cm² will not further facilitate the detection of warmth, and the threshold plateaus (Kenshalo et al, 1967).

The occurrence of thermally insensitive fields in the area of thermal stimulation may over-estimate the contribution of a central process of spatial summation in the detection of thermal stimuli (Green and Cruz, 1998). The sparse innervation of peripheral thermoreceptors results in sites insensitive to warm and cool temperatures (Green and Cruz, 1998; Defrin et al, 2009). When a thermal stimulus is presented on these insensitive fields alone, human
participants only detect the stimulus as it approaches noxious temperatures. Expanding the area of stimulation increases the chance of incorporating sites innervated by thermoreceptors, and detection thresholds decrease remarkably. In this scenario, the relationship between thermal thresholds and stimulation area does not rely on the activity of various peripheral fibres summat ing on a central neuron (Green and Cruz, 1998; Defrin et al, 2009). Still, the phenomenon of spatial summation is thought to reflect the convergence of various peripheral axons onto a common central afferent (Defrin et al, 2009). At low stimulus intensities, input from a larger area is more effective at activating central neurons. At higher (supra-threshold) stimulus intensities, however, the activity of fewer fibres (i.e., smaller area) is sufficient to elicit a response from the central neuron.

Spatial summation and discrimination are inversely related, such that, as the influence of summation diminishes, the perceptual ability to discriminate between one or two stimuli improves (Cain, 1973; Taus et al, 1975; Defrin et al, 2006). One study tested the ability to localize thermal stimuli using radiant heat (i.e., without mechanical clues), and found that it was relatively poor: An above-threshold stimulus delivered to either the front or back of the torso was mistakenly localized in 15% of trials (Cain, 1973). Yet, these participants were never unsure of the presence of a heat source.

The perception of pain is also dependent on the area of stimulation (Kojo and Pertovaara, 1987; Price et al, 1989; Douglass et al, 1992; Bouhassira et al, 1995; Defrin and Urca, 1996; Defrin et al, 2006; Defrin et al, 2009). Increasing the stimulation area on the forearm or hand lowers the HPT (Price et al, 1989; Douglass et al, 1992; Defrin and Urca, 1996; Defrin et al, 2006; Defrin et al, 2008a) and CPT (i.e., a painful response elicited by a higher absolute temperature) (Defrin et al, 2008a). When using a 0.25 cm\(^2\) thermode, Defrin and Urca (1996) reported the average HPT in their sample to be 47.7 °C. At stimulation areas greater than 15 cm\(^2\), the HPT was approximately 42 °C (Defrin and Urca, 1996; Defrin et al, 2006). Increasing the stimulation area also increases the perceived intensity of the stimulus (Price et al, 1989; Douglass et al, 1992; Defrin and Urca, 1996; Defrin et al, 2008b). Spatial summation of pain occurs across adjacent dermatomes (Douglass et al, 1992; Defrin et al, 2008a). A distance of 10 cm is required to effectively discriminate the presence of two noxious heat stimuli (Price et al, 1989; Defrin et al, 2006), and a separation of 10-20 cm to overcome the effect of spatial summation (Price et al, 1989; Defrin et al, 2006). However, the ability to localize a single
noxious stimulus is comparable to that for innocuous mechanical pressure (Ochoa and Torebjörk, 1989; Koltzenburg et al, 1993; Moore and Schady, 1995), and does not significantly change during a nerve block of myelinated A-β afferents (Koltzenburg et al, 1993).

1.3.4 Body Site Differences

Differences in thermal sensitivity at various body regions have been measured in relation to sensory thresholds, spatial summation (Kenshalo et al, 1967; Stevens et al, 1974), and thermoregulation (Nadel et al, 1973).

The detection of warm and cool stimuli applied to the face requires lower stimulus intensities than all other body sites (Kenshalo et al, 1967; Nadel et al, 1973; Stevens et al, 1974; Rolke et al, 2006). In turn, the torso and upper limbs have lower detection thresholds than the lower extremities to innocuous thermal stimuli. This is to be contrasted with differences across body sites in the perception of fine touch, where the face and extremities all show high tactile acuity (Stevens and Choo, 1998). Like in the fine touch pathway, differential sensitivity to thermal and noxious stimuli across body sites is assumed to reflect variations in innervation density at the periphery, and central integration.

Comparisons in thermoreception between the back and upper extremities are equivocal (Kenshalo et al, 1967; Stevens et al, 1974). Warm and cold detection threshold testing at the thenar eminence (i.e., the palmar surface at the base of the thumb) and the lower back reveals no significant difference at these two sites (Stevens and Choo, 1998). Conversely, the warm-cold difference limen (i.e., the temperature interval between perceiving warmth and cool) is smaller when tested on the thenar eminence than on the abdomen (Meh and Denislic, 1994). The forearm is more sensitive than the back for the detection of warmth through radiant or conducted heat onto small areas (< 9 cm²; Kenshalo, 1967). With large areas of stimulation (> 9 cm²), the WDTs across all body sites converge (Kenshalo et al, 1967). Similarly, when stimulating an area of 22 cm² to elicit a constant level of perceived warmth, other authors found that a similar irradiation level was needed at the back and forearm (Stevens et al, 1974).

The perceptual magnitude estimations in response to a fixed warmth stimulus are higher in response to stimulation of the back and abdomen than the thigh or calf (Stevens et al, 1974). The hand also has a lower WDT than the foot (Hagander et al, 2000). No difference is found between hairy and glabrous skin of the hand (Defrin et al, 2009).
The CDT is significantly lower at the thenar eminence than at the forearm, calf, or plantar surface of the foot (Greenspan et al, 1993; Hagander et al, 2000). Relative decreases in sensitivity to innocuous cold across body sites are accompanied by even greater declines in sensitivity to warmth (Stevens and Choo, 1998; Hagander et al, 2000).

For noxious thermal thresholds, differences across body sites other than the face (most sensitive), hand, and foot (least sensitive) have not been thoroughly documented, and may be harder to detect due to greater inter-individual variability in response (Riley III et al, 1998; Hagander et al, 2000; Rolke et al, 2006). Skin type may play a greater role in determining pain thresholds than comparisons between upper and lower extremities (Taylor et al, 1993; Pertovaara et al, 1996; Hagander et al, 2000). The glabrous skin of the hand and foot have lower CPTs (Harrison and Davis, 1999; Hagander et al, 2000), and possibly HPTs (Taylor et al, 1993; Hagander et al, 2000), than the volar surface of the arm and the dorsum foot, respectively.

1.3.5 Integration between Sub-modalities

Certain peripheral and central afferents are excited by specific stimulus modalities. This is in agreement with the “labelled-line” theory of somatosensation (Craig, 2003). However, there is cross-talk and integration between neuronal groups, as well as top-down modulation of spinal transmission. Wall and Melzack classically postulated a gating mechanism by which the perception of pain could be inhibited by concurrent innocuous mechanical stimulation (Wall and Melzack, 1965). In fact, dynamic mechanical stimuli (i.e., a changing pressure applied to the skin) can reduce the perception of thermal pain (Green and Pope, 2003). During painful intraneural stimulation, positioning a vibrational stimulus on the area of projected pain dampened the intensity of the perception (Bini et al, 1984). These same researchers reported that an innocuous cold stimulus had the next-best analgesic effect. No effective pain reduction was obtained by presenting a warm stimulus (Bini et al, 1984).

Convergent lines of evidence suggest that the threshold for cold pain depends on the integration of nociceptive and thermoreceptive channels (Figure 1-1). Activity of some polymodal nociceptors responding to cold stimuli begins at temperatures that are not perceived to be painful. The effectiveness of ascending nociceptive drive in producing the perception of cold pain is modulated (inhibited) by the concurrent activity at cold thermoreceptors. This inhibition can be temporarily released through a selective block of A-δ fibres, which results in cold allodynia (where individuals report pain in response to stimulation at higher absolute
temperatures) (Mackenzie et al., 1975; Wahren et al., 1989; Yarnitsky and Ochoa, 1990). At the same time, the ability to perceive cold is lost, and the pain assumes a hot burning quality (Wahren et al., 1989; Yarnitsky and Ochoa, 1990; Davis, 1998; Defrin et al., 2006).

A-δ fibre blocks do not affect the perception of warmth or heat pain (Fruhstorfer, 1984; Ochoa and Torebjörk, 1989; Wahren et al., 1989). However, as the block (induced by constriction) begins to affect unmyelinated afferents, the threshold for the detection of warmth increases, while the heat pain threshold remains unchanged (Yarnitsky and Ochoa, 1991). This likely reflects the higher density of nociceptors compared to warm thermoreceptors (Yarnitsky and Ochoa, 1991).

**Figure 1-1. Proposed circuitry underlying cold pain perception.** The figure depicts the suggested interaction between nociceptive (in green) and cold thermoreceptive (in red) pathways, and their response to a uniform cold stimulus. The green shapes symbolize nociceptive afferents and subsequent spinothalamic nociceptive neurons. Ascending cold thermoreceptive information is depicted in red. At the periphery, both nociceptive and cold thermoreceptive afferents are excited by the presence of a cold temperature stimulus impinged on the skin. Cold thermoreceptors also have ongoing activity at normal skin temperature. In turn, nociceptive and thermoreceptive afferents elicit activity at spinothalamic lamina I nociceptive HPC cells, and in lamina I Cold cells, respectively. The inhibitory role that thermoreceptive information has on this nociceptive channel results in this stimulus not being perceived as painful.
1.3.6 Paradoxical Heat during Cooling

Several investigators have noted that paradoxical sensations of warmth or heat (paradoxical heat sensations, PHS) can be elicited by the dynamic cooling of skin (Dyck et al., 1993; Greenspan et al., 1993; Harrison and Davis, 1999; Davis and Pope, 2002; Harju, 2002; Green and Pope, 2003; Rolke et al., 2006). The complementary phenomenon where heating is mistaken for cold rarely occurs (Green, 1977; Greenspan et al., 1993). PHS occurs during the cooling (Hämäläinen et al., 1982; Harrison and Davis, 1999; Susser et al., 1999; Green and Pope, 2003) or re-warming phase back to a thermally neutral baseline (Davis and Pope, 2002; Davis et al., 2004) of the stimulus. While PHS can occur following a decrease in temperature from thermal indifference (i.e., a baseline temperature perceived to be neither warm nor cool; 6-12% of trials), it is more frequent when the cooling ramp is preceded by a heating stimulus (30-66% of trials) (Hämäläinen et al., 1982; Hansen et al., 1996; Susser et al., 1999). The perception of PHS correlates in a time-locked manner with activation of the insular cortex (Davis et al., 2004). Reaction time measurements suggest that PHS are conducted through peripheral unmyelinated afferents (Hämäläinen et al., 1982; Susser et al., 1999).

