The Thermal Grill Illusion of Pain: Effects of Altering Placements of Warm and Cool Grill Elements

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 can elicit sensations of burning heat (the Thermal Grill Illusion, TGI). The TGI is thought to reflect changes in the central interactions between somatosensory sub-modalities (i.e. cold-inhibition of pain). Previous studies used multiple alternating warm and cool bars to elicit a TGI. The primary objective of this study was to evaluate the effects of altering the placements of warm and cool grill elements on the intensities of perceived pain and unpleasantness in 26 male subjects. Arranging the thermal grill elements in a warm flanking cool (WCW) pattern evoked significantly higher intensities of pain and unpleasantness than a cool flanking warm (CWC) pattern and a uniform warm stimulus. Conversely, CWC did not elicit significantly different intensities of perceived pain and unpleasantness than a uniform cool stimulus. This finding may reflect differences in the level of activation of central neurons.
Acknowledgments

Working towards a Master’s degree at the University of Toronto has undoubtedly been one of my most prosperous and rewarding experiences. I came to truly appreciate the vast field of neuroscience research and the accompanying painstaking efforts of scientists. I am proud to provide a small contribution to this field and am excited to witness the future scientific progression. This momentous opportunity allowed me to develop a deep interest in the neurosciences and I aspire to remain involved in this field.

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1 Introduction

1.1 Description and Purpose

The thermal grill illusion (TGI) was first characterized by Thunberg in 1895 (see Boring, 1942) as a paradoxical burning heat sensation elicited by the simultaneous application of interlaced innocuous warm and cool stimuli on the skin (a thermal grill stimulus, TGS). Recent research efforts attempted to uncover the physiological mechanisms underlying the TGI. To date, they are still a matter of debate. The most prominent theory (Craig et al., 1994) has postulated that the TGI reflects an interaction between the temperature and pain pathways. This theory is based on the concept that central cold thermoreceptive neurons normally inhibit the pain pathway, and that interlaced warm and cool stimuli reduce the activation of cold thermoreceptive neurons but not nociceptive neurons. Thus, according to this theory, the normal inhibition on the pain pathway by the cold thermoreceptive neurons is reduced and results in the perception of painful heat.

The paradoxical response to the TGS suggests that it may be an interesting and useful tool to study the somatosensory system, particularly how the pain and temperature pathways interact. This study sought to further characterize the importance of stimulus properties on the TGI. Most previous studies have utilized the typical alternating warm and cool configuration as a TGS. However, no studies have assessed the effect of altering the arrangements of warm and cool grill elements on the TGI or examined the ability of subjects to discern the temperature of the individual bars of the grill. In particular, the purpose of this study was to investigate the effects of alternating the placements of warm and cool bars of the TGS in a warm flanking cool pattern (i.e. a cool bar surrounded by warm bars) versus a cool flanking warm pattern.

1.2 Cutaneous Sensory System

The somatosensory system processes several sensory modalities, including touch, temperature, proprioception, kinaesthesia, itch, and pain (McGlone & Reilly, 2010). The system comprises receptors, peripheral afferents, and central neurons, which are responsible for the physiological detection and the perception of each stimulus modality. Distinct sets of neurons optimally respond to specific sensory modalities. Cutaneous afferents serve the perception of tactile (pressure, vibration, texture, and itch), thermal, chemical, and painful stimuli.
Proprioception is mediated primarily by afferents innervating the muscles and joints, which transmit joint and limb position information, although cutaneous skin stretch receptors also play a role.

Peripheral information from the skin sensory receptors and nerves is conveyed via sensory-modality defined pathways to various brain regions, notably through the dorsal columns and spinothalamic tracts (STT) amongst others. These two pathways differ in their receptor subset, spinal pathways, targets, and level of decussation within the central nervous system. The following literature review will focus strictly on the innervation of the cutaneous sensory system below the neck (i.e. exclusion of the trigeminal system) since this study only tested the forearm and calf.

1.3 Discriminative Touch
1.3.1 Peripheral Receptors and Afferents

The tactile component of the cutaneous system that serves the perception of pressure, vibration, and texture on the glabrous skin is subserved by functionally and morphologically distinct fast-conducting, large-diameter myelinated (A-β; conduction velocity: 35-75 m/s) afferents with specialized endings. These receptors, the Pacinian corpuscles (PC), Meissner corpuscles (RA), Merkel’s disks (SAI), and Ruffini endings (SAI) (reviewed in McGlone and Reilly, 2009) are responsive to low-threshold mechanical stimuli. The first two are classified as “rapidly adapting” as they respond only to the beginning and ending of a stimulus. The second two are classified as “slowly adapting” as they continuously fire for the duration of a stimulus. Pacinian corpuscles contribute in the detection of vibrations and are sensitive to slight indentations of the tissue. They have large and less defined receptive fields (RFs) and respond to vibration frequencies of 40-500 Hz. Meissner’s corpuscles are involved in the detection of light touch and tracking of an object as it moves along the skin. They have small and punctate RFs and have the highest sensitivity to lower frequencies of vibrations (2-40 Hz). Merkel’s disks are responsible for detecting maintained pressure. They have well-defined and spot-like RFs that are sensitive to indentation of the skin. Ruffini receptors have diffuse RFs that are sensitive to skin stretch. Their role in conscious touch perception is not well understood.
Both types of slowly adapting receptors (SAI and SAII) and also the PC receptors innervate the hairy skin. As well, the hairy skin contains two other classes of mechanoreceptors not present in the glabrous skin: hair units and unmyelinated (C-fibre; conduction velocity: 0.5-2 m/s) low-threshold mechanoreceptors. Hair units are known to innervate single or several follicles, which are activated by hair deflection. They have either large-diameter (A-β) or small-diameter (A-δ afferents; conduction velocity: 3-30 m/s) myelinated axons. There is also evidence for a population of C-fibres in the hairy skin that respond to light touch and slowly moving mechanical stimuli which may be involved with pleasurable touch (Johansson et al., 1988).

1.3.2 Central Projections

The dorsal column-medial lemniscus pathway is the main sensory pathway responsible for rostrally transmitting tactile and proprioceptive information from body areas below the neck (Cajal, 1952; Goodwin & Wheat, 2008; Willis & Coggeshall, 2004). The primary afferents ascend the white matter of the dorsal aspect of the spinal column forming the dorsal columns. First-order neurons travel up the dorsal columns through two separate tracts: the gracile fasiculus, consisting of those afferents innervating the lower half of the body (legs and trunk), and the cuneate fasiculus, consisting of those afferents innervating the upper half of the body (arms and trunk). These afferents synapse with second-order neurons in the medulla: the gracile nucleus and cuneate nucleus. The second-order neurons immediately decussate to form a contralateral tract, the medial lemniscus, and ascend the brainstem to the ventral posterolateral nucleus (VPL) of the thalamus. From the thalamus, third-order neurons travel up the internal capsule to synapse in the primary somatosensory cortex (SI) and other cortical regions, where conscious perception of touch is thought to be mediated (Cajal, 1952; Goodwin & Wheat, 2008; Willis & Coggeshall, 2004).

1.4 Innocuous Thermoreception and Pain

1.4.1 Peripheral Thermoreceptors

The cutaneous somatosensory system is able to detect a wide range of changes in ambient temperature. Thermosensation is mediated by primary afferents that transduce, encode, and transmit information regarding temperature. Thermoreceptive afferents have unencapsulated and morphologically-indistinct free nerve endings and are insensitive to mechanical deformation. Different classes of primary afferents have been identified that are involved in the innocuous
thermosensation of warmth and cold. Thermosensory fibres responding to innocuous warming and cooling will be discussed below, followed by a section characterizing nociceptive fibres.

1.4.2 Peripheral Afferents and Thermoreceptors Responsive to Cooling

Electrophysiological recordings in various mammalian species, including rats (Iggo, 1969), cats (LaMotte and Thalhammer, 1982), dogs (Iggo, 1969), monkeys (Bessou and Perl, 1969; Darian-Smith et al, 1973; Dubner et al, 1975; Hensel and Iggo, 1971; Iggo, 1969; Kenshalo and Duclaux, 1977; LaMotte and Thalhammer, 1982; Long, 1973; Long, 1977;), and humans (Campero et al, 2001; Konietzny and Hensel, 1983) have long identified A-δ ‘cold’ fibres that are exclusively activated by cooling. Cold fibres have a tonic discharge at normal skin temperatures, have spot-like RFs, ranging in diameter between 0.25 to 5 mm (Campero et al., 2001), and are mostly insensitive to mechanical stimulation (Darian-Smith et al., 1973; Kenshalo & Gallegos, 1967). A single cold afferent is also able to innervate multiple discrete RFs in both the hairy and glabrous skin (Bessou & Perl, 1969; Kenshalo & Duclaux, 1977). Cold afferents exhibit a bell-shaped stimulus response function, with maximal steady state activity between 20-30ºC and less activity at lower and higher temperatures (Darian-Smith et al., 1973; Kenshalo & Duclaux, 1977). Cold fibres have a low frequency of discharge or become silent at skin temperatures above 40ºC and below 17ºC.

Cold fibres exhibit a dynamic and graded response to rapid cooling shifts of 1-10ºC from a baseline temperature of 35ºC (Darian-Smith et al., 1973; Iggo, 1969). Cool stimuli elicit an initial transient period of high-frequency discharges in cold afferents, which decay in 2-15 seconds, followed by a slow adaptation to a steady-state activity (Darian-Smith et al., 1973). Rapid warm pulses transiently suppress cold fibre activity (Darian-Smith et al., 1973; Iggo, 1969).

Cold fibres also exhibit “paradoxical” activation upon noxious heat stimulation (Bessou & Perl, 1969; Campero et al., 2001; Kenshalo & Duclaux, 1977; Long, 1977). Typically, cold fibre afferent activity is inhibited with warming. However, stimulation with temperatures over 55ºC produces a transient discharge in cold fibres. Increasing the core body temperature also reduces the paradoxical heat threshold of cold fibres (Long, 1973; Long, 1977).
A population of ‘high-threshold’ cold receptors (HCR) has been identified in the monkey skin (LaMotte & Thalhammer, 1982). They are responsive to temperatures below 27°C, are insensitive to heat and mechanical stimuli, and have conduction velocities in the low A-δ to C fibre range. These afferents are unlike the classical ‘cold fibres’ in that they do not have ongoing activity at neutral temperatures, are able to adapt quickly and cease firing at steady state temperatures of 20-30°C, and have longer response latencies. It has been proposed that these fibres mediate innocuous cool perception at the cooler ranges of cold fibres. Innocuous cool stimulation has also been reported to activate unmyelinated, low-threshold mechanoreceptors (CLTMs) in the primate (Kumazawa & Perl, 1977). These C-fibres are activated by gentle touch and are insensitive to heat stimuli. They exhibit a small transient response to rapid cooling (>2°C/s), but not to static cool temperatures.

Microneurography studies in humans revealed a population of C-fibres that respond to innocuous cool temperature stimulation of the skin (Campero et al., 2001; Konietzny, 1984). They are activated by cold probes, display ongoing activity at normal temperatures, and do not respond to mechanical forces. Their role in innocuous cool perception is thought to be minimal. Human psychophysical studies have shown that A-δ nerve blocks prevent cool perception, implying that A-δ fibres are responsible for cool perception (Mackenzie et al., 1975; Ochoa and Torebjork, 1989; Wahren, 1989).