Two mechanisms have been hypothesized to explain PHS (Susser et al., 1999). In both scenarios, normal physiological functioning includes cold-evoked inhibition of nociceptive pathways. The close temporal proximity of warm and cool stimuli is thought to transiently suppress A-δ thermoreceptive afferents. In the first hypothesis, PHS was suggested to be a peripherally-mediated phenomenon, where the inhibition of C nociceptive afferents by A-δ thermoreceptive afferents is reduced. In the second mechanism, the modulation of nociceptive information by cold thermoreceptive neurons occurs centrally. Thus, the reduced activity of spinal Cold neurons leads to a decreased inhibition of pain. The occurrence of PHS was found to be more frequent amongst multiple sclerosis (MS) patients than healthy controls (Hansen et al., 1996). This finding led the researchers of that study to suggest that the mechanism underlying PHS is, in fact, a central phenomenon.

1.4 Thermal Grill Illusion (TGI) of Pain

Paradoxical heat sensations can also be investigated by the concurrent presentation of spatially interlaced innocuous warm and cool stimuli (TGS). In 1896, Thunberg created an apparatus consisting of two coiled tubes through which warm (43-45 °C) and cool (24 °C) water was perfused (see Boring, 1942). The application of this stimulus elicited perceptions of intense
“heat”, although the basic thermal components could still be discerned (Boring, 1942). Subsequent studies combining punctate warm (34-45 °C) and cold (2-30 °C) stimuli (Cutolo, 1918; Alston, 1920), or utilising a grill apparatus (Cutolo, 1918; Burnett and Dallenbach, 1927; Burnett and Dallenbach, 1928), were capable of recreating the paradoxical feeling of heat, which was characterized as stinging, burning, and, at times, painful (Cutolo, 1918; Burnett and Dallenbach, 1927).

Recent psychophysical studies have further described the TGI. Human participants reported feeling a hot, burning and stinging sensation in response to the TGS (often interlacing 18-22 °C and 38-42 °C) (Craig and Bushnell, 1994; Green, 2002; Fruhstorfer et al, 2003; Bouhassira et al, 2005; Leung et al, 2005). This sensation was often described as painful (Craig and Bushnell, 1994; Craig et al, 1996; Bouhassira et al, 2005; Defrin et al, 2008a; Kern et al, 2008a; Kern et al, 2008b). During stimulation with the TGS, the elicited paradoxical heat sensation co-localized most often with the cold probe (Alston, 1920; Defrin et al, 2008a). Meanwhile, the perceptual capacity to discern the thermal quality of the cold thermodes diminished (Craig and Bushnell, 1994; Green, 2002).

Interest in this phenomenon was rekindled by Craig and Bushnell, who evaluated it with respect to our current knowledge on thermoreceptive and nociceptive pathways (Craig and Bushnell, 1994). They proposed a physiological mechanism whereby the cold-evoked inhibition of nociceptive activity is released, and pain is “unmasked” (hence, the unmasking hypothesis). Electrophysiological recordings from dorsal horn lamina I STT neurons in anaesthetized cats demonstrated that the response of polymodal HPC neurons to the TGS was similar to the excitation seen in response to the cool temperature presented alone (Craig and Bushnell, 1994). In contrast, the activity of second-order Cold cells was attenuated by the concomitant presence of a warm stimulus. The resultant increase of HPC activity relative to the spinal Cold neurons mirrored the pattern of excitation produced by the presentation of a noxious cold stimulus (Craig and Bushnell, 1994). Craig et al. proposed that the integration of information transmitted by these two populations of lamina I neurons likely occurs at thalamic third-order neurons (Craig et al, 1996).

An alternate hypothesis suggests that the increased perceptual intensity elicited by the TGS results from a convergence of warm and cool signals onto lamina V WDR neurons (Green, 2002; Fruhstorfer et al, 2003). While WDR cells do respond to thermal stimuli, it is unclear
whether they receive both warm and cool thermoreceptive input. Like the unmasking hypothesis, this theory also hinges on the concurrent suppression of activity of lamina I Cold cells, since the perception of cold, but not warmth, is reduced during the TGS (Green, 2002). These two theories are not mutually exclusive: Green’s hypothesis was intended for small differentials between the warm and cool temperatures, where a perception of non-painful heat is produced. When the temperature differential is increased, HPC neurons are excited, and a painful illusion is produced. In fact, greater temperature differentials are more effective in eliciting either the non-painful (Burnett and Dallenbach, 1927; Green, 2002) or painful (Bouhassira et al, 2005; Leung et al, 2005) heat illusion. When the difference between the warm and cold temperatures is 11-15 °C, 15-40% of participants report pain; when that value is increased to 20 °C, paradoxical pain is felt by 70% of individuals (Bouhassira et al, 2005; Leung et al, 2005).

Different lines of evidence lend support to the unmasking hypothesis. The modulatory effect of thermoreceptive activity on nociceptive pathways has been previously alluded to in studies using A-δ blocks of peripheral cold thermoreceptors, which produces a alldynic response to cold in participants (described in section 1.3.5) (Wahren et al, 1989; Yarnitsky and Ochoa, 1990; Defrin et al, 2006). The parallels between these two perceptual phenomena suggest a similar decrease in the cold-evoked inhibition of pain (Craig and Bushnell, 1994). In fact, a PET imaging study indicated that the TGS elicits a pattern of brain activity resembling that produced by noxious, and not innocuous, stimulation (Craig et al, 1996).

The results from pharmacological studies have further characterized the central interactions between nociceptive and thermoreceptive projections. Kern et al. (2008a) found that the administration of morphine (known to suppress the activity of lamina I nociceptive neurons) produced correlated reductions in the CPT and the pain intensity reported in response to the TGS. These same authors found that the administration of ketamine, an NMDA agonist, specifically blocked the TGI without affecting innocuous and noxious thermal thresholds (Kern et al, 2008b). The cold-evoked inhibition of pain, then, may be mediated by glutamatergic channels. Together, these findings support a relationship between the thermoreceptive and nociceptive pathways in controlling the perceptual response to cold thermal stimuli. Like the CPT, the response to the TGS have been described as being variable between participants, with approximately 30% of individuals not reporting pain in response to the mixed stimulus
(Bouhassira et al., 2005; Leung et al., 2005; Defrin et al., 2008; Kern et al., 2008a; Kern et al., 2008b).

Thus, according to the unmasking hypothesis of the TGS, the elicitation of pain in response to the TGS depends on three physiological factors (Figure 1-2): First, it requires activity at STT polymodal nociceptive neurons. HPC neurons have been characterized as being sensitive to cold temperatures below 25°C (refer to section 1.2.2.3). Thus, HPC neurons would respond to the cold temperature component of a 20 °C/40 °C TGS. Second, there must be a concurrent decrease in the activity of lamina I Cold neurons. STT Cold neurons have large RFs, which contrast the small, punctate RFs of thermoreceptive primary afferents (refer to section 1.2.2.3). Activity of peripheral cold thermoreceptors is suppressed by the presentation of warm temperatures against the area of skin they innervate (section 1.2.2.1.1). Thus, the TGS causes a reduction in the convergent input onto spinal Cold cells. Third, thermoreceptive and nociceptive activity is believed to integrate at a common supra-spinal target. STT Cold cells have an inhibitory role in this integration. Compared to the uniform cold stimulus, the activity of Cold cells is reduced in response to the TGS, thus also reducing the inhibition onto this nociceptive pathway.

Craig (2008) speculated that the unmasking mechanism underlying the TGI mirrors the patho-physiology of some neuropathic pain patients. There are meaningful similarities between the painful TGI amongst healthy individuals and the complaints of patients suffering from neuropathic pain due to spinal cord injury (SCI). Amongst this population of pain patients, it has been proposed that damage to the STT is a necessary, but not sufficient, precondition for the presence of pain (Defrin et al., 2001; Ducreux et al., 2006). Spontaneously painful areas colocalize with the body regions of maximal thermal deficits (Defrin et al., 2001; Ducreux et al., 2006). To date, only two case studies have been published where central pain patients were tested with the TGS: In the first, a patient with complex regional pain syndrome I (CRPS I) experienced an intolerable burning sensation on her affected hand when it was placed on the TGS. The second study concerns an MS patient with cold hyperalgesia and allodynia, who reported less pain in response to the TGS than to the cool component (20 °C) itself (Morin et al., 2002). Craig (2008) further proposed to use the TGS as an investigative tool into the mechanisms of pain. The TGS has potential value for studying the interactions between the nociceptive and thermal sensory systems. In particular, the TGS could be used to imitate
symptoms of cold alldynia in healthy volunteers. Similarly, Kern et al. (2008b) discussed the potential of using the TGS as a tool to uncover the physiological mechanisms of analgesics.

Interlaced warm and cool stimuli

**Figure 1-2. The unmasking hypothesis.** This figure depicts the response of nociceptive (green) and cold thermoreceptive (red) afferents and neurons to the TGS. The painful response elicited by the TGS depends on at least three factors: First, it requires activity at lamina I polymodal nociceptive cells. HPC cells will be activated by the presence of the cool temperature bars. Concurrently, the activity of Cold spinal cells must be effectively reduced. Cold cells can have large RFs, which contrast the small, punctate RFs of peripheral thermoreceptors. Activity of some of these peripheral cold afferents will be suppressed by the presentation of warm temperatures against their RFs. This will lead to a significant decrease in the convergent input onto spinal Cold cells. Lastly, at the point of integration between thermoreceptive and nociceptive activity, there must be a reduction in the inhibition inflicted by cold thermoreceptive information onto the common supra-spinal target. In this manner, the nociceptive channel is disinhibited.

1.5  Rationale

The goal of this study was to further describe the TGI amongst healthy subjects while investigating the physiological factors which underlie this phenomenon. The unmasking hypothesis suggests that the TGI depends on the functioning of cold thermoreceptive pathways and, in particular, on the degree of spatial summation of peripheral cold thermoreceptive information onto spinal Cold neurons. Previous studies researching cold thermoreception have described significant body site differences (reviewed in section 1.3.4) (Kenshalo et al, 1967; Dyck et al, 1993; Greenspan et al, 1993; Meh and Denislic, 1994; Yarnitsky and Sprecher, 1994; Stevens and Choo, 1998; Hagander et al, 2000; Harju, 2002; Rolke et al, 2006). Briefly,
compared to the upper extremity, the lower extremity requires a higher intensity stimulus for the
detection of cold when using a fixed stimulus probe. Conversely, using a fixed temperature, the
lower extremity necessitates a larger area of stimulus for the detection of a cold stimulus. These
psychophysical findings insinuate suggest varying degrees of peripheral innervations and central
convergence of thermoreceptive information across body sites. Past studies using the TGS had
only tested the upper extremity. Testing body sites other than the palm and forearm could
potentially further elucidate the mechanisms underlying the TGI. Assuming the TGS depends on
the central convergence of peripheral thermoreceptive and nociceptive activity, these previous
psychophysical findings on innocuous cold thermoreception suggested that a TGS with a fixed
spatial arrangement would not be perceived similarly at all body sites. Instead, it was assumed
that the TGI would differ across body sites in terms of the number of individual reporting pain,
and the intensity of the pain perceived. Specifically, if the TGS leads to a decrease in the cold-
evoked inhibition of pain by reducing the primary thermoreceptive input onto STT Cold cells,
then differences across body site in peripheral innervation density and/or central RFs should
alter the efficacy of the TGS in eliciting pain. The TGI was expected to be more effectively
elicited in areas that where the RFs of innocuous cold thermoreceptive afferents are spatially
smaller, because of a more drastic reduction of cold thermoreceptive information by the
presence of warm temperature bars.

This study also sought to investigate the factors that may contribute to the inter-
individual variability in response to the TGS. Particular attention was paid to the relationship
between the CPT and the TGI, since the proposed integration of thermoreceptive and
nociceptive activity has been implicated with both phenomena. If this integration occurs as
hypothesized, a more effective inhibition of pain pathways by spinal Cold afferent activity could
result in higher CPT and a weaker response to the TGI. Thus, CPTs were expected to correlate
with the intensity of response to the TGS.
2 Methodology

This study’s ethics protocol was reviewed and approved by the Research Ethics Board of the University of Toronto (REB #23556). Approval extended to the consent form, questionnaires, and data collection sheets.

2.1 Subjects

Fifty participants were recruited through advertisements posted throughout the St. George campus of the University of Toronto (refer to Appendix A). Candidates were screened by telephone to assess eligibility. Individuals were included if they were between 19-30 years of age, non-smokers, generally healthy, and fluent English-speakers. Exclusion criteria were the following: difficulties in hearing or comprehension of English; existing pain; a history of chronic pain; the diagnosis of a neurological, psychiatric, or systemic illness; and/or the regular use of psychoactive drugs. During the telephone screening interview, participants were advised to wear comfortable clothes on the test date, and were asked to refrain from consuming caffeinated products immediately before testing.

Prior to participation, each volunteer was informed on the purpose of the study and its experimental procedure. Testing only proceeded after written consent was obtained (refer to Appendix B). Importantly, participants were told that they would be exposed to three thermal stimuli: one where all bars of the thermal grill apparatus (i.e., the TG) were set to a cool temperature (20 °C), one where all bars were set to a warm temperature (40 °C), and one where warm and cool temperature bars were interlaced (20 °C/40 °C). It was also stated that the last stimulus (the TGS) “may produce a paradoxical heat illusion in some individuals”; however, volunteers were kept blind to the fact that this heat sensation is often accompanied by pain. At the end of the testing session, participants received monetary compensation.

2.2 Experimental Procedure

All testing was conducted at the Medical Science Building of the University of Toronto, in a room held at a constant ambient temperature. Participation entailed a single testing session, which lasted approximately an hour and a half. All testing was conducted by the author. Testing sessions were scheduled between 10:00 am to 3:00 pm.
After giving consent to the study, participants were asked to provide information regarding their birth date, height, and weight. Females were also asked to disclose the date their last menses began, and the length in days of their menstrual cycle.

2.2.1 Questionnaires

Participants were requested to complete two questionnaires. The first, the Edinburgh Handedness Inventory (Oldfield, 1971), is a 12-item list which describes various everyday tasks or objects (e.g., holding a toothbrush). By using a scale from 0-2 (0 = no preference; 1 = preference; 2 = strong preference), participants were instructed to indicate their inclination in using either their right or left hand. A relative percentile score was calculated by subtracting points assigned to the left hand from those assigned to the right hand, and dividing that value from the total of points assigned throughout the questionnaire. Thus, all final scores ranged from -100 to +100, indicating strict use of the left and right hand, respectively.

The Pain Catastrophizing Scale (PCS) (Sullivan, 1995) is a 13-item questionnaire on thoughts and feelings towards painful situations. The scale is further subdivided into three categories: rumination (4 items), magnification (3 items), and helplessness (6 items). Participants were asked to rate from zero (“not at all”), to four (“all the time”), the various items according to how much they agree with the given statement. Scoring of the subscales involved a summation of the points assigned by the participants to the items that correspond to each of the three categories; the global score is an addition of all points, with a maximum possible score of 52.

2.2.2 Body Sites Tested

Thermal testing was conducted unilaterally on four regions of the non-dominant side of the body. These included: the palmar surface of the hand and the plantar surface of the foot (two glabrous skin areas), the mid-calf and the lower back (two regions of hairy skin) (Figure 2-1). Thus, the tested body sites spanned the upper extremity, the torso, and the lower extremity.
Figure 2-1. Body sites tested using the Medoc and TG apparatuses. The palm, lower back, mid-calf, and plantar surface of the foot were tested. Testing was always conducted on the non-dominant side of the body.

2.2.3 Determination of Thermal Thresholds

For the first 30 participants, the testing session included the determination of thermal thresholds. Due to equipment availability, we were unable to collect threshold information from the remaining 20 participants. Thermal threshold testing was conducted at each of the four body sites (the palm, back, calf, and foot) in the following order: warm detection (WDT), cold detection (CDT), heat pain (HPT), and cold pain (CPT). The method of limits protocol was employed (Yarnitsky et al., 1995). A 3 × 3 cm computer-controlled Peltier-type thermal stimulator (TSA 2001; Medoc, Israel), set at the baseline temperature of 32 °C, was placed against the skin. When indicated by the experimenter, the temperature of the thermode dynamically changed from the baseline temperature at a predefined rate. For the warm and cold detection threshold trials, the rate was 0.5 °C/s; for the heat and cold pain threshold trials, the rate was 1.0 °C/s. The subject signalled that a threshold was reached by clicking on a response unit, at which point the temperature of the thermode stopped changing, reversed direction, and returned to baseline at a rate of 1 °C/s for the WDT and CDT, and 8 °C/s for the HPT and CPT. Before the beginning of threshold testing, participants were instructed on what each of the four thresholds are, how to respond, and two trial runs were conducted on the forearm for each of the thresholds so they could become familiar with the sensation of the changing thermal intensity. During testing, three runs were conducted for the determination of WDTs and CDTs, and five for HPTs and CPTs. The results from the different runs were averaged together to determine
each threshold. For the CPT and HPT, only the middle three runs where included in the calculation. The Medoc apparatus did not deliver thermal stimuli above 51 °C or below 0 °C. For statistical purposes, in trials where participants did not respond before these extreme temperatures were reached, the corresponding limit was recorded as the threshold for that trial. However, a note was made indicating that, in fact, the participant had not yet reached their actual threshold.

2.2.4 The TG Apparatus

In past studies, the interlaced TGS was produced by as few as two, and as many as 16, thermal elements (Craig and Bushnell, 1994; Craig et al, 1996; Heavner et al, 1997; Green, 2002; Fruhstorfer et al, 2003; Bouhassira et al, 2005; Leung et al, 2005; Defrin et al, 2008a; Kern et al, 2008a; Kern et al, 2008b). The thermal elements were often arranged in rows; however, the authors using 16 elements arranged them in a 4 x 4 array. The spacing between the thermal stimulators ranged between 2 mm – 10 mm. The elements were often rectangular in shape, varying in width between 0.75, 0.8, 1, and 1.2 cm.

The TG apparatus employed in the study was similar to that used by other researchers (Figure 2-2). The thermal stimuli were produced by a TG composed of six aluminum bars (220 mm x 13 mm x 6 mm) spaced 4 mm apart (the thermal grill apparatus, TG; Rehabilitation Engineering Laboratory, Toronto Rehab – Lyndhurst Centre, Toronto, Ontario). There were three Peltier elements for each of the bars, connected in parallel, which cooled or heated the aluminum element based on the temperature set by the experimenter through a computer interface (based on LabView 7.1; National Instruments). Resistance Temperature Detector (RTD) elements acted as real-time temperature sensors and provided continuous feedback information to the system.
Figure 2-2. The thermal grill (TG) apparatus. Six aluminum bars were arranged in parallel and spaced 4 mm apart. Each of the bars were cooled and heated by three Peltier elements.

2.2.5 Testing Using the TG

The following protocol employed the TG and was tested on all participants. Three stimuli were tested at each of the body sites: a warm stimulus (all bars set to 40 °C); a cool stimulus (all bars set to 20 °C); and the thermal grill stimulus (TGS, where bars set to 40 and 20 °C were interlaced). These temperatures were chosen based on the results from past studies, which indicate that the combination of 20 and 40 °C effectively elicited a painful TGI amongst most of their participants (Burnett and Dallenbach, 1927; Craig and Bushnell, 1994; Bouhassira et al, 2005; Leung et al, 2005).

The presentation of each of the stimuli was static; thus, the temperature of the bars was pre-set and maintained constant as participants made contact with the grill. Participants were comfortably seated on a chair. When testing the palm, participants placed their hand on the TG while resting the weight of their arm on a ledge of the TG apparatus. A similar procedure was used when testing the calf and foot. To stimulate the back, the TG was placed against the back support of the chair, and participants were instructed to lean onto the grill.

Participants made contact with the grill for 60 s. During this time, participants completed one of two tasks (refer to Table 2-1): During the first run, participants were asked to continuously rate the intensity of pain they were experiencing using an on-line, computer-interfaced visual analogue scale (on-line VAS). The horizontal scale was displayed on a monitor, and was anchored at zero by “No Pain” and at 100 by “Worst Pain”. Participants were
 instructed to move a mouse right or left in response to increasing or decreasing pain, respectively. In response to this movement, a red bar depicted along the VAS would increase or decrease in length, providing a visual depiction of where the cursor laid along the scale.

During the second run, participants were given short response sheets at 5, 25, and 45 s (found in Appendix C). On these forms, participants were asked to assess the level of unpleasantness they were feeling at that particular moment. To do this, they used an 11-point numeric rating scale (U-NRS), with anchors at zero and 10 of “not unpleasant” and “as unpleasant as you can imagine”, respectively. Also, they were asked to describe the predominant thermal quality of their perception of the thermal stimulus by choosing one of the following six options: “neutral”, “warm”, “cool”, “hot”, “cold”, or “a mixture of warm and cool”. Lastly, the sheet provided them with a list of the pain descriptors from the short McGill Pain Questionnaire (Melzack, 1987), namely, “throbbing”, “shooting”, “stabbing”, “sharp”, “cramping”, “gnawing”, “burning”, “aching”, “heavy”, “tender”, “splitting”, “tiring/exhausting”, “sickening”, “fearful”, “punishing/cruel”, “annoying”, “tingling”, “troublesome”, as well as the options of “neutral”, or “other”. They were informed that they were allowed to select as few or as many words that described their sensation at the moment. The participants were familiarized with the on-line VAS and the forms before testing, since trials for both types of runs were conducted on the forearm with the TG set at room temperature.