Large myelinated afferents (A-β mechanoreceptors) have also been found to respond to cooling in cats and monkeys. Particularly, about half of the slowly adapting mechanoreceptors (SA fibres; Merkel discs and Ruffini endings) respond to cooling from 14.5 to 33°C (Cahusac & Noyce, 2007; Duclaux & Kenshalo, 1972; Hensel & Zotterman, 1951; Iggo & Muir, 1969; Tapper, 1965). However, these afferents are not believed to play a prominent role in cool perception as their response is negligible in comparison to that elicited by mechanical stimulation (Johnson et al., 1973) and their activity likely only mediates tactile sensation.

1.4.3 Peripheral Afferents and Thermoreceptors Responsive to Warming

Peripheral thermoreceptive afferents responsive to innocuous warm temperatures have been described in the monkey (Darian-Smith et al., 1979a; Darian-Smith et al., 1979b; Duclaux and Kenshalo, 1980; Hensel and Iggo, 1971; LaMotte and Campbell, 1978). Warm afferents are unmyelinated (C-fibres) (Darian-Smith et al., 1979; Duclaux & Kenshalo, 1980; Hallin et al.,
Warm fibres respond to warming, are insensitive to mechanical stimulation (Hensel & Iggo, 1971; LaMotte & Campbell, 1978), and are inhibited by cooling (Darian-Smith et al., 1979). Warm fibre activation follows a bell-shaped stimulus response function, similar to that of cold fibres. Warm fibres display graded, steady-state firing frequencies at temperatures between 30-40°C (Darian-Smith et al., 1979; Hensel & Iggo, 1971; LaMotte & Campbell, 1978) and exhibit lesser activity at higher and lower temperatures (Duclaux & Kenshalo, 1980; Hensel & Iggo, 1971). Above 40°C, some warm fibres continue to fire with increasing rates as temperature increases to the noxious range, while another class of warm fibres reach a maximal firing frequency at 40-43°C and then level off with increasing temperatures (Duclaux & Kenshalo, 1980; Hensel & Iggo, 1971).

Warm fibres display strong sensitivity to warming. With rapid warming, an initial burst of activity is seen followed by an adaptation to the steady state temperature (Darian-Smith et al., 1979). More intense warming pulses elicit a higher dynamic firing frequency and a shorter latency in activation. However, when the upper range of the warming pulse is greater than 45°C, the dynamic response of the warm fibres plateaus or changes into short bursts with a subsequent cessation in firing (Darian-Smith et al., 1979; LaMotte & Campbell, 1978). With repetitive application of a warming stimulus at an inter-stimulus interval shorter than 60 seconds, the activity of the warm afferents decreases (Darian-Smith et al., 1979). There have been no reports of “paradoxical” activation of warm fibres upon innocuous or noxious cold stimulation.

Warm afferents have spot-like RFs that are less than 1 mm in diameter (Darian-Smith et al., 1979; Duclaux & Kenshalo, 1980; Hallin et al., 1982; Hensel & Iggo, 1971; LaMotte & Campbell, 1978). Warm afferents are relatively sparse in comparison to cold afferents and are more difficult to isolate (Hallin et al., 1982; Iggo, 1969).

1.4.4 Primary Afferent Nociceptors

Thinline myelinated and unmyelinated nociceptive afferents have been characterized in monkeys (Davis et al, 1993; Georgopoulos, 1976; Georgopoulos, 1977; Meyer et al, 1991; Treede et al, 1995; Treede et al, 1998), and in humans (Adriaensen et al, 1983; Campero et al,
They terminate as unspecialized “free nerve endings” in the skin. Nociceptors respond selectively to mechanical, thermal and/or chemical stimuli that are tissue threatening or damaging. They are either responsive to one modality or are polymodal. Typically, primary afferent nociceptors are silent without noxious stimulation (Bessou and Perl, 1969; Georgopoulos, 1976; Georgopoulos, 1977; Hallin et al, 1981; LaMotte and Campbell, 1978; Simone and Kajander, 1997). Generally, nociceptors are first classified according to the morphological properties (unmyelinated, C-fibres versus small myelinated, A-δ fibres) of the axons associated with them.

Typically, the sensation of pain follows nociceptor activation. However, pain is more complex and not to be confused with nociception. It involves a conscious sensory experience, defined as an “unpleasant sensory and emotional experience associated with actual or potential tissue damage” (Merskey & Bogduk, 1994). Pain is therefore a multi-faceted phenomenon, having sensory-discriminative, cognitive-evaluative, and affective-motivational components (Treede et al, 1999). The following information will focus strictly on the characteristics of primary afferent nociceptors.

The largest population of nociceptors responds to more than one modality, i.e. polymodal, including noxious mechanical, thermal, and/or chemical stimuli (Bessou & Perl, 1969; Georgopoulos, 1976; LaMotte & Thalhammer, 1982). Another class of nociceptors is the thermally insensitive, high-threshold mechanoreceptors. They represent approximately 50% of A-δ and 10-30% of C-nociceptive fibres in the hairy skin of cats and glabrous skin of monkeys (Adriaensen et al., 1983; Bessou & Perl, 1969; Georgopoulos, 1976). There is also a small population of unimodal nociceptors identified in the hairy and glabrous skin of monkeys that are strictly sensitive to either noxious cold or heat stimulation (Georgopoulos, 1976; Meyer et al., 1991; Treed et al., 1998). In addition, there are mechanically-insensitive nociceptive afferents (MIAs) that have extremely high mechanical thresholds (around 150 g/mm²) or are mechanically insensitive (Davis et al., 1993; Meyer et al., 1991). Some MIAs are responsive to heat, whereas others are not. An injection of a mixture of inflammatory mediators was found to sensitize some of these afferents to mechanical stimuli (Davis et al., 1993; Meyer et al., 1991).
The A-δ nociceptive fibres respond to intense mechanical and/or thermal stimuli and have RFs in clusters of 5-20 small sensitive spots over an area of 2-3 mm in diameter (Bolanowski et al., 1988). A-δ mechano-sensitive afferents (A-MSA) have high mechanical thresholds (36 g/mm² in comparison to 0.1-1 g/mm² for A-β mechanoreceptors) (Bessou & Perl, 1969; Davis et al., 1993; Georgopouloos, 1976; Jarvilehto et al., 1976; Schmidt et al., 1997) and are sensitive to noxious cold and heat (Simone & Kajander, 1996; Simone & Kajander, 1997). The firing frequency of A-MSA fibres increase linearly with a decrease in temperature and are believed to encode the initial pricking sensation with a noxious cold stimulus (Cain et al., 2001; Davis, 1998; Simone & Kajander, 1997). Furthermore, teased fibre recordings in monkeys have provided further classification of A-δ nociceptors into two types in response to heat stimuli (Treede et al., 1995; Treede et al., 1998). Type I A-δ mechano-heat nociceptive afferents (AMHs) have high heat thresholds (>53ºC), display a delayed response to heat (~5 s), and slowly increase their firing rate during noxious heat application. Type II AMHs have lower heat thresholds (~46ºC), respond immediately (<1 s) to heat, and their response rapidly decays. Type II AMHs have either high mechanical thresholds or are mechano-insensitive. They are not found in the glabrous skin, which may account for the fact that the first (faster) part of the dual heat pain experience is absent on the glabrous skin (Treede et al., 1995). However, recent studies evoked sharp sensations in the glabrous skin with laser and contact heat stimuli, although of lower intensity than in the hairy skin (Hashmi & Davis, 2010; Iannetti et al., 2004; Towell et al., 1996).

Most of the C-fibre nociceptive afferents are polymodal and are activated by moderately intense mechanical, noxious heat, cold, and/or various chemical irritant stimuli (Bessou & Perl, 1969; Campero et al., 1996; Georgopouloos, 1976; Kumazawa & Perl, 1977; LaMotte & Thalhammer, 1982; Simone & Kajander, 1996). They have extremely variable RF sizes that vary with body site (Bessou & Perl, 1969; Bromm et al., 1984; Georgopouloos, 1976; Hallin & Wiesenfeld, 1981; Meyer et al., 1991; Schmidt et al., 1997; Van Hees & Gybels, 1981). Some nociceptive afferents were reported to have non-continuous RFs (Georgopouloos, 1976; Hallin & Wiesenfeld, 1981; Schmidt et al., 1997). Their thresholds for noxious cold are reported to vary from -10 to 20ºC and display a linear increase in firing rate with increasingly noxious cold stimuli (Cain et al., 2001; Campero et al., 1996; Simone & Kajander, 1996). C-fibre heat thresholds are reported to range from 37-49ºC (Schmidt et al., 1997; Treede et al., 1990). Stimulus response functions for heat in polymodal nociceptive C-fibres in monkeys match the...
stimulus response function for heat in psychophysical studies in humans (Croze et al., 1976; Georgopoulos, 1976; Meyer & Campbell, 1981; Tillman et al., 1995), suggesting that nociceptive C-fibres encode noxious heat perception (LaMotte & Campbell, 1978). Furthermore, studies in the human peroneal nerves showed that about 50% of C-fibres respond to both mechanical and noxious heat stimuli, 25% are insensitive to mechanical and heat stimuli, 15% are insensitive to heat, and 10% sensitive to only heat (Schmidt et al., 1995).

With repeated stimulation, the number of evoked action potentials of A-δ and C-fibre nociceptive afferents are reduced (Adriaensen et al., 1983; LaMotte & Campbell, 1978; Peng et al., 2003; Price et al., 1977; Treede et al., 1998). However, continuous exposure to suprathreshold noxious heat leads to lowered thermal and mechanical thresholds in both A-δ and C-fibre nociceptive afferents in cats and monkeys (Bessou & Perl, 1969; LaMotte & Campbell, 1978).

Polymodal A-δ and C-fibre nociceptive afferents also respond to chemical stimuli, such as capsaicin, bradykinin, histamine, and mustard oil (Adriaensen et al., 1983; Davis et al., 1993; Meyer et al., 1991; Schmelz et al., 1996; Schmidt et al., 1997). Generally, the sensitivity of these afferents to chemical agents is relatively low in comparison to that of noxious heat and mechanical stimuli (Bessou & Perl, 1969; Campero et al., 1996; Georgopoulos, 1976; LaMotte & Campbell, 1978; Van Hees & Gybels, 1981). Yet, an intradermal injection of various chemical inflammatory mediators elicited a strong response to mechanically-insensitive nociceptive afferents (MIAs) (Davis et al., 1993; Meyer et al., 1991). As well, chemical mediators were able to sensitize nociceptive afferents and subsequently lower mechanical and thermal thresholds (Davis et al., 1993; Schmelz et al., 1996; Serra et al., 2004; Yeomans et al., 1996).

### 1.4.5 Spinal Cord Termination Patterns

The grey matter of the spinal cord consists of ten laminae (I-X) identified by Rexed (1952): The dorsal horn consists of laminae I-VI, the intermediate zone consists of laminae VII and X, and the anterior/ventral horn consists of laminae VIII-IX. All primary afferents innervating the body below the neck converge onto distinctive laminae of the spinal dorsal horn based on morphological and functional criteria (Han et al., 1998). Information from primary afferents (unmyelinated and thinly myelinated) regarding temperature, pain, and crude touch enters the dorsal horn via collaterals from the posterolateral (Lissauer’s) tract (LaMotte, 1977;...
Light & Perl, 1977; Sugiura et al., 1986). Most second-order dorsal horn neurons project to the brain through the spinothalamic tract, or through other ascending tracts such as the spinomesencephalic and spinoreticular tract. About 85% of second-order neurons from the dorsal horn immediately decussate before ascending to higher centres (Apkarian & Hodge, 1989).

Lamina I, also known as the marginal zone, is the major primary afferent termination site of A-δ and C-fibres (Gobel et al., 1981; Sugiura et al., 1988; Sugiura et al., 1989). The neurons in lamina I receive input from nociceptors, thermoreceptors, and/or polymodal afferents sensitive to strong mechanical stimuli and/or noxious cold and heat (Han et al., 1998).

Several distinct types of neurons have been identified in lamina I of the dorsal horn. Nociceptive-specific (NS) cells respond only to noxious mechanical (e.g., pinch) and/or heat stimuli, primarily receive A-δ input, and have no ongoing discharge (Christensen & Perl, 1970; Craig, 2003b; Dostrovsky & Craig, 1996; Ferrington et al., 1987; Price et al., 1978; Willis et al., 1973; Zhang et al., 2006). Another group of nociceptive neurons termed HPC cells are responsive to noxious heat, pinch, and cold stimuli, and respond to temperatures below 25ºC and above 45ºC (Christensen & Perl, 1970; Craig & Bushnell, 1994; Craig, 2003b; Davidson et al., 2008; Dostrovsky & Craig, 1996; Ferrington et al., 1987; Han et al., 1998; Zhang et al., 2006). HPC cells are thought to mainly receive C-fibre inputs and have little ongoing discharge (Craig, 2003b). Another class of lamina I neurons are wide(dynamic)-range (WDR) cells, which respond to both innocuous and noxious cutaneous stimuli (Andrew & Craig, 2011; Dostrovsky & Craig, 1996; Ferrington et al., 1987). These neurons can be polymodal and are sensitive to a wide range of mechanical stimuli including pressure and brushing. High-threshold cutaneous mechanoreceptors in the A-δ range also have a strong input to lamina I (Fyffe & Light, 1984; Mense & Prabhakar, 1986). These second order lamina I nociceptive neurons ascend via the contralateral spinothalamic tract to the thalamus and other brain regions and have an important role in pain perception and localization from the opposite side of the body (Craig, 2003b; Ferrington et al., 1987).

There are also thermoreceptive-specific lamina I neurons that are specifically sensitive to innocuous cooling or to warming. Cooling-specific neurons (COLD) are excited by small temperature drops below skin temperature, increase their firing rate with colder temperatures, and are unresponsive to mechanical stimuli (Dostrovsky & Craig, 1996; Hellon & Misra, 1973; Kumazawa & Perl, 1978; Zhang et al., 2006). Similar to classical cold afferents, they display a
tonic and graded response to cooling which plateaus around 15°C (Craig & Bushnell, 1994; Dostrovsky & Craig, 1996; Zhang et al., 2006). In addition, they also display paradoxical activation with noxious heat stimulation above 44°C (Dostrovsky & Craig, 1996; Zhang et al., 2006). Ascending projections of COLD cells in the spinothalamic tract have been identified in the cat (Craig & Kniffki, 1985; Craig & Hunsley, 1991; Craig & Bushnell, 1994) and monkey (Price et al., 1978). COLD neurons were found to have large receptive fields that covered parts of the entire limb. In contrast, Zhang (2006) reported that no NS cells had receptive fields as large.

Thermoreceptive-specific WARM cells in lamina I are selectively activated by cutaneous innocuous warming and receive input from C-fibre thermoreceptors (Andrew & Craig, 2001). They have thresholds around the 35-37°C range and discharge linearly with graded warming stimuli and their response plateaus or decreases in the noxious heat range (Andrew & Craig, 2001). WARM cells have moderately sized RFs either as small as the glabrous pads of the hindlimb or as large as all of the glabrous and hairy skin of the plantar hindlimb in the cat (Andrew & Craig, 2001). Dorsal horn lamina I thermoreceptive neurons have large RFs in comparison to the small punctate RFs of peripheral thermoreceptors, suggesting that dorsal horn neurons receive convergent input from many primary afferents (Andrew & Craig, 2001; Dostrovsky & Craig, 1996; Hellon & Mitchell, 1975; Zhang et al., 2006).

Lamina II neurons of the dorsal horn (substantia gelatinosa) receive primary afferent input primarily from unmyelinated sensory fibres that enter through the Lissauer tract (Pearson, 1952; Scheibel & Scheibel, 1968; Szentagothai, 1964). Responses to both high- and low-threshold mechanical stimuli have been characterized (Sugiura et al., 1986). As well, evidence that primary afferent nociceptors terminating in lamina II have been found (Dhaka et al., 2008). Small myelinated fibres (A-δ) also make synaptic contact with neurons in lamina II (Kumazawa & Perl, 1978; Sugiura et al., 1986; Yoshimura & Jessell, 1989). In addition, the ventral part of the substantia gelatinosa has been found to have considerable input from large-myelinated (A-β) afferent fibres (Scheibel & Scheibel, 1968; Szentagothai, 1964). Only a few of the neurons originating in lamina II project supraspinally up the white matter (Willis et al., 1973). Most of neurons in lamina II are interneurons and have connections to both lamina I and V neurons (Li et al., 1999). Lamina II is thought to have a role in nociceptive processing. In fact, lamina II receives dense descending inhibitory inputs from the brain stem and thus, could have an
important role in modulating the excitability of nociceptive transmission (Hori et al., 1996; Kawasaki et al., 2003; North & Yoshimura, 1984).

Lamina III is the site of termination for primarily large myelinated (A-β) fibres that enter from the medial division of the dorsal root (Beal & Fox, 1976; Hamano et al., 1978; Scheibel & Scheibel, 1968; Szentagothai, 1964). Some C-fibres have been found to terminate in lamina III, although their role does not seem to be significant in comparison to that of the large fibres (Sugiura et al., 1986). Many of the large myelinated (A-β) fibre inputs arise from Pacinian corpuscles and rapidly and slowly adapting mechanoreceptors that respond to vibration, hair brush, and innocuous pressure (Brown, Rose, & Snow, 1978; Kumazawa & Perl, 1977; Light & Perl, 1977; Price et al., 1978; Willis et al., 1973).

Lamina IV neurons have small cutaneous RFs and receive convergent input from many different cutaneous afferents (Wall, 1967; Brown et al., 1978). These neurons are excited by mechanoreceptors with A-β fibres, which are not responsive to noxious stimulation, and have been termed low-threshold mechanoreceptors (LTMs) (Wall, 1967; Price et al. 1978). Lamina V receives input from both muscle afferent fibres, thinly myelinated nociceptive fibres, and A-β mechano-sensitive afferents (Brown & Fyffe, 1979; Light & Perl, 1977). Neurons in lamina IV-VI respond to cutaneous stimulation, but only lamina VI neurons respond to movement (Wall, 1967). The cells in lamina V habituate to repeated light pressure stimuli, show a graded response to increasing intensities of mechanical stimuli, and have larger RFs than lamina I and IV cells (Wall, 1967). As well, lamina IV and V consist primarily of WDR neurons and have been found to respond to noxious and innocuous mechanical stimuli and noxious heat stimuli (Price et al., 1978; Willis et al., 1973).

1.4.6 Projections to Thalamus and Cortex

Axons of the spinothalamic tract (STT) ascend through the contralateral, anterolateral quadrant of the spinal cord. A large portion of STT neurons originate from the cervical and lumbar enlargements of the spinal cord (Apkarian & Hodge, 1989). The majority of axons that ascend in the lateral STT originate from lamina I cells (Apkarian & Hodge, 1989), however, there are also some inputs from deeper layers. In contrast, the majority of anterior STT axons originate from the deeper laminae (Apkarian & Hodge, 1989).
Neurons of the spinothalamic tract project to the medial and/or lateral thalamus and are strongly activated by volleys in cutaneous A-δ and C-fibres (Giesler et al., 1981). Neurons projecting to either the lateral or both lateral and medial thalamus originate in the dorsal horn. In contrast, cells terminating in the medial thalamus were found to mainly lie in the intermediate zone or ventral horn (Giesler et al., 1981).

The lateral thalamus and the medial thalamus are each believed to mediate different dimensions of pain: the sensory-discriminative aspect of pain has been attributed to the lateral, whereas the affective-motivational component of pain has been attributed to the medial (Treede et al., 1999). The lateral system receives strong input from afferents originating in laminae I and V (Apkarian & Hodge, 1989). Both the ventroposterior lateral (VPL) and inferior nuclei (VPI) in the lateral system are substantial relay stations for neurons originating in laminae I, IV and V of the STT (Apkarian & Hodge, 1989). Craig and colleagues have also provided evidence for a lamina I relay in the posterior aspect of the ventral medial nucleus (VMpo) and ventrocaudal medial dorsal nucleus (MDvc), which is described below.

The medial thalamus, which is responsible for the affective-motivation aspect of pain, also receives nociceptive information. Neurons in the medial thalamus respond to noxious stimulation and can code its intensity (Bushnell & Duncan, 1989). Most of the neurons in this area were classified as high-threshold nociceptive (62%) and WDR (25%) neurons. They have large, bilateral, and complex RFs that can span the entire body and face (Dostrovsky & Guilbaud, 1990; Giesler et al., 1981). In monkeys, neurons in the medial thalamus are modulated by changes in attentional state and by analgesics (Bushnell & Duncan, 1989).

Lamina I neurons also project to the posterior thalamus (Apkarian & Hodge, 1989; Craig, 2006; Davidson et al., 2008). Input to this area is mainly nociceptive, although some neurons have been found to respond to innocuous temperatures (Davidson et al., 2008; Zhang et al., 2006). In primates (including humans), the posterior aspect of the ventral medial nucleus (VMpo) receives strong lamina I input from both nociceptive cells (NS and HPC) (Dostrovsky & Craig, 1996) and innocuous WARM and COOL cells (Blomqvist et al., 2000; Craig & Hunsley, 1991; Craig & Bushnell, 1994). Input from the STT that is relayed through the VMpo projects to the dorsal anterior insula (Craig et al., 1995). The insula is involved in a variety of functions, including interoception (Craig, 2003a) and visceral sensation (Augustine, 1996) and is activated
by both innocuous and noxious thermal stimulation (Casey et al., 1996; Craig et al., 1996; Ostrowsky et al., 2002). Reports of cool, warmth, and pain can be elicited with microstimulation of the VMpo in humans (Davis et al., 1999; Lenz et al., 1993). The insula is thought to have a role in sensory integration of pain, taste, tactile, and vestibular input (Treede et al., 1999). Thus, it has been suggested that the VMpo is a lamina I STT thalamocortical relay nucleus in primates that subserves pain and temperature processing (Blomqvist et al., 2000; Craig et al., 1994).