Table 2-1. Psychological data collected during testing with the TG.

<table>
<thead>
<tr>
<th>Run type</th>
<th>Task completed during run (total duration 60s)</th>
<th>Task completed after the run</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-line VAS Runs</td>
<td>Continuous rating of pain</td>
<td>Full Response Form</td>
</tr>
<tr>
<td>Descriptive Runs</td>
<td>Short Response Form (5 s)</td>
<td>Short Form (25 s)</td>
</tr>
<tr>
<td></td>
<td>Short Form (45 s)</td>
<td></td>
</tr>
</tbody>
</table>

At the end of all runs, participants were asked to complete a longer form (Appendix C), which included a yes/no question on whether or not they had felt pain, a rating of the intensity of pain felt throughout the trial, similar questions with regards to unpleasantness, the list of descriptors mentioned above, a choice on the predominant thermal quality of the stimulus, and a question on whether they ever felt the urge to withdraw from the grill. Participants were also invited to provide their own comments on their sensations regarding the grill.
2.2.6 Order of Presentation

A diagram depicting the study design is shown in Table 2-2. The order in which the four body sites were tested was pseudo-randomized, as was the order of presentation of the warm stimulus, cool stimulus, and TGS. All runs for a certain stimulus were performed across the four body areas before moving on to the next stimulus. Thus, there were at least 3 minutes in between testing at a particular body site. The TGS was never presented as the first stimulus.

Table 2-2. Order of presentation of stimuli, and of testing body sites

<table>
<thead>
<tr>
<th>Body Site</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stimulus A</strong></td>
<td>On-line VAS Runs</td>
<td>Descriptive Runs</td>
<td>On-line VAS</td>
<td>Descriptive</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Stimulus B</strong></td>
<td>On-line VAS</td>
<td>Descriptive</td>
<td>On-line VAS</td>
<td>Descriptive</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Stimulus C</strong></td>
<td>On-line VAS</td>
<td>Descriptive</td>
<td>On-line VAS</td>
<td>Descriptive</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

2.3 Statistical Analysis

Statistical tests are specified in-text. Distributions are described by their means \((M)\) and their standard deviation \((SD)\). As indicated in the captions, the error bars depicted in the figures represent the standard error of the mean \((SEM)\).

Generally, group comparisons of the data collected in response to the three TG stimuli across the four body sites were performed by a 3 x 4 repeated-measures ANOVA. Sex was always included as a between-subject factor. Where the assumption of sphericity was violated, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. Significant effects were further investigated by performing post-hoc contrasts comparing the TGS to the uniform warm or cool stimuli (for the main effect of stimulus type), and the palm to the other tested regions (for the main effect of body sites). Comparisons between two groups were done by performing Student’s \(t\)-tests (for independent groups) or paired-sample \(t\)-test (for related groups).

To compare the likelihood of two response outcomes within a single sample, binomial tests were performed. To compare the likelihood of an outcome between two independent groups (e.g., non-responders vs. responders), \(\chi^2\) tests were performed. To compare the likelihood of an outcome across paired data, McNemar \(\chi^2\) tests were performed.
When group sizes fell below 20 individuals, normality was not assumed. Thus, non-parametric tests were used. Non-parametric tests were also employed to analyze the threshold data. This was due to the ceiling-effect caused by some participants not responding to the thermal stimuli before the maximum temperature of the thermode was reached. To compare the response between two unrelated groups, Mann-Whitney $U$ test were performed. With three independent groups, Kruskal-Wallis one-way ANOVAs were performed. With two related groups, Wilcoxon signed-ranks tests were performed. With three related groups, Friedman two-way ANOVAs were performed.

Results were considered significant at $\alpha = 0.05$. When multiple comparisons (k) were made, a family-wise correction of the critical significance level was performed ($\alpha_{\text{adjusted}} = \alpha / k$). Reported $p$ values correspond to a two-tailed test of significance.
3 Results

3.1 Subjects

Fifty healthy individuals were recruited. However, one testing session was incomplete, and the data from that participant were removed from the sample. The following analyses include data from the remaining 49 testing sessions. The mean age of participants was 23 years (SD = 3). Twenty-five participants were female. The average score from the Pain Catastrophizing Scale was 17.5 (SD = 8.70), and there was no statistically significant difference in those scores between male and female participants (females: $M = 18.0$, $SD = 9.02$; males: $M = 16.8$, $SD = 8.46$; Student’s $t$-test; $t(47) = 0.483; p = 0.632$). The majority of participants were right-handed ($n = 45$; for all participants, their Edinburgh Laterality Quotient score was in accordance with their preferred handedness).
3.2 Thermal Thresholds across Body Sites

The WDT, CDT, HPT, and CPT were measured at the four body sites of the first 29 participants, and are depicted in

![Box plots showing thermal thresholds across body sites](image)

Figure 3-1. At each of the body sites, a number of participants reported cold pain at temperatures above 20 °C (n = 6, 6, 6, and 8; for the palm, back, calf, and foot, respectively); similarly, some participants reported heat pain at temperatures below 40 °C (n = 4, 6, 2, and 2; for the palm, back, calf, and foot; respectively). Conversely, some participants did not report pain by the time the thermode reached its temperature limits for these two tests (51 °C and 0 °C for the heat pain threshold and cold pain threshold, respectively; HPT: n = 1 and 2 for the calf and foot, respectively; CPT: n = 2, 10, 9, 1 for the palm, back, calf, and foot, respectively).

The four thermal thresholds significantly differed between body sites (Friedman’s Test; \(\chi^2(3, 27) = 35.8, 23.8, 30.4, \text{and } 27.6\), for the WDT, CDT, HPT, and CPT; \(p < 0.001\) at all
sites). For each threshold measure, body site differences were further explored by comparing participants’ responses on the back, calf, and foot to those on the palm (Wilcoxon signed-ranks test; $\alpha_{adj} = 0.004$). Participants had higher WDTs on the calf and foot than on the palm (calf vs. palm: $Z = 4.04$; foot vs. palm: $Z = 4.48$; $p < 0.001$ for both comparisons). Similarly, the measured CDTs were significantly higher on the calf and foot than on the palm (calf vs. palm: $Z = 4.07$, $p < 0.001$; foot vs. palm: $Z = 3.02$, $p = 0.003$). No significant differences in the WDT and CDT were found between the palm and the back (WDT: $Z = 0.75$, $p = 0.45$; CDT: $Z = 0.64$, $p = 0.52$).

Compared to the palm, the HPT was significantly lower when measured on the back (palm vs. back: $z = 3.97$, $p < 0.001$). The CPTs measured on the calf were significantly higher than the CPTs of the palm (palm vs. calf: $z = 3.39$, $p = 0.001$).

![Figure 3-1. Thermal thresholds at the four body sites.](image)

*Figure 3-1. Thermal thresholds at the four body sites.* Box-plots of the WDT, CDT, HPT, and CPT are depicted across the four body sites (P = Palm, B = Back, C = Calf, F = Foot). The bottom, middle,
and top lines of the box represent the 25th, 50th, and 75th percentile value, respectively. The whiskers represent the minimum and maximum values. Wilcoxon signed-ranks tests were conducted to compare the thresholds of the back, calf, and foot to that of the palm (** p < 0.01, *** p < 0.001). The critical significance level was corrected for multiple comparisons.

3.3 Pain and Unpleasantness Elicited by the TGS

Of the 49 participants, 43 perceived pain and/or unpleasantness in response to the TGS (87%). Painful responses to the TGS were elicited at the palm, back, calf, and foot. Participants were significantly more likely to report pain at least once in response to the TGS than the uniform warm and cool stimuli (McNemar test; $\chi^2(1) = 12.2$ and 18.0 for warm vs. TGS and cool vs. TGS, respectively; $p < 0.001$ for both comparisons). Conversely, at each of the four body sides, approximately 20-30% of this study’s sample reported feeling neither pain nor unpleasantness in response to the TGS ($n = 10, 10, 15$, and 14 for the palm, back, calf, and foot, respectively; “non-responders”). There were an equal number of males and females non-responders.

Two 3 x 4 repeated-measures ANOVA with stimulus type (warm, cool, and TGS) and body site of stimulation (palm, back, calf, and foot) as within-subject factors were performed. Sex was included as a between-subject factor. In one analysis, the outcome measure was the pain intensity reported through the P-NRS. In the other analysis, the outcome measure was the participants’ perceived unpleasantness ratings (U-NRS). For both measures, there was a main effect of stimulus type (P-NRS: $F(1.64, 76.9) = 22.2$; U-NRS: $F(1.95, 91.5) = 23.4$, $p < 0.001$ for both analyses; Figure 3-2 and Figure 3-3). Subjects rated the pain sensation produced by the TGS as significantly higher than that elicited by the warm or cool stimulus alone (TGS vs. warm: $F(1, 47) = 23.2$, $p < 0.001$; TGS vs. cool: $F(1, 47) = 32.2$, $p < 0.001$). Similarly, the unpleasantness of the sensation produced by the TGS was rated as significantly greater than the two uniform stimuli (TGS vs. warm: $F(1, 47) = 18.3$, $p < 0.001$; TGS vs. cool: $F(1, 47) = 46.3$, $p < 0.001$).

There were significant differences across the four sites in the average unpleasantness and pain ratings (P-NRS: $F(2.33, 109.5) = 7.81$; U-NRS: $F(2.53, 118.8) = 15.0$, $p < 0.001$ for the main effect of body site in both ANOVAs). The highest mean pain intensity rating was in response to the application of the TGS on the back, which was significantly greater than the mean pain intensity rating obtained in response to stimulation of the calf and the foot with the TGS (paired-samples $t$-test; calf vs. back: $t(48) = 2.74$, $p = 0.009$; foot vs. back: $t(48) = 2.43$, $p =
The application of the TGS to the back was also rated as significantly more unpleasant than when it was applied to the palm, calf or foot ($t(48) = 2.17, p = 0.035$; $t(48) = 3.49, p = 0.001$, and $t(48) = 2.83, p = 0.007$; respectively).

The interactions between the two main within-subject factors (i.e., body site and stimulus type) of the repeated-measures ANOVAs were also not significant (P-NRS: $F(3.9, 183.9) = 1.52, p = 0.20$; U-NRS: $F(4.02, 188.8) = 2.02, p = 0.37$ for data from the unpleasantness NRS).