The ventrocaudal part of the medial dorsal nucleus (MDvc) also receives input from lamina I STT cells and projects to the anterior cingulate cortex (ACC) (Craig et al., 1996; Vogt, 2005). The nociceptive neurons in the ACC have large, complex, and sometimes bilateral RFs that can cover the entire body (Sikes & Vogt, 1992; Yamamura et al., 1996). Although the ACC does not seem to play a major role in the sensory-discriminative coding aspect of pain, some intensity coding for noxious mechanical stimuli has been reported (Bushnell & Duncan, 1989; Sikes & Vogt, 1992; Yamamura et al., 1996). The ACC is believed to play a prominent role in affective-motivational pain processing (Treede et al., 1999). Imaging studies have found the ACC to be activated upon noxious mechanical and thermal stimuli (Treede et al., 1999). The STT input into the MDvc in humans is believed to be phylogenetically similar to the medial thalamic submedial nucleus (Sm) in the rat and cat (Craig Jr & Burton, 1981; Craig & Hunsley, 1991; Craig & Bushnell, 1994). The Sm receives thermoreceptive and nociceptive information from lamina I of the spinal cord (Craig & Hunsley, 1991; Dostrovsky & Guilbaud, 1988; Dostrovsky & Guilbaud, 1990). However, the Sm is only activated by nociceptive stimuli and not innocuous thermal stimuli.

The medial thalamic centre median (CM), central lateral (CL), and parafascicular (Pf) nuclei also receive input from laminae I, V, and deeper laminae of the STT (Apkarian & Hodge, 1989; Giesler et al., 1981). These nuclei have wide frontal basal projections, suggesting a role in the aversive behavior to pain (Craig Jr & Burton, 1981). However, their role is not fully understood.

Cells from the VPL and ventroposterior medial nucleus (VPM) project to both the primary and secondary somatosensory cortex (SI and SII) (Treede et al., 1999). Nociceptive neurons in SI have small RFs and display a somatotopic organization along the post-central gyrus (Lamour et al., 1983). These cells are able to encode graded intensities and location of
nociceptive stimuli (Kenshalo Jr et al., 1988; Kenshalo et al., 2000; Treede et al., 1999). As well, SI responds to innocuous warming and cooling of the skin (Chatt & Kenshalo, 1977; Duclaux et al., 1974).

The secondary somatosensory cortex (SII) contains few nociceptive neurons (Treede et al., 1999). Some have found these relatively sparse neurons to have large RFs, respond only to noxious mechanical stimuli, and encode stimulus duration of nociceptive stimuli (Dong et al., 1989; Whitsel et al., 1969). Dong et al. (1994) reported that about half of the heat nociceptive neurons encoded the intensity of stimulation. It has been postulated that SII does not have a strong role in the sensory-discriminative aspect of pain, but more of a role in spatially directed attention towards noxious stimulation (Dong et al., 1994).

1.5 Psychophysical Studies on Thermal Stimulation Induced Sensations

1.5.1 Detection of Innocuous and Noxious Thermal Stimuli

Measuring the thresholds for detection of thermal stimuli is common in many psychophysical studies and can be used to assess thermoreceptive and nociceptive function. Typically, in human studies, a contact thermode is placed onto the skin, which usually has a resting temperature of 32-34°C, and the temperature of the thermode is increased or decreased until the subject feels warmth (warmth detection threshold, WDT), cold (cold detection threshold, CDT), heat pain (heat pain thresholds, HPT), or cold pain (cold pain thresholds (CPT). These values have been reported to vary across subjects in the range between: 33-36°C, 28-32°C, 39-46°C, 10-25°C for WDT, CDT, HPT, and CPT, respectively (Greenspan et al., 1993; Hagander et al., 2000; Harju, 2002; Meh & Denislic, 1994; Rolke et al., 2006; Taylor et al., 1993; Wahren et al., 1989; Wasner & Brock, 2008; Yarnitsky & Ochoa, 1990a). The CDT displays the least variance, while the CPT shows the highest variance (Rolke et al., 2006). Previous studies yielded inconsistent results in the comparison of responses to noxious heat. Some have reported lower HPT in females (Arendt-Nielsen & Bjerring, 1998; Feine et al., 1991; Meh & Denislic, 1994) while others have reported no sex difference in HPT (Clausen & King, 1950; Kenshalo, 1986; Fellingim et al. 1996). Measurements of thermal thresholds also have been found to vary with: testing paradigm, body area, size of contact thermode, and age. Varying
resting/baseline skin temperature was not found to affect threshold measurements (Hagander et al., 2000; Meh & Denislic, 1994; Pertovaara et al., 1996).

1.5.2 A-δ and C-Fibre and their Contribution to the Perception of Pain

In humans, brief noxious heat stimulation can produce what is known as the first pain-second pain experience. The difference in the perception of first and second pain is attributed to the difference in conduction velocity properties of A-δ and C-fibre afferents. First pain is typically “sharp” and localized to the site of stimulation or injury, whereas the second pain that follows is a more dull and diffuse “burning” sensation that can persist well after stimulation (Price et al., 1977). The sharp first pain is experienced on the hairy skin and has been also reported to occur in the glabrous skin (Hashmi & Davis, 2010; Iannetti et al., 2004; Towell et al., 1996).

Price (1977) reported that first pain progressively decreased in perceived intensity with repeated heat stimulation having inter-stimulus intervals of less than 3 s on the same area. In contrast, second pain increased with repetitive stimulation. He posed that the temporal suppression of first pain is related to the suppression of A-δ heat nociceptors, whereas, the temporal summation of second pain is due to the summation within the central nervous system (Price et al., 1977). Both NS and WDR neurons have been found to show summated responses to repeated C-fibre activation and could account for this summation (Price et al., 1977). However, Craig (2003) speculated that NS lamina I STT neurons are associated with first pain, whereas the HPC lamina I neurons have been associated with second pain. The activity of HPC cells coincided with the perceptual increase in second pain intensity with repetitive stimulation (Craig, 2004).

1.5.3 Spatial Summation and Discrimination

Many studies have reported reciprocity between the stimulus area and the intensity of stimulation needed to detect suprathreshold warmth, non-painful cold, and pain (Bouhassira et al., 2005b; Defrin & Urca, 1996; Defrin et al., 2006b; Defrin et al., 2009; Douglass et al., 1992; Kojo & Pertovaara, 1987; Price et al., 1989). With a greater thermal stimulation area, lower stimulus intensity is needed for the detection of warmth, cold, and pain (Hardy & Oppel, 1937; Kenshalo & Gallegos, 1967). This phenomenon is known as spatial summation and plays a significant role in innocuous and noxious thermal perception. Larger stimulation areas increase
the perceived intensities of stimuli (Defrin & Urca, 1996; Defrin et al., 2008; Douglass et al., 1992; Price et al., 1989). However, with stronger intensities of thermal stimulation, the relative contribution of stimulus area to detection decreases (Stevens & Marks, 1979). For example, an area greater than 10 cm² of warmth stimulation will not further decrease WDT. For pain detection, increasing stimulation area reduces the HPT and CPT in the forearm and hand (Defrin & Urca, 1996; Defrin et al., 2006a; Defrin et al., 2009; Douglass et al., 1992; Price et al., 1989). Defrin and Urca (1996) reported that with a smaller 0.25 cm² probes, the average HPT was 47.7°C, while with a larger area of stimulation (15 cm²), the HPT was 42°C. Spatial summation is thought to be due to the convergence of peripheral axons onto a common central neuron (Defrin et al., 2009; Douglass et al., 1992; Green & Zahachuk, 2001; Torebjork et al., 1984). At higher intensities of stimulation, the activity of fewer fibres from a small area is sufficient to activate a central neuron. In contrast, with lower stimulus intensities, a larger area is necessary to provide sufficient input to activate central neurons.

Spatial summation occurs in both the hairy and glabrous skin (Defrin et al., 2009; Iannetti et al., 2006; Stevens & Choo, 1998; Towell et al. 1996). Defrin (2009) reported that spatial summation of CDT in the glabrous skin was greater than that in hairy skin. Moreover, spatial summation occurs both within and between dermatomes (Defrin et al., 2008; Douglass et al., 1992; Marks & Stevens, 1973). With heat-induced pain, separating two thermal probes over a range of 0-10 cm has no effect on the spatial summation of pain intensity and unpleasantness (Price et al., 1989).

On the other hand, spatial discrimination, the perceptual ability to discriminate between one or two stimuli, improves as the contribution of spatial summation lessens. Thermal stimuli are commonly applied concurrently with mechanical stimuli, i.e. application with a thermode. However, without mechanical cues, the localization of thermal stimuli is poor (Cain, 1973; Green, 1977; Green, 1978; Nathan & Rice, 1966; Simmel & Shaprio, 1969). The ability to localize radiant heat is relatively poor (Cain, 1973). With radiant heat stimulation, a suprathreshold warmth stimulus was incorrectly localized in 14% of trials (Cain, 1973). Also, the “two-point” threshold for radiant heat was found to be 4.5 cm (Von Bekesy, 1965). The “two-point” threshold is greater when thermal stimuli are placed across multiple dermatomes (Lee et al., 1996). The accuracy of localization increases with the intensity of stimulation (Nathan & Rice, 1966; Simmel & Shaprio, 1969). The ability to localize cool stimuli is greater than that of
warming stimuli. With nociceptive stimulation, localization ability is much greater. Studies reported localization errors from 6.7-23 mm in the forearm for nociceptive stimuli elicited by laser stimuli (Moore & Schady, 1995; Schlereth et al., 2001; Trojan et al., 2006).

1.5.4 Body Site Differences

Body site variations in “thermal sensitivity” in terms of spatial summation and thermal thresholds have been evaluated in previous studies.

The thermal sensitivity of the face (forehead and cheek) is consistently shown to have the highest sensitivity of all body sites, i.e. lowest sensory thresholds (Kenshalo et al., 1967; Meh & Denislic, 1994; Stevens et al., 1974; Stevens & Choo, 1998). In contrast, the lower extremities have the least thermal sensitivity, whereas the upper body and torso have intermediate sensitivity (Stevens & Choo, 1998). These variations in thermosensitivity are believed to reflect the differences in innervation density and receptive field size of peripheral thermoreceptors.

In general, the level of sensitivity to warmth in one region parallels that of cold (Stevens & Choo, 1998). More intense warm and cold sensations to the same stimulation size were induced in the forearm than in the calf (Stevens & Choo, 1998). Kenshalo (1967) found that the forearm is more sensitive than the back to small areas of radiant warmth stimulation. However, with larger areas of warmth stimulation, body site variance lessens. The ability to estimate the magnitude of warmth is greatest on the cheek, less on the forearm and back, and lowest on the calf (Stevens et al., 1974). For warmth detection, there were no differences found in the hairy and glabrous skin (Defrin et al., 2009; Defrin 2009). For cold detection, lower threshold values were reported in the thenar eminence of the hand than at the forearm or calf (Greenspan et al., 1993; Hagander et al., 2000; Hagander et al., 2000).

There have been only a few studies on the variation of thermal pain thresholds with body site. Meh (1994) reported slightly higher HPT and CPT in the forearm than in calf. CPTs were lower in the glabrous skin of the hand and foot compared to the hairy skin of the volar surface of the arm (Harrison & Davis, 1999).
1.5.5 Integration between Modalities

Thermosensation and nociception involve both specific labeled-line and convergent somatic activity (Craig, 2003b). The labeled-line theory of somatosensation holds that peripheral afferents and central neurons are activated by specific stimulus modalities. However, there is interaction between neuronal groups and top-down modulation of spinal transmission. In 1965, Melzack and Wall (1965) introduced their “gate control” theory of pain in which dorsal horn nociceptive neurons are inhibited by the activity of large-diameter fibres, those responding to touch, pressure, and vibration (Melzack & Wall, 1965). Indeed, vibratory and dynamic mechanical stimuli can reduce thermal pain to a greater extent than static stimuli (Bini et al., 1984; Green & Pope, 2003). Bini et al. (1984) reported that an innocuous cold stimulus was also able to dampen the intensity of perceived thermal pain. Nerve compression and ischaemic block studies, which eliminated the contribution of A-δ afferents, resulted in a lowered CPT, i.e. warmer threshold temperature (Fruhstorfer, 1984; Wahren et al., 1989; Yarnitsky & Ochoa, 1990a). As well, A-δ blocks consistently produce temporary cold allodynia, which is believed to be due to the loss of inhibition of the pain pathway by cold fibre input (Davis, 1998; Mackenzie et al., 1975; Wahren et al., 1989; Yarnitsky & Ochoa, 1990a).

Further evidence of interaction between thermoreceptors and nociceptors arises from the phenomenon of low-threshold nociception (LTN). For example, Green (2008) reported that nociceptive sensations could be evoked by cooling or heating the skin to mild temperatures between 29ºC and 37ºC. Because touch and dynamic tactile stimuli can inhibit pain, thermode contact with the skin may reduce the probability of discovering LTN sites (Green et al., 2008). Although the neurophysiological basis of LTN spots is unclear, Green (2008) stated these results provide evidence that primary afferents sensitive to innocuous warming and cooling relay to the nociceptive system.

Warm stimuli, unlike cold, have not been reported to reduce pain perception. In addition, warm perception is not affected by A-δ fibre block (Fruhstorfer, 1984; Ochoa & Torebjork, 1989; Wahren et al., 1989). However, when a compression-ischaemia block is maintained long enough to interfere with the conduction of unmyelinated nerves (C-fibres), the WDT increases, while HPT is unaffected (Yarnitsky & Ochoa, 1991). The authors suggested that this difference
demonstrated that the warmth sensation depends more on spatial summation than heat-induced

1.5.6 Paradoxical Heat

Paradoxical heat sensations (PHS) can be elicited in approximately 10-12% of
individuals upon innocuous cooling of the skin (Davis & Pope, 2002; Dyck et al., 1993; Green &
Pope, 2003; Greenspan et al., 1993; Harju, 2002; Harrison & Davis, 1999; Rolke et al., 2006)
and re-warming back to baseline temperatures (Davis & Pope, 2002; Davis et al., 2004).
Preheating the skin has been found to increase PHS up to 35% of individuals (Chery-Croze &
Duclaux, 1980; Greenspan et al., 1993; Hamalainen et al., 1982; Hansen et al., 1996; Susser et
al., 1999). PHS is common during nerve fibre blocks (Davis, 1998; Fruhstorfer, 1984; Mackenzie
et al., 1975; Wahren et al., 1989; Yarnitsky & Ochoa, 1990a) suggesting that unmyelinated C-
fibres are responsible for this paradoxical perception. Indeed, the peripheral conduction velocity
of afferents giving rise to PHS are in the C-fibre range (Susser et al., 1999). There is a higher
occurrence of PHS in patients with central and peripheral neuropathic disorders, such as uraemic
polyneuropathy (Yosipovitch et al., 1995) and multiple sclerosis (Hansen et al., 1996). An fMRI
study reported that the activation of the right insular cortex coincided with the individual’s
perception of PHS (Davis et al., 2004). Moderate cooling is known to activate both A-δ and C
fibre polymodal nociceptors. Thus, there is a possibility that polymodal nociceptors could
facilitate PHS (Campero et al., 1996; Georgopoulos, 1976; LaMotte & Thalhammer, 1982). One
group of authors suggested that PHS might reflect a malfunction of the cold-sensing pathway
disinhibition of the heat-sensing channel, at peripheral, central, or both levels (Susser et al.,
1999).

Another population of C-fibres was recently identified that responded to innocuous
cooling and sensitized by menthol (Campero et al., 2009). These cold-sensitive C-fibres (termed
Type 2 C-fibres or C2) also responded to temperatures up to 48°C and to a stronger magnitude
following sensitization with menthol. Half of these identified C2 fibres had thresholds to heating
below 38°C. These differed from A-δ cold fibres in that they fired to temperatures as cold as 0°C,
whereas typical A-δ cold fibres ceased activity at around 14°C. It is possible that C2 fibres are
responsible for the perception of paradoxical heat.
1.6 Thermal Grill Illusion of Pain

In 1896, Thunberg constructed a coiled instrument (which he termed the “heat grill”) from two copper tubes which warm (44°C) and cold (24°C) water was continuously circulated. Upon application of this apparatus to the skin, he described the perception “as if the temperature were suddenly raised and a feeling of ‘hot’ ensued”. Following, he regarded this “heat” sensation to be an imperfect fusion of warmth and cold, in which both warmth and cold could be still be separately perceived (Boring, 1942). Subsequently, Cutolo (1918) built a similar heat grill from apposed glass tubes of cold (13°C) and warm (44°C) water that was capable of recreating the paradoxical heat perception in which he described was like pain, a ‘sting’, or a ‘smack’ (Cutolo, 1918). Alston (1920) also demonstrated that the “warm-cold fusion” occurred when he stimulated spots as far apart as 10 to 15 cm (Alston, 1920). In addition, Burnett and Dallenbach (1927) found that the ‘heat’ sensation varied with the temperatures of the cold and warm water in the grill (Burnett & Dallenbach, 1927).

Recent psychophysical studies have further characterized the TGI. Many human subjects have described the perception of heat along with a burning and stinging sensation in response to an interlaced warm and cool thermal stimulus (Bouhassira et al., 2005a; Craig & Bushnell, 1994; Fruhstorfer et al., 2003; Green, 2002; Leung et al., 2005). The perceived sensation has also been reported as painful (Bouhassira et al., 2005a; Craig & Bushnell, 1994; Craig et al., 1996; Defrin et al., 2008; Kern et al., 2008; Kern et al., 2008) and thus the term “illusion of pain” is frequently used in the current literature to describe the sensation.

In 1994, Craig and Bushnell conducted an electrophysiological and psychophysical study to evaluate the mechanisms underlying the TGI (Craig & Bushnell, 1994). They proposed a physiological model of central disinhibition where the cold-evoked inhibition of the nociceptive channel is reduced when the warm elements took the place of alternate cooling elements in the grill. As a result, the nociceptive channel becomes “unmasked” (thus, referred to as the unmasking hypothesis). Electrophysiological recordings of dorsal horn lamina I spinothalamic tract cells in the lumbosacral cord of anesthetized cats revealed that the addition of interlaced warm (40°C) bars to a cool (20°C) stimulus reduced the activity of COLD cells, but not of HPC cells (Craig & Bushnell, 1994). Thus, the TGS shifted the relative pattern of activity in favor of the HPC (C polymodal) channel. This relative shift in pattern of activity resembled the pattern of
excitation produced by the presentation of a noxious cold stimulus according to an extrapolation of their psychophysical data (Craig & Bushnell, 1994). Craig and Bushnell (1994) believe that the integration of these nociceptive and thermoreceptive channels may be performed by neurons in the thalamus or cortex.

The modulatory effect of thermoreceptive activity on nociceptive pathways provides supporting evidence to the unmasking hypothesis. For example, the cold allodynic response demonstrated in subjects during A-δ blocks bear much similarity to the sensation induced by the TGI and may also involve the reduced cold-evoked inhibition of pain (Defrin et al., 2006a; Wahren et al., 1989; Yarnitsky & Ochoa, 1990a).

Subsequent studies by Green (2002) and Fruhstorfer (2003) demonstrated that the sensation produced by interlaced warm and cool stimuli is not always accompanied by pain. Mild temperatures (cooling to 29-32°C and warming to between 35-40°C) were able to produce a sensation of non-painful heat (Fruhstorfer et al., 2003; Green, 2002). Green (2002) maintained that this finding could not be directly explained by Craig and Bushnell’s hypothesis, which depends on the unmasking of the nociceptive pathway. Green (2002) suggested that the activity in the warm and cold fibres summated as they converged on lamina V WDR neurons (Green, 2002). Indeed, it has been previously shown that some WDR neurons are activated by mild cooling (28°C) (Khasabov et al., 2001) and can respond to warmth (Khasabov et al., 2001; Maixner et al., 1986; Willis Jr, 1985). Convergent input to WDR neurons could increase activity of these neurons to levels normally produced by intense heating or cooling. However, the role of WDR neurons in thermoreception is unclear. Both Green’s and Craig’s hypotheses are not mutually exclusive and could act over different but overlapping temperature ranges. Green’s hypothesis explains perceptions of heat produced by smaller differentials between warm and cool stimuli. Only when cooling and heating are sufficiently intense to directly activate temperature-sensitive nociceptors, will the sensation be perceived as painful. A subsequent positron emission topography (PET) imaging study demonstrated the brain activity response to the TGS (20°C/40°C) resembles activity evoked by only noxious (not innocuous) stimulation (Craig et al., 1996).

Furthermore, greater temperature differentials produce higher reports of “heat” or pain. At low temperature differentials (22/38°C and 24/36°C), only 20% of subjects experienced a “hot
burning pain” sensation (Leung et al., 2005). With higher temperature differentials (18/42°C and 20/40°C), about 70% of volunteers reported a “hot burning pain” sensation (Leung et al., 2005). Bouhassira (2005a) reported that the intensity of painful sensations was directly related to the magnitude of the difference (5-25°C) between the cool and warm stimuli of the thermal grill. Other stimulus parameters such as the number of stimulating bars (2-6) and the distance between bars (2-10 mm) did not affect the TGI (Li et al., 2009).

Pharmacological studies provided further insight into the nociceptive and thermal nature of the TGI (Kern et al., 2008a, Kern et al., 2008b). Systemic administration of morphine (0.1 mg/kg; opioid receptor agonist) and ketamine (0.2 mg/kg; NMDA antagonist) produced reductions in the intensity of the paradoxical pain elicited by the TGS, whereas naloxone (0.1 mg/kg; opioid receptor antagonist) did not. This suggested that the mechanisms underlying the TGI involve both the opioidergic and glutamatergic systems, but not the tonic endogenous opioid systems. Ketamine and naloxone administration did not affect physiological pain and non-painful thermal sensations, whereas morphine produced reductions in cold pain thresholds (Kern et al., 2008a, Kern et al., 2008b).

In neuropathic pain patients, there is a loss of warm and cool sensory capability in the regions to which ongoing pain is referred (Defrin et al., 2008; Ducreux et al., 2006). Craig (2008) notes that the burning pain referred to the areas of reduced warm/cool sensation resembles the TGI response. In accordance with the unmasking theory, Craig posits that in central pain patients the loss of thermosensation releases (or disinhibits) integrated polymodal nociceptive activity (Craig, 2008). Only two case studies testing central pain patients with the TGS have been published. In one study, a patient with complex regional pain syndrome type I (CRPS I), possessing cold allodynia, experienced an intolerable burning sensation on her affected hand with thermal grill stimulation (Heavner et al., 1997). The second study tested a multiple sclerosis patient, who exhibited heat hyperalgesia and cold allodynia and reported less pain with the thermal grill stimulation than to the cool component (20°C) alone (Morin et al., 2002).