Sex was not a significant between-subject factor (P-NRS: $F(1, 47) = 0.55, p = 0.46$; U-NRS: $F(1, 47) = 0.19, p = 0.67$). In fact, the main effect of sex was never significant in any of the ANOVA analyses performed for this study, nor were any of the interactions between the factor of sex and the factors of body site and/or stimulus type significant.

Figure 3-2. Perceived pain intensity in response to the three stimuli when applied to the palm (P), back (B), calf (C), and foot (F). The bar graph displays the mean pain intensity rating (P-NRS, 0-10); error bars depict the standard error of the mean (SEM). Pain was significantly higher in response to the TGS than either the uniform warm or cool temperatures ($** p < 0.001$). On average, greater pain intensity ratings were elicited in response to stimulation of the back with the TGS than stimulation of the calf and foot.
Figure 3.3. Perceived unpleasantness in response to the three stimuli when applied to the palm (P), back (B), calf (C), and foot (F). The bar graph displays the mean perceived unpleasantness (U-NRS, 0-10); error bars signify the SEM. The TGS was perceived as significantly more unpleasant than the other two uniform stimuli (*** $p < 0.001$). The unpleasantness perceived in response to the TGS applied to the back is significantly greater than that reported following stimulation of the palm, calf, and foot.

3.4 Continuous Ratings of Pain and Unpleasantness

The average and maximum pain intensity ratings recorded using the on-line visual analogue scale (on-line VAS; avgVAS and maxVAS, respectively) were analyzed by performing 3x4 repeated-measures ANOVAs like the ones described above (within-subject factors: stimulus type, and body site tested; between-subject factor: sex). The main effect of stimulus type was significant (avgVAS: $F(1.59, 74.6) = 17.0$, $p < 0.001$; maxVAS: $F(1.69, 79.5) = 21.6$, $p < 0.001$). Both the average and maximum pain ratings were significantly higher when presenting the warm and cool temperatures in an interlaced manner (TGS vs. warm: $F(1, 47) = 17.6$, $p < 0.001$, and $F(1, 47) = 24.0$, $p < 0.001$; TGS vs. cool: $F(1, 47) = 22.6$, $p < 0.001$, and $F(1, 47) = 29.4$, $p < 0.001$; for the avgVAS and maxVAS values, respectively).

At the five second time point, the continuous pain ratings in response to the TGS were significantly higher than those in response to the warm and cool stimuli alone (main effect of stimulus type: $F(1.68, 79.1) = 6.24$, $p = 0.005$; TGS vs. warm: $F(1, 47) = 7.93$, $p = 0.007$; TGS vs. cool: $F(1, 47) = 7.55$, $p = 0.008$). At 60 s, the pain reported in response to the TGS was still rated as significantly higher than that reported in response to the uniform stimuli (main effect of
stimulus type: $F(1.53, 71.9) = 15.8, p < 0.001$; TGS vs. warm, $F(1, 47) = 16.5, p < 0.001$; TGS vs. cool: $F(1, 47) = 20.2, p < 0.001$).

Figure 3-4. Graphs of the continuous pain ratings in response to the three thermal stimuli applied to each body site. Using an on-line VAS (0-100), a rating was obtained every 100 ms. The line graphs depict the continuous rating of pain over 60 s, averaged across the 49 participants. The error bars represent the SEM for the 15, 30, 45, and 60 s time points. By the 5 s mark, the pain intensity ratings in response to the TGS were significantly higher than from either the warm or cool stimulus at all body sites. At 60 s, the pain felt in response to the TGS remains significantly higher than that felt in response to the uniform stimuli.

The main effect of body site was significant in the four analyses involving the average VAS ratings, the maximum VAS ratings, and the VAS ratings at 5 and 60 s (avgVAS: $F(2.52, 118.2) = 7.06, p < 0.001$; maxVAS: $F(2.48, 116.6) = 8.25, p < 0.001$; VAS at 5 s: $F(2.2, 103.2) = 7.63, p = 0.001$; VAS at 60 s: $F(2.60, 122.3) = 6.07, p = 0.001$). In fact, while pain intensity
ratings in response to the TGS showed a similar temporal progression across the four body sites during the first 15 s, two patterns emerged as the stimulus continued. Towards the end of the stimulus, the pain intensity ratings in response to the TGS applied to the back and palm were higher than those of the calf and foot. Comparing the average pain intensity ratings between zero and 15 s, and that between 45 and 60 s revealed that the pain intensity ratings of the TGS significantly increased during the second time-span for the palm and back (paired t-test; palm: $t(48) = 3.60, p = 0.001$; back: $t(48) = 3.19, p = 0.002$). There was no significant difference during these two time periods in the pain ratings for calf and foot (calf: $t(48) = 0.82, p = 0.41$; foot: $t(48) = 1.12, p = 0.27$).

![Figure 3-5. Average pain intensity rating (on-line VAS) during 0-15 s and 45-60 s. Mean pain ratings during these time periods were calculated by averaging all the ratings obtained between 0-15 s and 45-60 s, respectively. Error bars depict the SEM. Pain ratings in response to the TGS applied to the palm and back, but not the calf and foot, significantly increased during the second time period compared to the first (** p < 0.01).](image)

### 3.5 Thermal Quality and Characteristics of the TGS

When asked to choose words from the s-MPQ that describe the sensation elicited by the three stimuli, participants selected significantly more words in response to the TGS than either the warm or cool stimuli (repeated-measures ANOVA; main effect of stimulus type: $F(1.73,$
81.2) = 28.8, p < 0.001; TGS vs. warm: $F(1, 47) = 43.3, p < 0.001$; TGS vs. cool: $F(1, 47) = 28.3, p < 0.001)$. The warm and cool stimuli were perceived as “neutral” (48.3%), “tingling” (15%), or “annoying” (15%). Instead, in approximately a third of all runs, the response to the TGS was characterized as “burning” and “sharp” (Table 3-1). The descriptor “burning” was rarely used to describe the uniform stimuli; rather, it was uniquely selected to describe the perception elicited by the TGS.

Table 3-1. Descriptors chosen to describe the three thermal stimuli. Descriptors were chosen from a list provided to participants of words from s-MPQ. Participants were free to choose as few or as many words from the list as they thought appropriate. The frequency reported in brackets indicates the percent of participants that chose a particular descriptor. “TGS responders” refers to participants that reported pain and/or unpleasantness in response to the application of the TGS at a particular body site.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Body site</th>
<th>All participants – Descriptors (%)</th>
<th>TGS responders only – Descriptors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cool</td>
<td>Palm</td>
<td>Neutral (42.9) Annoying (22.4)</td>
<td>Neutral (38.5) Annoying (25.6)</td>
</tr>
<tr>
<td></td>
<td>Back</td>
<td>Neutral (34.7) Sharp (30.6)</td>
<td>Neutral (30.8) Sharp (30.8)</td>
</tr>
<tr>
<td></td>
<td>Calf</td>
<td>Neutral (55.1) Tingling (12.2)</td>
<td>Neutral (50.0) Tingling (17.6)</td>
</tr>
<tr>
<td></td>
<td>Foot</td>
<td>Neutral (49.0) Sharp (16.3)</td>
<td>Neutral (45.7) Annoying (20.0)</td>
</tr>
<tr>
<td>Warm</td>
<td>Palm</td>
<td>Neutral (57.1) Tingling (16.3)</td>
<td>Neutral (56.4) Tingling (20.5)</td>
</tr>
<tr>
<td></td>
<td>Back</td>
<td>Neutral (40.8) Sharp (22.4)</td>
<td>Neutral (38.5) Sharp (28.2)</td>
</tr>
<tr>
<td></td>
<td>Calf</td>
<td>Neutral (65.3) Tingling (12.2)</td>
<td>Neutral (61.8) Tingling (17.6)</td>
</tr>
<tr>
<td></td>
<td>Foot</td>
<td>Neutral (55.1) Tingling (14.3)</td>
<td>Neutral (55.9) Tingling (20.6)</td>
</tr>
<tr>
<td>Mix</td>
<td>Palm</td>
<td>Burning (44.9) Sharp (26.5)</td>
<td>Burning (53.8) Sharp (33.3)</td>
</tr>
<tr>
<td></td>
<td>Back</td>
<td>Sharp (40.8) Burning (30.6)</td>
<td>Sharp (48.7) Burning (38.5)</td>
</tr>
<tr>
<td></td>
<td>Calf</td>
<td>Neutral (44.9) Burning (28.6)</td>
<td>Burning (38.2) Neutral (35.3)</td>
</tr>
<tr>
<td></td>
<td>Foot</td>
<td>Burning (36.7) Sharp (24.5)</td>
<td>Burning (51.4) Sharp (34.2)</td>
</tr>
</tbody>
</table>

Participants correctly identified the thermal quality of the warm stimulus in 92% of runs (describing it as “warm” or “hot”; otherwise, the stimulus was either described as “neutral” or as a “mixture of warm and cool”). All participants correctly identified the thermal quality of the cool stimulus (describing it as “cool” or “cold”; except $n = 1$ who described the cool stimulus as “hot” when it was applied to the foot). Participants reported mixed sensations of warm and cool during the majority of run testing the TGS (56%). However, when they identified a single predominant thermal quality, participants most frequently described the stimulus according to
the warm component, i.e., as either “warm” or “hot” (count of “warm” or “hot” vs. “cool” or “cold”: Binomial tests; \( p = 0.003, p = 0.029, p = 0.023, p = 0.052; \) for the palm, back, calf, and foot, respectively). Plotting the thermal quality participants perceived at 5, 25, and 45 s revealed a similar pattern. A large proportion of participants were capable of discerning the mixed components of the TGS at all three time points. The response of other participants would fluctuate during the 60 s stimulation period. In these situations, participants more frequently felt a predominantly warm or hot sensation than cool or cold (Binomial test; \( p = 0.001, \) for the palm, back, calf; \( p = 0.134 \) for the foot) (Figure 3-6).

**Figure 3-6.** Progression of the perceived thermal quality of the TGS. Participants were asked to describe the predominant thermal quality of the TGS at 5, 25, and 45 s. The temporal progression of the thermal quality of the stimulus was coded as fixed combinations (e.g., when the participant initially indicated mixed sensations that progressed to a predominantly hot sensation, this was coded as “mix → hot”). Combinations where the participants indicated consistently cold or cool sensations are all located to the left of the graph. This is followed by combinations of cold and mixed sensations; mixed sensations; hot and mixed sensations; finally, combinations indicating predominantly hot sensations are found on the right-hand side of the graph. Following stimulation with the TGS at the palm, back, and calf, combinations which included predominantly warm or hot sensations were significantly more frequent than those that included predominantly cool and cold sensations (**\( p < 0.01 \)).