Craig proposes that the thermal grill is a valuable investigative tool to study the dysfunctional neural basis and mechanisms of thermosensory integration in central pain patients (Craig, 2008). As well, he mentions the potential for the thermal grill to become a diagnostic tool
for central pain (Craig, 2008). Kern et al. (2008b) also noted that the “central mechanism underlying the thermal grill illusion is pharmacologically distinguishable from the neural mechanisms underlying both innocuous thermal sensations and noxious thermal sensations”. Kern et al. (2008b) discussed the potential for the thermal grill to unravel the physiological mechanism of analgesics.

Furthermore, subjects with depression reported increased unpleasantness and pain intensity to the TGS (Boettger et al., 2011; Pinerua-Shuhaibar et al., 2011). Similarly, a sad mood induction, but not neutral or elevated mood inductions, in healthy volunteers increased pain intensity and unpleasantness to the TGS (Boettger et al., 2011; Pinerua-Shuhaibar et al., 2011). This demonstrates an association between minor depression and sadness with an increased TGI response, possibly due to enhanced central processing of nociceptive C-fibre polymodal activity.

Another group reported that the middle finger immersed in cold (14°C) water felt significantly hotter when the outer fingers (second and fourth fingers) were immersed in warm (43°C) water than in 29°C water (Kammers et al., 2010). This suggests a release of inhibitory nociceptive input normally evoked by the cold due to the warm water. When both hands were thermally stimulated in this same fashion and touched with each other (corresponding fingers touched), the perceived temperature of the middle finger was reduced. The authors proposed that self-touch enhanced the coherence of cognitive body representation (Kammers et al., 2010). This provides further evidence that the TGI is a central phenomenon. This evidence also points to the possibility of the involvement of SII, since neurons in SII of the primate have bilateral RFs on the hands or arms (Iwamura, 2000; Whitsel et al., 1969), integration across multiple fingers (Fitzgerald et al., 2006a, Fitzgerald et al., 2006b), and a somatotopic coding of thermal and nociceptive input (Kammers et al., 2010).

1.7 Rationale and Aims

The goal of this study is to further characterize the stimulus properties of the TGI amongst human subjects while investigating the physiological underpinnings underlying this phenomenon. Previous studies examined the effect of altering various stimulus parameters on the TGI (discussed in section 1.8). For example, researchers used a TGS of varying number of elements (from 2 to 15) (Boettger et al., 2011; Bouhassira et al., 2005a; Craig & Bushnell, 1994;
Craig et al., 1996; Defrin et al., 2008; Fruhstorfer et al., 2003; Green, 2002; Heavner et al., 1997; Kern et al., 2008a; Kern et al., 2008b; Leung et al., 2005; Li et al., 2010; Morin et al., 2002; Pinerua-Shuhaibar et al., 2011) and altered the spacing between elements (from 2 to 10 mm) (Li et al., 2010). These properties did not affect the occurrence of the TGI. Previous studies used various fixed combinations of warm and cool element temperatures, whereas, others have based warm and cool temperatures on HPT and CPT values (Bouhassira et al. 2005, Kern et al., 2008a, Kern et al., 2008b). In addition, greater temperature differentials between warm and cool temperatures 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) TGI. However, there have been no reports of the effect of altering the placements of warm and cool grill elements on the TGI. Previous studies always used an equal number of alternating warm and cool elements to elicit a TGI. Investigating the importance of bar configuration on the TGI response could potentially further elucidate the underlying mechanisms. Assuming the TGI depends on the release of inhibition (or unmasking) of the nociceptive pathways normally induced by the cold thermoreceptive pathways (Craig et al., 1994), increasing or decreasing the relative activity of HPC to COOL channels should alter the TGI. Thus, increasing the warm-to-cool stimulation within a TGS should increase relative HPC to COOL channel activation. Therefore, the specific aim of this study was to test if configuring the thermal grill elements in a warm flanking cool pattern (WCW, greater warm-to-cool stimulation area) versus a cool flanking warm pattern (CWC, lesser warm-to-cool stimulation area) would evoke higher intensities of pain than a CWC pattern of stimulation.

This study also sought to characterize body site differences on the TGI to help further understand thermoreceptive function across body sites. Specifically, the response to thermal stimulation of the volar forearm and posterior calf were examined to compare thermal sensitivity between the upper and lower extremities. Another area of interest was the subject’s ability to perceive the coolness or warmth of each individual bar of a TGS, which has not been reported in previous TGI studies.
2 Methodology

The Research Ethics Board of the University of Toronto reviewed and approved the study’s ethics protocol (REB file# 07-044). Approval extended to the consent form, questionnaires, and data collection sheets.

2.1 Subjects

Twenty-six subjects were recruited through flyers posted in buildings around the University of Toronto, St. George campus area (Appendix A). Candidates were screened through telephone for eligibility. Subject inclusion criteria were the following: males between the ages of 19-30, non-smokers, and fluent in the English language. Due to previous reports of variability in noxious thermal perception between males and females and changes in pain sensitivity across the female menstrual cycle, a male population was chosen (Bajaj et al., 2001; Fellingim et al., 1997; Giamberardino et al., 1997; Hapidou & Catanzaro, 1988; Hellstrom & Lundberg, 2000; Riley et al., 1999; Soderburg et al, 2006). Both left-handed and right-handed participants were accepted. 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 interview, subjects were asked to refrain from drinking caffeinated products prior to testing.

Testing took place in a quiet laboratory room that was in the Medical Sciences Building (Room 3302) at the University of Toronto and that was free from temperature fluctuations, drafts, and noises. The temperature of the room was maintained at 22-24°C. Participation consisted of a single testing session lasting approximately an hour and a half. Participants were first informed of the purpose of the study. Informed consent was obtained prior to any testing (Appendix B). Participants were told that they would be exposed to different combinations of thermal stimuli, but were not told what sensations these might produce. All recruited subjects fully participated in the study and were compensated twenty dollars at the end of testing.
2.2 Skin Temperature Measurement

Skin temperature was measured immediately before all thermal stimulus applications to ensure that skin temperature at the testing location was between 32ºC and 34ºC. A thermocouple (BAT-12; Physitemp Instruments Inc.) was taped onto the centre of the intended stimulus site. If skin temperature was below 32ºC, subjects would be asked to rest their body area of stimulation onto a water-circulated warming pad until skin temperature reached over 32ºC. If skin temperature was over 34ºC, subjects were asked to allow their forearm or calf to air-cool until skin temperature dropped below 34ºC.

2.3 Thermal Grill Construction and Testing

Previous studies have produced the TGS with an apparatus composed of 2 to 16 rectangular metal bars or tubes of alternating innocuous warm and cool temperatures (Bouhassira et al., 2005a; Craig & Bushnell, 1994; Craig et al., 1996; Fruhstorfer et al., 2003; Green, 2002; Heavner et al., 1997; Kern et al., 2008; Kern et al., 2008; Leung et al., 2005). Depending on the design of the apparatus, the width of the individual bars was between 0.75 cm and 1.2 cm. The spacing between each thermal element ranged from 2 mm to 10 mm.

The thermal grill (TG) apparatus in the current study (Figure 2-1) was similar in construction to that used by some previous researchers. For example, Bouhassira (2005) and Kern (2008) used a thermal grill consisting of 6 copper surfaced bars (1.2 x 16 cm) controlled by thermoelectric Peltier elements and spaced 2 mm apart. The thermal grill in this study consisted of six 220 mm (length) x 13 mm (width) x 6 mm (thickness) aluminum bars spaced 4 mm apart (from the edge of one bar to the edge of the next) (Rehabilitation Engineering Laboratory, Toronto Rehabilitation Institute – Lyndhurst Centre, Toronto, Ontario). Individual bar temperatures were controlled by three Peltier elements on each bar, connected in parallel, and controlled electronically 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 on each bar and provided continuous feedback information to the system.

For the purpose of this study, only five bars were used for sensory testing. The sixth bar was repositioned to the furthermost position from the subject and set to the neutral skin
temperature of 33°C. Subject’s contact with this bar was not controlled. For technical reasons, the bar could not be removed entirely for the study. The thermal grill was placed on a 66 cm table so that the subjects could comfortably place their volar forearm on the thermal grill for stimulus application. For stimulus application on the posterior calf, the thermal grill was placed on a 44 cm high stool so that the subjects could comfortably rest their calf on the thermal grill. Stimulus application was to the subject’s non-dominant side so that they were able to fill in questionnaires using their dominant hand (mentioned in Section 2.5.4). Subjects were not given any instruction concerning the pressure to be exerted on the thermal grill. All timing was conducted using a stopwatch visible to the subjects. Subjects were verbally instructed when to apply/remove their body area to/from the stimulus. The subjects were seated in a comfortable high-backed chair during testing. All testing was conducted by Jason Lam.

![Figure 2-1. The thermal grill (TG) apparatus.](image)

Five aluminum bars were arranged in parallel and spaced 4 mm apart. Each of the bars was cooled and heated by three Peltier elements. The uppermost bar (set to 33°C) was repositioned away from the subjects.

### 2.4 The Six Thermal Configurations

The warm and cool bars of the thermal grill apparatus were maintained at 40°C and 20°C, respectively. These innocuous temperatures were used by several previous researchers to effectively evoke the TGI response (Bouhassira et al., 2005a; Craig & Bushnell, 1994; Leung et al., 2005). The innocuous warm and cool temperatures used in this study were well below the generally accepted heat pain and cold pain thresholds of 45°C and 15°C for most individuals,
respectively, although a small percentage of individuals have unusually low pain thresholds in this range (Greenspan et al., 1993; Hagander et al., 2000; Harju, 2002; Meh & Denislic, 1994; Rolke et al., 2006; Taylor et al., 1993; Wahren et al., 1989; Wasner & Brock, 2008; Yarnitsky & Ochoa, 1990b).

A total of six different bar configurations (two experimental configurations and four control configurations) were used in this study. All configurations consisted of five bars. The experimental configurations had a single centre bar between two sets of contiguous bars of the alternate temperature as follows: Configuration CWC - centre bar 40°C, outer 4 bars 20°C; Configuration WCW – centre bar 20°C, outer 4 bars 40°C.

The control configurations were as follows: WWW - all five bars set to 40°C; CCC - all five bars set to 20°C; NWN - centre bar 40°C, outer bars set at a neutral skin temperature of 33°C, NCN - centre bar set at 20°C, outer bars set at 33°C. Configurations WWW and CCC were uniformly warm and uniformly cool. Configurations NWN and NCN were intended to control for the tactile input of the 5 bars in the experimental configurations. The order of delivery of the six different temperature configurations was pseudo-randomized so the two experimental configurations were never presented as the first stimulus. The experimenter was not blind to the order of thermal stimuli presentation because manual input was required to change bar temperatures.

2.5 Measures

The measures used in this study are described below. The order of presentation for the following measures will be discussed in Section 2.6.

2.5.1 Evaluation of the Initial Perception

The first stimulus in each run was a short (5s) stimulus, immediately after which the subjects completed a one-page questionnaire (Appendix C, Questionnaire – 5s Post-Stimulus). Subjects were asked to 1) subjectively describe their perception of the stimulus, 2) declare whether or not they perceived a thermal quality, and 3) declare whether or not the entire stimulus area was perceived as a uniform temperature.
2.5.2 Concurrent Pain and Unpleasantness Ratings

To calculate the perceived intensity of pain and unpleasantness during the stimulus period, subjects were asked to concurrently rate the intensity on an electronic VAS. The electronic VAS was presented through a computer interface (based on LabView 7.1; National Instruments). To rate pain and unpleasantness intensities, subjects used a computer mouse to control a sliding element along a horizontal line on the computer screen. The line had no demarcations other than two endpoint descriptors with text anchors; “no pain” and “worst pain imaginable”, or “not unpleasant” and “As unpleasant imaginable”.

Concurrent pain and unpleasantness ratings were obtained during the second and third stimulus in the run and were of 30 seconds in duration followed by a 60 second rest period. For each configuration, subjects rated pain intensity during the first 30 second stimulus and rated intensity of unpleasantness during the second identical 30s stimulus (Figure 2-2). The subject was instructed to continuously report pain/unpleasantness intensity of the sensation evoked by the TGS during stimulus application by moving the mouse horizontally while observing the results on the screen. Recording of cursor movement on the VAS scale was manually initiated as soon as the stimulus was applied to a body site and manually stopped after 30 seconds. Data points were automatically recorded electronically every 100 milliseconds and exported as an Excel file. The profile of the intensity ratings was graphed for visualization. The pain concurrent VAS ratings (P-10VAS) and unpleasantness concurrent VAS ratings (U-10VAS) were calculated from the average of the last 10 seconds of stimulation. Pilot tests indicated concurrent VAS ratings to plateau after approximately 10-15 seconds of thermal stimulation. To investigate pain and unpleasantness intensities after the plateau, the average VAS ratings during the last 10 seconds of stimulation were used for analysis. Mean values were compared by two repeated-measures ANOVA (for P-10VAS and for U-10VAS) tests with configuration and body site (forearm and calf) as within-subject factors. A Tukey’s HSD post-hoc test with Sidak correction for multiple comparisons was conducted to evaluate significant differences between specific configurations.
2.5.3 Post-Stimulus Rating of Perceived Intensity and Quality of the Thermal Stimuli

For each configuration at each site the overall perceived intensity and quality of the perception was rated immediately after the fourth stimulus in the run (Figure 2-2). The subject completed a one-page questionnaire (Appendix C, Questionnaire – 30s Post-Stimulus) as follows: first they were instructed to choose a word from a list (neutral, warm, cool, hot, cold, or a mix of warm and cool) that best described the predominant perceived thermal sensation; next, they were asked to declare whether or not the sensation evoked by that thermal configuration felt unpleasant. If the subject declared that the sensation seemed unpleasant, they continued on to rate the intensity of perceived unpleasantness on a 10 cm VAS, with texts as anchors: “Not unpleasant” and “As unpleasant imaginable”. If the response was “no”, they were asked to leave the VAS blank. The subject was then asked to declare whether or not the evoked sensation was painful. If so, they continued on to rate the overall perceived pain intensity on a 10 cm VAS, with texts as anchors: “No pain” and “Worst pain imaginable”. If the response was “no”, they were asked to leave the VAS blank. To compare the mean pain and unpleasantness intensity ratings for each configuration, two repeated-measures ANOVAs with configuration and body site (forearm and calf) as within-subject factors were performed for each. One evaluated pain (P-VAS) and a separate analysis evaluated unpleasantness (U-VAS).

The subjects then completed the McGill Pain Questionnaire Short-Form (MPQ-SF) (Melzack & Torgerson, 1971). The MPQ-SF was modified by adding the word “neutral” to the choices of words to help subjects describe a neutral sensation. Subjects were allowed to choose any quantity of words to fully help describe their sensation. Subjects were also allowed to provide any additional comments concerning any aspects of their sensation. Finally, the subjects were asked to declare if they ever felt the urge to withdraw from the grill.

2.5.4 Discrimination of Coolness/Warmth of Individual Bars in the Grill

To determine the influence of bar configuration on the ability to perceive the coolness or warmth of each bar in the configuration, subjects were asked to complete a questionnaire rating the perceived coolness/warmth of each bar on a 20 cm horizontal line with a 5 point ordinal scale (Appendix C, Questionnaire - Individual Bar Ratings of Coolness/Warmth). This task was completed during the last stimulus period in each run (Figure 2-2). The midpoint on the
scale was labeled “neutral”. An arrow defined the left 10 cm of the scale as “More cool” and the right 10 cm as “More Warm”. Four intervals (5 cm each) were labeled from left to right as follows: “Cold Pain”, “Cool”, “Warm” and “Heat Pain” with an illustrative colour key background. Subjects were given a one-page questionnaire with five of the scales described above clearly labeled so that each scale represented a single bar in the stimulus configuration. They were familiarized with the scale before the task began. They were asked to rate the perceived sensation at each bar by placing the appropriate mark on the temperature spectrum scale representing that bar. Subjects had the option to circle the text “Unsure”, which was adjacent to the VAS for each of the five bars if they were uncertain of their sensation at that particular bar. Subjects were given as much time needed to complete the questionnaire.

The exact location of the subject’s mark was measured in millimeters. Marks to the left of neutral point were given negative values and marks to the right of neutral point were given positive values. Subjects were asked to rate their temperature sensation on the VAS of each bar in a randomized order. Thermal stimuli were always applied on the subject’s non-dominant side so that they were able to indicate their rating on the VAS during stimulation with their dominant hand.

To compare the configurations where the temperature of the centre bar remained the same while the temperatures of the outer bars were warm (40ºC), cool (20ºC), or neutral (33ºC), two repeated-measures ANOVA tests were performed with configuration (first analysis: WWW, NWN, CWC; second analysis: CCC, NCN, WCW) and body site (forearm and calf) as within-subject factors to compare coolness/warmth ratings at the centre bar.

### 2.6 Order of Presentation

For each of the six bar configurations, two runs were conducted: one at the volar forearm and the other at the posterior calf. Testing of the forearm always preceded testing of the calf. Alternating the presentation of stimulus sets to the forearm and calf reduced consecutive thermal stimulation at one particular body site. As illustrated in Figure 2-2, each run tested one thermal configuration and consisted of the following sequence of stimulus periods and specific ratings for each as follows: 1) 5s thermal stimulus application and a one-page questionnaire (Section 2.5.1; Appendix C, Questionnaire – 5s Post-Stimulus), 2) 30s thermal stimulus
application with concurrent VAS measuring perceived pain intensity (Section 2.5.2), 3) 30s thermal stimulus application with concurrent VAS measuring perceived unpleasantness (Section 2.5.2), 4) 30s thermal stimulus application followed by a one-page questionnaire (Section 2.5.3; Appendix C, Questionnaire – 30s Post-Stimulus), and 5) thermal stimulus with temperature rating of each of the 5 bars (Section 2.5.4; Appendix C – Individual Bar Rating of Coolness/Warmth). Inter-stimulus periods were at least 60s.

At the beginning of the experimental session, a test run was conducted to familiarize the subjects with the testing protocol and the various measures used. All bars were set to 33ºC (approximate skin temperature).

![Graphical representation of a single run](image)

**Figure 2-2. Graphical representation of a single run.** On and off “steps” indicate thermal stimulus application and measures given are denoted by arrows. Vertical arrows indicate when the measure was taken, whereas horizontal arrows indicate that the measure was taken throughout the whole time period indicated.

### 2.7 Statistical Analysis

PASW Statistics 18.0.0 was used for statistical analysis. Comparisons of the data between different configurations across two body sites were performed using a repeated-measures ANOVA. Where the assumption of sphericity was violated, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. Post-hoc contrasts were performed to observe significant differences between the six thermal stimuli configurations and body sites. To compare two groups, a Student’s *t*-test (for independent groups) or a paired-sample *t*-test (for related groups) was performed. Binomial tests were conducted to compare the likelihood of two response outcomes within a single sample. $\chi^2$ tests were conducted to compare the likelihood of an outcome between two independent groups. To compare the response between two unrelated groups, Mann-Whitney *U* test was performed.
Significance values were set at $p = 0.05$. A Sidak correction of the critical significance level was used for multiple comparisons ($k$). ($p_{\text{adjusted}} = p / k$). Reported $p$ values correspond to a two-tailed test of significance.
3 Results

3.1 Subjects

Twenty-six male subjects (mean age: 23.5, SD = 2.9) were recruited for the study. All participants completed testing sessions and were included in the analysis. The majority of subjects were right-handed (n = 23).

3.2 Description of the Initial Perception (5s Stimulus)

For each configuration, there were a total of 52 runs (26 subjects; 2 body sites). When subjects were asked “What did the stimulus feel like?” they spontaneously provided only thermal terms to subjectively describe all of the six configurations (e.g., hot, warm, cold, cool, mix, or neutral). The majority of subjects perceived the uniformly warm configuration (WWW) as either “warm” or “hot” (98%; 51/52) (Figure 3-1). The control configuration NWN was most often described as “warm” (41/52) with remaining subjects reporting neutral or mixed thermal sensations. Configuration CCC was consistently reported to feel “cold” or “cool” (96%; 50/52) with the exception of one subject who reported that it felt “warm” at both the forearm and calf. Interestingly, configuration NCN was only identified as “cold” or “cool” in 15 of the 52 runs. In the remaining runs, NCN was described as “mixed” (17/52) or as “warm” or “hot” (15/52). Thus, after a 5s stimulus, there was a clear difference in the range of thermal percepts evoked by a single cold bar (NCN) that included a paradoxical warm sensation, which was not apparent in response to a single warm bar (NWN).

The experimental configuration CWC was identified as “mixed” (26/52), “cool” or “cold” (20/52), or “hot” or “warm” (6/52) (count of “cool” or “cold” vs. “warm” or “hot” reports: Binomial tests; p = 0.077 for forearm; p = 0.118 for calf). Configuration WCW was identified as either “mixed” (31/52) or “warm” or “hot” (21/52) (count of “warm” or “hot” vs. “cool” or “cold” reports: Binomial tests; p < 0.001 for forearm; p = 0.001 for calf). Thus, after a 5s stimulus, WCW was never perceived as cool or cold, whereas CWC could be perceived in some cases as warm and in some as cool. Similar findings were reported after the 30s stimuli.
Figure 3.1. Reports of perceived thermal sensations after a short five-second stimulus. The colored boxes within each column represent the number of subjects who reported each quality (hot, warm, cold, cool, mix, neutral, or other) for each configuration.

Subjects were asked to identify whether or not the thermal percept evoked by each configuration was uniform. Two participants did not respond to this question, so four runs were excluded from this analysis (thus, total of 48 runs). The thermal percept evoked by CCC was consistently declared as uniform (94%; 45/48), whereas that evoked by WWW was identified as uniform in 75% (36/48) of the runs. For the non-uniform configurations (NWN, NCN, CWC, WCW), it is simpler to report the numbers of subjects who perceived the non-uniform configurations as uniform since this indicates that subjects did not perceive the contrasting temperature of the centre bar. A uniform thermal perception was reported as follows: NCN: 9/48, WCW: 13/48, CWC: 13/48, whereas NWN was identified as uniform in 34/48, implying that in 71% of the NWN presentations, the subjects were unable to perceive the unique temperature of the centre bar. However, this was only true in 19% of the NCN presentations.