Responders and non-responders were compared in terms of how they described the TGS. At all body sites, non-responders chose significantly fewer descriptors from the S-MPQ to describe the interlaced stimulus than responders did (Mann-Whitney test; \( U = 48.0; U = 33.0; U = 94.0; U = 25.0; p < 0.001 \) at all body regions). Non-responders were also less likely to describe the perception produced by the TGS as “burning” (\( \chi^2(1, 49) = 9.42, p = 0.002; \chi^2(1, 49) \)
= 5.54, \( p < 0.019; \chi^2(1, 49) = 4.41, p = 0.036; \chi^2(1, 49) = 11.4, p = 0.001 \); for the palm, back, calf, and foot, respectively. Responders and non-responders were equally likely to identify the mixed thermal components of the TGS. While responders frequently described the stimulus as predominantly “hot”, non-responders were more likely to employ the qualifier “warm” to describe the thermal quality of the TGS. This trend was observed at the four body sites, but only reached statistical significance at the back and foot (\( \chi^2(1, 49) = 6.78, p = 0.009; \chi^2(1, 49) = 10.0, p = 0.002 \)).

3.6 Variance in Response

The response to the TGS between individuals was varied: pain intensity ratings ranged from zero (i.e., “no pain”) to scores of nine out of 10 points (10 = “worst pain imaginable”). Volunteered comments from individuals not feeling pain or unpleasantness to the TGS reflect this variability. Non-responders perceived the TGS to be “mostly warm”. While most non-responders refrained from making comments on valence of the stimulus (e.g., one participant stated it was “neither bothersome not pleasant – neutral”), a couple of these participants characterized the TGS as “therapeutic” and “soothing”; another individual described it as “pleasant”. In contrast, participants who did report pain and/or unpleasantness often referred to the hot quality of the stimulus, describing it as “more hot than cold; pinching heat”, “very hot sensation”, “hot and sharp”. Responders described their experience with the TGS as “uncomfortable”, “unpleasant”, “awkward”, “shocking”, “painful” and a sensation they “didn’t like/disliked”.

In contrast, the pain ratings (P-NRS ratings) in response to the TGS at the four body sites are all moderately correlated amongst one another (Table 3-2). Similarly, the perceived unpleasantness ratings (U-NRS) in response to the TGS across body sites are all moderately or highly correlated. This suggests that the within-subject responses were fairly consistent across body sites. In fact, on average, the ratings of pain and unpleasantness in response to the TGS were significantly less variable within individuals, across the four body sites, than the ratings between participants (comparing the average between-subject variance at a single body site vs. the average within-subject variance across body sites; Student’s t-test; P-NRS: \( t(4.24) = 4.75, p = 0.008 \); U-NRS: \( t(12.9) = 3.65, p = 0.003 \)). The average variance within subjects is 61.8% less than that between subjects for the P-NRS ratings (1.99 compared to 5.20), and 55.8% less for the ratings of perceived unpleasantness (2.95 and 6.67; respectively).
Table 3-2. Correlations across body sites of pain and unpleasantness ratings in response to the TGS. Reported values represent Pearson’s $r$ (** $p < 0.01$; *** $p < 0.001$).

<table>
<thead>
<tr>
<th></th>
<th>P-NRS</th>
<th></th>
<th></th>
<th>U-NRS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm</td>
<td>-</td>
<td>0.61</td>
<td>***</td>
<td>0.56</td>
<td>***</td>
<td>0.50</td>
<td>***</td>
</tr>
<tr>
<td>Back</td>
<td>---</td>
<td>0.52</td>
<td>***</td>
<td>---</td>
<td>0.53</td>
<td>***</td>
<td>---</td>
</tr>
<tr>
<td>Calf</td>
<td>---</td>
<td>0.60</td>
<td>***</td>
<td>---</td>
<td>0.72</td>
<td>***</td>
<td>---</td>
</tr>
<tr>
<td>Foot</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.7 Thermal Thresholds and the TGS

A Spearman’s correlation was calculated for the four different thresholds and the pain intensity ratings (P-NRS) elicited by the TGS at the four body sites. The only threshold that was consistently significantly correlated with these ratings was the CPT ($\rho = 0.584$, $p = 0.001$; $\rho = 0.589$, $p = 0.001$; $\rho = 0.457$, $p = 0.015$; $\rho = 0.515$, $p = 0.005$; for the palm, back, calf, and foot, respectively).

The relationship between the CPT and pain intensity ratings in response to the TGS was further investigated. In particular, the CPT of some participants was measured as being higher than 20 °C; it was possible that these subjects were maintaining the relationship between the CPT and the pain intensity ratings. To isolate the effect of these individuals, subjects were assigned to one of three groups according to their CPT. At each body site, participants who reported a CPT above 20 °C (~ 25% of participants) were assigned to group A. Subjects whose CPT was below 20 °C were distributed into groups of equal size. Thus, group M comprised those individuals with a threshold above the median CPT of the remaining sample (~ 37%). Lastly, members of Group B had a threshold below that median (~ 38%). At all body sites, pain perceived in response to the TGS was found to be significantly different across these three groups (Kruskal-Wallis ANOVA; $\chi^2(2) = 8.00$, $p = 0.018$; $\chi^2(2) = 10.1$, $p = 0.006$; $\chi^2(2) = 6.23$, $p = 0.044$; $\chi^2(2) = 11.3$, $p = 0.003$; at the palm, back, calf, and foot, respectively) (Figure 3-7).

Importantly, participants in Group M rated the TGS as significantly more painful than participants in Group B (Mann-Whitney test; $U = 30.0$, $p = 0.038$; $U = 29.5$, $p = 0.034$; $U = $
31.0, \( p = 0.044; \ U = 14.5, \ p = 0.056; \) for the palm, back, calf, and foot, respectively). A similar relationship was not found when comparing the response of these three groups to the cool stimulus alone.

Figure 3-7. Box-plots of pain intensity ratings in response to TGS for the three groups A, M, and B. The bottom, middle, and top lines of the box represent the 25\textsuperscript{th}, 50\textsuperscript{th}, and 75\textsuperscript{th} percentile value, respectively. The whiskers represent the minimum and maximum values. The groups were defined by their CPT. Group A included all subjects whose CPT was above 20 °C at a particular body site. Group M consisted of participants whose CPT was below 20 °C, and above the remaining median; Group B included individuals whose CPT was below the remaining median. Pain elicited by the TGS was significantly different between these groups. Specifically, individuals in group M felt more pain in response to the TGS than individuals in group B (* \( p < 0.05 \) for the palm, back, and calf; \( p = 0.056 \) for the foot).
4 Discussion

The present findings confirm that the simultaneous application of interlaced warm and cool stimuli can elicit a paradoxical perception of thermal pain and unpleasantness. The TGS was perceived as significantly more painful and unpleasant than its component warm and cool stimuli alone. In addition, significantly more pain-related verbal descriptors were chosen to describe the TGS versus the uniform thermal stimuli. The TGI was most often described as a “burning” sensation.

This is the first study to show that the TGI can be elicited at body sites other than the upper extremity. There were significant differences across body sites in the response to the TGS, with stimulation of the palm and back eliciting more intense pain and unpleasantness than the calf and foot. Following a similar pattern, the CDT and WDT were both significantly lower on the palm than on the calf and foot.

Out of a sample of 49 individual, six participants reported never feeling pain or unpleasantness in response to the TGS. Males and females responded to the TGS in a similar fashion. Intensity ratings of TGS-induced pain correlated with cold pain thresholds. Thus, by demonstrating that TGI relates to both thermoreceptive and nociceptive functioning, the results from this study are in line with the unmasking theory.

4.1 TGI as a Painful Experience

In this study, the burning sensation attributed to the TGS was coupled with reports of pain and unpleasantness. Whether the TGI is experienced as painful has been a matter of debate (Craig and Bushnell, 1994; Craig et al, 1996; Green, 2002; Fruhstorfer et al, 2003; Bouhassira et al, 2005; Leung et al, 2005). Results from this thesis lend support to the hypothesis that subjects do, in fact, frequently describe the TGI as a painful and/or unpleasant sensation. Conversely, participants who did not report pain or unpleasantness in response to the TGS were also less likely to report feeling the burning sensation characteristic of the TGI.

Participants were required to rate the TGS, as well as the component temperatures, for pain intensity and perceived unpleasantness, with the option of indicating the experience as non-painful or not unpleasant (i.e., the rating of 0 on the numeric scales). Craig and Bushnell reported that the uniform 20 °C and 40 °C stimuli were rarely rated as painful by their 11
participants (Craig and Bushnell, 1994). None of the 13 individuals that participated in Leung et al.’s study commented on feeling pain in response to the presentation of thermodes set to 18 °C, 20 °C, 24 °C, 36 °C, 40 °C, or 42 °C (Leung et al., 2005). In this study’s protocol, participants were required to report on feelings of pain and/or unpleasantness in response to the uniform stimuli. Mild pain and unpleasantness was often described. This may reflect the prolonged period of stimulation. However, compared to the warm and cool temperatures alone, pain in response to the TGS was elicited more frequently, and rated as more intense.

4.2 Perceptual Characteristics of TGS Fluctuate Over Time

Given the option to describe the TGS as a combination of both warm and cool, the majority of participants expressed feeling mixed thermal sensations in response to this stimulus. When one predominant thermal quality was indicated, participants most often described the TGS as hot or warm. Information obtained over the 60 s runs indicated that the elicited thermal sensations often fluctuated between being predominantly hot or warm, and being a mixed stimulus. In his original study, Thunberg described an imperfect blend of temperatures (Boring, 1942). Alston (1920) stated that his participants experienced a fluctuation between detecting both warm and cool, and feeling “heat”. Recent studies on the TGS (using milder temperatures) have not systematically addressed the issue of whether the elicited perception is uniform. Craig and Bushnell (1994), as well as Green (2002), reported that the ability to detect the thermal quality of “cold” diminished during the simultaneous presentation of both warm and cool temperatures. When comparing the perception elicited by the TGS after 3 s of stimulation to that produced by a uniform stimulus, participants matched the TGI to painfully hot stimuli that surpassed the temperature of the warm component (Leung et al., 2005). Following 10 s of stimulation, the sensation evoked by the TGS still resembled hot (and not cold) stimuli; however, the temperatures of these stimuli were not significantly higher than the warm component of the TGS. Those authors suggested that the diminished noxious nature of the TGS may reflect adaptation of the central process underlying the TGI, and should be contrasted to the stable nature of the pain elicited by uniform noxious temperatures. Bouhassira et al. (2005) reported that their participants described mixed sensations in response to the TGS over the 30 s of application. The current data insinuates that, in fact, the TGI does fluctuate over time in terms of its thermal quality. The TGI changed from feeling uniformly hot or warm to being a composite perception of warm and cool, and vice versa. However, the pain induced by the TGS did persist throughout the 60 s of stimulation.
4.3 TGI Elicited More Consistently at the Palm and Back

The TGS, which had fixed spatial and thermal characteristics, elicited pain on the palm, back, calf, and foot. There were small, but significant differences in the ability to elicit the TGI response across the four body sites. On average, higher pain and unpleasantness ratings to the TGS were obtained in response to stimulation of the palm and back. Fewer participants felt pain in response to the TGS applied to the calf and foot.

In their unmasking hypothesis, Craig et al. proposed that the TGS produces a particular pattern of activity in central thermoreceptive and nociceptive channels. Body site differences could have resulted from: differences in the activation of peripheral thermoreceptive, and/or nociceptive afferents; differences in the central recruitment of thermoreceptive channels, or nociceptive channels; and/or body site differences at the level of nociceptive and thermoreceptive integration (e.g., the efficacy of the cold-evoked inhibition of pain).

Thermal threshold data indicated that the lower extremity had higher innocuous thermal thresholds (WDT and CDT) than the palm and back. The body site differences in the pain intensity felt in response to the TGS and those seen in the measurement of innocuous thermal thresholds may have a common physiological basis. However, warm afferents are not thought to play a role in the TGI. Thus, the body site differences found in this study are more likely related to the body site differences found in cold detection. Since the findings did not indicate a divide between glabrous versus hairy skin, there was no evidence to suggest that the body site differences were due to variations in the type of peripheral afferent innervation. Instead, the results from this study suggest body site differences in peripheral innervation density and/or central convergence of cold thermoreceptive input onto central neurons.

The shift in relative activity of HPC and Cold cells produced by the TGS depends, amongst other factors, on the degree of spatial summation of cold input onto central Cold neurons (Craig, 2002). Whether or not a cold temperature is perceived to be painful depends on the supra-spinal integration of central nociceptive and cold thermoreceptive channels (Wahren et al, 1989; Yarnitsky and Ochoa, 1990; Defrin et al, 2006). Both innocuous and noxious cold stimuli lead to activity in Cold and HPC cells. Compared to a uniform cold stimulus, the TGS elicits less firing in second-order Cold cells due to the presence of the warm bars. The activity of HPC cells, however, remains unchanged (Craig and Bushnell, 1994). In this manner, the TGS
mimics the pattern of activation produced by a noxious cold stimulus in lamina I HPC and Cold neurons. Thus, according to the unmasking hypothesis, the TGS is perceived as painful because the activity of Cold cells is effectively reduced by the presence of the warm stimuli on their RFs (Craig, 2002).

Unlike peripheral thermoreceptors that have small, punctate RFs, spinal thermoreceptive cells have large RFs (Andrew and Craig, 2001). Spinal Cold cells likely receive convergent peripheral input (Hellon and Mitchell, 1975). Psychophysical studies have consistently found that, when comparing the threshold detection of cold at the leg to that of the arm or torso, a larger area of stimulation is required for a fixed stimulus intensity (or, conversely, a higher stimulus intensity for a fixed stimulus area) (Kenshalo et al., 1967; Dyck et al., 1993; Greenspan et al., 1993; Meh and Denislic, 1994; Yarnitsky and Sprecher, 1994; Stevens and Choo, 1998; Hagander et al., 2000; Harju, 2002; Rolke et al., 2006). Presumably, the leg has sparser peripheral innervation, and wider central convergence, than the upper body.

Central convergence of thermoreceptive information facilitates the detection of thermal stimuli near threshold (28-32 °C). However, with higher stimulus intensities (supra-threshold temperatures; e.g., 20 °C), spatial summation is not necessary for the activation of ascending central neurons and for the subsequent perception of cold. Instead, the activity of a single peripheral afferent may be sufficient for the successful recruitment of downstream central neurons (summarized in Green and Zaharchuk, 2001). During stimulation with the TGS, central Cold neurons receiving widely convergent input may be activated by the presence of the supra-threshold cold temperature (20 °C) somewhere on their large RF. In this scenario, the warm component of the TGS would be less effective in disinhibiting the pain pathway. Conversely, central thermoreceptive neurons with smaller RFs, and receiving less convergent information, are likely to be more affected (silenced) by the presence of the warm stimulus. The fact that the TGI was more often elicited at the palm and back compared to the calf and foot may be related to differences in the RF size of central afferents. To continue exploring this hypothesis, the spatial distribution of the TGS should be modified so that a larger portion of the stimulus area is dedicated to either the cold or warm temperature (e.g., having a middle cold bar flanked by two warm bars at each side; and vice versa). Based on the reasoning above, a larger stimulus area dedicated to the warm stimulus may facilitate the suppression of Cold cells, and the unmasking of pain, in body sites like the calf and foot. In fact, when using three thermodes to stimulate the
index, middle, and ring finger, Green described a high proportion of synthetic heat reports when a cold stimulus was flanked by two warm thermodes, but not during the opposite configuration (Green, 1977).

4.4 Relationship between TGI and thermal thresholds

The intensity of the painful response to the TGS significantly correlated to the CPT at all body sites. This finding suggests that the CPT and TGI share a common physiological mechanism. It has been hypothesized that cold thermoreceptive channels (ascending lamina I Cold information) modulate the effect of nociceptive (lamina I HPC) pathways (discussed in the previous section). Both the CPT and TGI would be affected by this integration. This relationship may partially explain the inter-individual variability that we and others have observed in the response to the TGS. The CPT is also highly variable between individuals (refer to section 1.3.1) (Rolke et al, 2006). Various lines of evidence suggest a relationship between cold pain, cold thermoreception, and the TGI. Following the administration of morphine, there is a significant correlation between the reductions in the CPT and the lower pain intensity felt in response to the TGS (Kern et al, 2008b). Craig and Bushnell (1994) related the disinhibition of pain due to the TGS to the cold allodynia experienced during myelinated nerve blocks. During myelinated nerve blocks, the CPT is raised as cold thermosensitivity is lost (Wahren et al, 1989; Yarnitsky and Ochoa, 1990). Similarly, amongst individuals with central neuropathic pain after injury to the spinal cord, the painful areas co-localize with areas of maximal thermosensory deficits (Defrin et al, 2001; Ducreux et al, 2006). Together, these results suggest a modulatory role for innocuous thermoreceptive input in the perception of pain.

4.5 Inter-individual Variability in Response to the TGS

The results from this study also indicate that the TGI is a highly variable phenomenon between individuals, in terms of frequency, intensity, and quality of the percept. Six participants (representing 12% of the sample) did not report either pain or unpleasantness to the TGS. Conversely, three participants (6%; 14% of responders) rated the pain as high as 9/10 on an 11-point pain NRS. Amongst non-responders, the weak intensity of their experience with the TGS was also confirmed by a lower likelihood to attribute words related to pain to the TGS, as well as a decreased likelihood to describe the TGS as either “hot” or “burning”. Previous researchers identified within their samples a similar proportion of non-responders (Bouhassira et al, 2005; Leung et al, 2005; Defrin et al, 2008a; Kern et al, 2008a; Kern et al, 2008b). Of remaining
studies using the TGS, Craig and Bushnell (1994) reported that ten of their 11 participants rated the TGS as painful. Green (2002), using milder temperature combinations (e.g., 27 °C and 40 °C), managed to elicit non-painful heat sensations. Participants of Fruhstorfer et al.’s (2003) study, were not explicitly required to assess their response to the TGS with respect to pain and did not describe the mixed stimulus as painful. The consistency in the proportion of non-responders across these different studies poses an interesting question as to what makes individuals more or less prone to feeling pain in response to the TGS.

Data from studies reporting on pain thresholds also point to considerable inter-individual variability (Taylor et al., 1993; Meh and Denislic, 1994; Hagander et al., 2000; Rolke et al., 2006; Wasner and Brock, 2008). This variance in thermal thresholds is highest at the lower limb (Meh and Denislic, 1994; Hagander et al., 2000; Rolke et al., 2006). Variability in pain responses across individuals have been attributed to genetic, physiological, as well as psychosocial factors (Mogil, 1999; Fillingim and Ness, 2000; Coghill et al., 2003; Seminowicz and Davis, 2006; Diatchenko et al., 2007). In contrast, test-retest paradigms indicate that individuals’ thermal pain thresholds are stable over periods of 1-21 days (Yarnitsky et al., 1995; Wasner and Brock, 2008). The analysis conducted in this study comparing the variance between and within individuals is based on similar calculations by Rolke et al. (2006) on their quantitative sensory testing data. Those authors reported that the lowest variance was between intra-individual thresholds at bilateral body sites. In the present study, inter-individual variance was also significantly higher than intra-individual variance in response to the TGS.

Pain and unpleasantness ratings in response to the TGS were not significantly different between females and males. Describing sex differences is important because of the higher incidence of several chronic pain states amongst females compared to males (Greenspan et al., 2007). Females are thought to be more sensitive than males to heat pain stimuli, and temporal summation of pain (Meh and Denislic, 1994; Fillingim et al., 1998; Rolke et al., 2006; Defrin et al., 2008a). Results from previous studies are equivocal on the importance of sex on the perception of paradoxical heat, with one study indicating a greater likelihood for females to report feeling paradoxical heat (Hämäläinen et al., 1982), while another finding no difference (Susser et al., 1999). Past studies using the TGS with similar numbers of males and females have not found significant differences between the two sexes (Bouhassira et al., 2005; Defrin et al.,
Both sexes were equally likely to be non-responders (Bouhassira et al., 2005). Thus, if sex differences do exist in response to the TGS, the effect is likely to be quite small.

4.6 Comments on the Methodology

Methodological concessions were made to facilitate comparisons between the stimuli and across body sites. Participants recorded information regarding their perceptions through provided pain and unpleasantness scales, as well as lists of descriptors (refer to response forms in Appendix C: Response Forms). They were also encouraged to provide further detail regarding their experience with the different stimuli in writing. Previous authors have questioned the influence that presenting a pain scale and pain-related descriptors may have on participants (Fruhstorfer et al., 2003). The response forms may have pushed participants to evaluate the TGS with respect to pain. However, the pain-related questions could also have had the opposite influence, i.e., participants rating the pain produced by the thermal stimuli more conservatively because the stimuli did not prove to be as painful as the expectations created by the forms. It is impossible to discern whether none, some, or all participants experienced either bias. However, it is important to stress that all participants were explicitly informed that they could report their perception of the stimuli as non-painful. Furthermore, the findings from this study suggest that the scales provided to participants were appropriate to capture the variability in response across our sample.

Using the same component temperatures for the TGS across all body sites and individuals meant that, for some trials, the cold temperature or the warm temperature fell either below or above pain thresholds, respectively. Previous authors have suggested that these uniform stimuli (20 °C and 40 °C) would be perceived as painful by certain participants (Bouhassira et al., 2005; Leung et al., 2005; Kern et al., 2008a; Kern et al., 2008b). To offset this possibility, some researchers have defined the cool and warm temperature with respect to the CPT and HPT, respectively (e.g., CPT +2 °C/HPT -2 °C) (Bouhassira et al., 2005; Kern et al., 2008a; Kern et al., 2008b). Having fixed temperatures across body sites and individuals increased the comparability of responses. Our decision to use the temperature 20 °C and 40 °C was based on previous studies which indicate that this temperature combination is effective in eliciting the TGI (Craig and Bushnell, 1994; Bouhassira et al., 2005; Leung et al., 2005). With milder temperature combinations, a smaller proportion of participants experience pain in response to the TGS (Bouhassira et al., 2005). Temperatures below 20 °C and above 40 °C (e.g.,
a combination of 18 °C/42 °C), however, approach mean pain thresholds reported by other authors (Wahren et al., 1989; Yarnitsky and Ochoa, 1990; Greenspan et al., 1993; Taylor et al., 1993; Meh and Denislic, 1994; Hagander et al., 2000; Harju, 2002; Rolke et al., 2006; Wasner and Brock, 2008).

4.7 Using the TGS as a Research Tool

It was proposed that the TGS can be used to mimic the burning pain experienced by neuropathic pain patients (Craig, 2008), as well as to evaluate the sensory effects of analgesic agents (Kern et al., 2008a; Kern et al., 2008b). Since neuropathic pain can occur in any region of the body, the findings from this study are an important step forward in adapting the TGS for pain research. In fact, it was not necessary to modify the thermal grill characteristics to elicit the TGI at the palm, back, calf, and foot. The following arguments can be advanced in favour of optimizing the TGS for research: First, the composite temperatures of the TGS do not represent a physical harm to the participant, and the TGS is ideal for prolonged testing paradigms. Second, the TGS is likely to reflect a modulatory relationship between cold thermoreception and nociception that is not described by traditional quantitative sensory testing (e.g., threshold testing). However, our results suggest significant variability across body sites and between participants. As mentioned above, stimulus variables (including the component temperatures, and the spatial distribution of warm and cool) could be modified in the attempt to make the TGI more consistent.

5 Conclusions

This study shows for the first time that the TGI can be elicited in body sites other than the palm and forearm. The response to a fixed TGS varied across body sites and individuals. These differences may relate to underlying differences in thermoreceptive and nociceptive functioning, and their interactions. Future studies should attempt to manipulate the TGS in an attempt to further elucidate the mechanisms of the paradoxical pain response elicited by this mixed stimulus.
References


Craig, AD (2008). Can the basis for central neuropathic pain be identified by using a thermal grill?. *Pain*.


Green, BG & Pope, JV (2003). Innocuous cooling can produce nociceptive sensations that are inhibited during dynamic mechanical contact. Exp Brain Res 148, 290-299.


Appendices
Appendix A  Recruitment Flyer

A pilot study to evaluate the perceptions produced by simultaneous combination of warm and cool stimuli

Graduate Student: Maria Brunello
Supervisors: Judi Hunter, PhD; Jonathan Dostrovsky, PhD

We are investigating a unique sensation that individuals’ experience during the simultaneous application of warm and cool stimuli. It is well known that when these stimuli are applied in a specific pattern of alternating warm and cool bars (thermal grill, TG) they produce a paradoxical heat illusion.

The purpose of this pilot study is to evaluate the range of responses to the thermal grill in a group of young healthy volunteers.

VOLUNTEERS ARE NOW NEEDED!!

You qualify if you are fluent in English, between the ages of 19 and 30, and are generally healthy.

Unfortunately we cannot accept you as a participant if you have a systemic, neurological, or skin disease; a history of diabetes or arthritis; or if you are currently experiencing recent or chronic pain. You will also need to pass a screening interview where you will be asked about these criteria.

What you need to know about the study:
It will involve a two-hour testing session, scheduled at your convenience, at the Medical Science Building, room 3302.
Testing consists of 1) warm, cool, cold, and heat threshold testing and 2) the simultaneous presentation of six thermal stimuli
Testing will be conducted on various body areas: palm, back, calf, and foot

YOU WILL BE COMPENSATED FOR YOUR TIME ($20).

If you are interested or have any further questions, please contact Maria Brunello to arrange your screening telephone-interview: 416-978-5289 or thermal.perception.study@gmail.com
Appendix B  Consent Form

Study Title: A pilot study to evaluate the perceptions produced by simultaneous combination of warm and cool stimuli.

Graduate Student: Maria Eugenia Brunello

Supervisors: Judi Hunter, PhD; Jonathan Dostrovsky, PhD

Contact Information: Please contact Maria Brunello: 416-978-5289 or me.brunello@utoronto.ca

Funding Organization: Canadian Institute of Health Research, CIHR

Introduction
Before agreeing to participate in this research study, it is important that you read and understand this research consent form. This form provides all the information we think you will need to know in order to decide whether you wish to participate in the study. If you have any questions after you read through this form, ask your questions to a study doctor or study personnel. You should not sign this form until you are sure you understand everything in the form.

Purpose
We are investigating unique sensation that individual’s experience during the simultaneous application of pleasant warm and cool stimuli. It is well known that when these stimuli are applied in a specific pattern of alternating warm and cool bars (thermal grill) they may produce a paradoxical heat illusion in some individuals. The purpose of this pilot study is to evaluate the range of responses to the thermal grill amongst a group of young health volunteers.

Subject Criteria
In order to be accepted into the study, you must:
Be between the ages of 19 – 30 years
• Have no hearing or language problems
• Have no systemic illness (e.g., diabetes, arthritis) or neurological disease
• Have no history of chronic pain
• Have no current pain or recent painful injury
• Have no skin disease, rash, or skin hypersensitivity or contact allergies
• Not be taking medication that may affect the results of the study (e.g., Tylenol, tobacco use)

Description of Study
Part 1: Once it has been determined that you meet the criteria for the study, you will complete the one page questionnaire that asks about your age, gender, education, and the criteria listed above.

Part 2: This will be followed by sensory testing. This testing measures your response to cool and warm temperatures. A small stimulator (1” x 1”) will be placed on your skin and the temperature will slowly increase or decrease. You will be asked to press a button as soon as you sense the change in temperature. This will be repeated 5 times for the cool stimulus and 5 times for the warm stimulus. This procedure will be repeated again and you will be asked to press a
button as soon as you sense that the cool stimulus become cold or the warm stimulus becomes hot. As soon as you press the button the temperature change will stop progressing. This sensory testing will be repeated on your palm, back, calf, and foot.

**Part 3:** The thermal stimuli will be delivered by a group of six metal bars, each the size of a pencil. The temperatures of the six bars may be: 1) all warm; 2) all cool or 3) they may be adjusted to the some are warm and some are cool. You will be asked to describe the sensation that you feel when you the six bars touch your skin. You will be asked to then describe how intense you feel that this sensation is. This will be repeated in the same body areas tested in part 2.

**Assurance of Confidentiality, Voluntary Participation, & Withdrawal**

- Your name and any other identifying information will remain confidential and all identifying material will be locked in a cabinet at the office of Dr. Dostrovsky, accessible only by the investigators listed at the top of this form.
- A number will be assigned to you and will be used to maintain privacy and confidentiality in any report or presentation given at the conclusion of this study.
- Your participation in this study is completely voluntary and you may withdraw your consent to participant at any time with no repercussion or undesirable effects on your future.

**Benefits**

There will be no direct benefit for participating in this study. However, the results of this study may benefit individuals living with chronic neuropathic pain.

**Potential Harm**

Other than the inherent time that participation involves, there are no known physical, emotional, or social harms associated with participation in this study.

**Confidentiality**

Confidentiality will be respected. Your name and any other identifying information will remain confidential and all identifying material will be locked in a cabinet at the office of Dr Dostrovsky, accessible only by the investigators listed at the top of this form. A number will be assigned to you and will be used to maintain privacy and confidentiality in any report or presentation given at the conclusion of this study.

**Compensation**

Participants will be rewarded $20 after successful completion of the study.

**Further Information**

If you have questions about your rights as a research participant, please contact the Office of Research Ethics at ethics.review@utoronto.ca or 416-946-3273.

**Dissemination of Findings**

If you wish, upon completion of this study, you will receive a brief summary of the study findings.

**Documentation of Informed Consent**
Your signature certifies that the content and meaning of the information on this consent form have been fully explained to you. It also means that you have read and understood the information presented above and you have decided to participate. Your signature also certifies that you have had all your questions answered to your satisfaction. Please contact the Site Investigator if you have any additional questions throughout this study. You will receive a copy of this consent form.

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Appendix C  Response Forms

Full Form

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<th>Site</th>
<th>Condition</th>
<th>Trial</th>
<th>Skin Temp.</th>
<th>Room Temp.</th>
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</table>

What was the predominant thermal quality of the stimulus?
- Neutral
- Cool
- Warm
- Hot
- Cold
- Mix of warm and cool

Was the sensation ever unpleasant?
- Yes
- No

Unpleasantness:

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| Unpleasant |   |   |   |   |   |   |   |   |   |   | As unpleasant as you can imagine

Was the sensation ever painful?
- Yes
- No

Rate the pain:

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| Pain   |   |   |   |   |   |   |   |   |   |   | Worst Pain

Choose the words that best describe the sensation you experienced:
- Neutral
- Throbbing
- Burning
- Aching
- Sharp
- Heavy
- Stabbing
- Tender
- Cramping
- Splitting
- Gnawing
- Tiring/Exhausting
- Sickening
- Fearful
- Punishing/Cruel
- Annoying
- Tingling
- Troublesome
- Other _______

Did you feel the urge to withdraw from the grill?
- Yes
- No

Comments
Short Form

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**Thermal sensation:**
- Neutral
- Warm
- Cool
- Hot
- Cold
- Mix of warm and cool

**Quality:**
- Neutral
- Throbbing
- Shooting
- Stabbing
- Sharp
- Cramping
- Gnawing
- Burning
- Aching
- Heavy
- Tender
- Splitting
- Tiring/Exhausting
- Sickening
- Fearful
- Punishing/ Cruel
- Annoying
- Tingling
- Troublesome
- Other ________

**Unpleasant?**

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