3.3 Concurrent Ratings of Pain and Unpleasantness

Five subjects who rated the concurrent pain and unpleasantness intensity as zero during the entire 30s stimulus period for all six configurations were excluded from the following analyses. Twenty-one subjects rated one or more configurations as painful (VAS > 0). Non-responders to the 30s stimuli are described in Appendix E.
3.3.1 Temporal Profile of the Intensity Ratings

The average time course of the perceived intensities of pain and unpleasantness during stimulus application for all subjects is illustrated in Figure 3-2. Of the six configurations, WCW evoked the highest average pain and unpleasantness throughout the 30s stimulus period at both body sites. This was clearly illustrated for pain intensity. The intensity of the pain evoked by WCW did not decrease across the entire stimulus period.

![Figure 3-2. Time course of average VAS ratings for each configuration.](image)

3.3.2 Average Late Phase Intensities of Concurrent Pain and Unpleasantness

The average perceived late phase intensity of pain (P-10VAS) and unpleasantness (U-10VAS) during stimulus application for all subjects averaged across the final 10s of the 30s concurrent response is illustrated in Figure 3-3. For analysis of the entire concurrent recording,
see Appendix F. There was a main effect of configuration for perceived pain (P-10VAS: F(5, 16) = 5.004, p = 0.006) and unpleasantness (U-10VAS: F(5, 16) = 6.87, p = 0.001). In both analyses, configuration WCW evoked the greatest response. There was also a main effect of body site for both pain and unpleasantness intensities. Ratings were higher in the calf (P-10VAS: F(1, 20) = 8.75, p = 0.008; U-10VAS: F(1, 20) = 10.4, p = 0.004). There was no significant interaction between the two main within-subject factors (configuration and body site) of the repeated-measures ANOVAs (P-10VAS: F(5, 16) = 2.663, p = 0.062; U-10VAS: F(5, 16) = 1.294, p = 0.315).

Post-hoc analysis of the main effect for configuration (Table 3-1) revealed that WCW elicited significantly higher pain intensity than all other configurations including the experimental configuration CWC (P-10VAS; p = 0.005) and the control configurations (Table 3-1). Similarly, perceived late phase intensity of unpleasantness for WCW was significantly greater than all other configurations with the exception of that evoked by WWW (U-10VAS; p = 0.069). In contrast, configuration CWC did not evoke significantly different late phase intensities of pain or unpleasantness than any of the control configurations except NWN.

Figure 3-3. Average late phase intensities of pain and unpleasantness for each configuration. The bar graphs display the concurrent intensity (VAS, 0-100) ratings for pain (left graphs) and unpleasantness (right graphs).
(right graphs) averaged across the final 10 seconds of the 30 second stimulus period (n = 26). Error bars represent standard error. Ratings during forearm stimulation are shown in the top two graphs; ratings during calf stimulation are shown in the bottom two graphs.

Table 3-1. P-values from post-hoc analysis of the concurrent intensity (concurrent VAS) rated during the last 10 seconds of stimulus application. P-values from comparison of pain intensity ratings are in the upper table and p-values from comparison of unpleasantness intensity rating are in the lower table. The bolded numbers highlight significant p-values (p<0.05).

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<thead>
<tr>
<th>Pain</th>
<th>WWW</th>
<th>NWN</th>
<th>CWC</th>
<th>WCW</th>
<th>NCN</th>
<th>CCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWW</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NWN</td>
<td>0.326</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CWC</td>
<td>0.990</td>
<td></td>
<td><strong>0.021</strong></td>
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<tr>
<td>WCW</td>
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<td><strong>0.001</strong></td>
<td><strong>0.005</strong></td>
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<td>NCN</td>
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<td>0.368</td>
<td><strong>0.001</strong></td>
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<td>CCC</td>
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<td>0.107</td>
<td>1.000</td>
<td><strong>0.002</strong></td>
<td><strong>0.530</strong></td>
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<th>CWC</th>
<th>WCW</th>
<th>NCN</th>
<th>CCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NWN</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CWC</td>
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<td></td>
<td><strong>0.011</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WCW</td>
<td>0.069</td>
<td><strong>0.000</strong></td>
<td><strong>0.034</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCN</td>
<td>0.727</td>
<td>0.607</td>
<td>0.069</td>
<td><strong>0.001</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCC</td>
<td>0.988</td>
<td>0.117</td>
<td>0.671</td>
<td><strong>0.005</strong></td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

3.4 Pain and Unpleasantness Intensities: Rated Immediately Post-Stimulus

Data of five subjects who rated the pain and unpleasantness intensities at zero for all six configurations were excluded in the following analyses. Twenty-one subjects rated one or more configurations as painful (VAS > 0).

3.4.1 Incidence of Perceived Pain for Each Bar Configuration

Figure 3-4 shows the number of VAS scores that were greater than zero for each bar configuration (total of 126 stimulus applications; 21 subjects, 6 configurations). The frequencies of painful and unpleasant responses significantly differed between configurations (Friedman’s Test; $\chi^2(5, 21) = 34.5$, p < 0.05). Configurations CWC and WCW elicited the highest frequency of painful and unpleasant responses. Stimulation to the calf elicited significantly more reports of
unpleasantness (81) compared to stimulation of the forearm (49) ($\chi^2(1) = 7.877, p = 0.005$), but this was not true for pain (44 reports in the calf vs. 31 in the forearm; $\chi^2(1) = 2.253, p = 0.133$).

![Figure 3-4. Frequency of pain and unpleasantness reports for each configuration.](image)

The coloured boxes within each column represent the number of times a response greater than zero was reported for pain or unpleasantness on the 10 cm VAS. For each column, the total number of responses is 126.

### 3.4.2 Comparison of Intensity Ratings of Bar Configurations

The “most painful” and the “most unpleasant” configurations were determined from post-hoc comparison of individual subject intensity ratings for each of the six configurations. Figure 3-5 illustrates, for each configuration, the number of subjects who rated that configuration as producing the most intense pain or unpleasantness. Configuration WCW most often received the highest pain intensity ratings in response to stimulation at both the forearm ($\chi^2(5) = 31.77, p = 0.000$) and the calf ($\chi^2(5) = 35.0, p = 0.000$). Similarly, WCW also most often received the highest ratings of unpleasantness at both the forearm ($\chi^2(5) = 15.0, p = 0.01$) and the calf ($\chi^2(5) = 33.57, p = 0.000$ for calf).
3.4.3 Intensities of Perceived Pain and Unpleasantness for Bar Configurations

The mean overall perceived intensity of pain and unpleasantness rated immediately after stimulus application is illustrated in Figure 3-6. There was a main effect of configuration for both pain (P-VAS: F(5, 16) = 5.223, p = 0.005) and unpleasantness (U-VAS: F(5, 16) = 10.053, p = 0.000). There was a significant effect of body site for unpleasantness (U-VAS: F(1, 20) = 14.295, p = 0.001), but not pain (P-VAS: F(1, 20) = 4.269, p = 0.052). There was no significant interaction between the two main within-subject factors (configuration and body site) of the repeated-measures ANOVAs (P-VAS: F(5, 16) = 1.112, p = 0.345; U-VAS: F(5, 16) = 0.886, p = 0.444).

A Tukey’s HSD post-hoc test with a Sidak correction for multiple comparisons was conducted to analyze further the differences between configurations (Table 3-2). The most striking comparisons were for intensities of perceived unpleasantness. WCW elicited higher intensity ratings for unpleasantness (p = 0.041), but not pain, (p = 0.809), than the contrasting experimental configuration CWC. However, both WCW and CWC were rated at significantly
higher pain and unpleasantness intensities than any of the control configurations, except CCC (see Table 3-2).

Figure 3-6. Perceived intensity of pain (left) and unpleasantness (right) rated immediately after a 30s stimulus to the forearm and calf; error bars represent SEM.

Table 3-2. P-values from post-hoc analysis of the intensity of overall perceived pain and unpleasantness in response to each bar configurations. P-values from the comparison of pain intensity ratings are in the upper table and p-values from comparison of unpleasantness intensity rating are in the lower table. The bolded numbers indicate significant p-values (p<0.05).
3.5 Perceived Qualitative Characteristics: Rated Immediately Post-Stimulus

3.5.1 McGill Pain Questionnaire-Short Form

Participants selected significantly more words from the modified MPQ-SF in response to configuration WCW than all other configurations except configuration CWC (repeated-measures ANOVA; main effect of configuration: F(5, 21) = 3.999, p = 0.011). The frequency of the most commonly selected words is shown in Table 3-3. “Burning” was chosen most often for WCW (36.5%), whereas the word “neutral” was chosen most often for all other configurations. “Burning” was also chosen for CWC (26.9%) and WWW (5.8%). The word “tingling” was also frequently chosen for each configuration (Table 3-3).

<table>
<thead>
<tr>
<th>Config.</th>
<th>Descriptors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWW</td>
<td>Neutral (51.9) Tender (9.6) Burning (5.8)</td>
</tr>
<tr>
<td>NWN</td>
<td>Neutral (73.1) Tender (11.5) Tingling (9.6)</td>
</tr>
<tr>
<td>CWC</td>
<td>Neutral (28.8) Burning (26.9) Tingling (15.1) Sharp (13.5)</td>
</tr>
<tr>
<td>WCW</td>
<td>Burning (36.5) Neutral (25.0) Sharp (21.2) Throbbing (19.2) Cramping (15.4) Tingling (13.5)</td>
</tr>
<tr>
<td>NCN</td>
<td>Neutral (65.4) Tingling (21.2) Sharp (9.6)</td>
</tr>
<tr>
<td>CCC</td>
<td>Neutral (50.0) Tingling (17.3) Aching (13.5)</td>
</tr>
</tbody>
</table>

**Table 3-3. Descriptors chosen to describe the six thermal stimuli.** Descriptors were chosen from words on the MPQ-SF modified by adding one additional word choice “neutral”. Participants were free to choose as many descriptors as they felt necessary. The bracketed numbers represent the percentage of participants who chose that particular descriptor.

3.5.2 Subjective Description of Overall Perception

Only six of the 26 participants provided additional comments on the quality of their sensation. One subject described configuration CCC as “colder as time goes by”. Another described configuration NWN as “pleasant”. One participant characterized configuration CWC as “strange”, while another identified it as “aching/numbing”. Two comments from separate individuals on the quality of configuration WCW were “on the edge of serious discomfort” and “felt a cold bar at the beginning, but then after a few seconds I predominantly felt warm”.  

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Few subjects declared that they had experienced the urge to withdraw from any of the thermal stimuli (Table 3-4). The urge to withdraw was declared most often after the WCW stimulus (forearm, n = 4; calf, n = 3) and never after CCC. Three subjects declared this after CWC stimulation to the forearm.

<table>
<thead>
<tr>
<th>Body Site</th>
<th>Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWW</td>
</tr>
<tr>
<td>Forearm</td>
<td>1</td>
</tr>
<tr>
<td>Calf</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3-4. Counts of subjects, for each configuration, who indicated the urge to withdraw from the thermal stimuli.

3.6 Perceived Coolness/Warmth of Individual Bars During Application of Each Configuration

Figure 3-7 display the data from configurations where the temperature of the centre bar was kept constant while the temperatures of the outer bars were either warm (40°C) or cool (20°C) or neutral (33°C). When the centre bar was cool (20°C) (configurations CCC, NCN and WCW), there was a significant effect for configuration (F(2, 20) = 11.638, p = 0.000) and body site (F(1, 21) = 4.597, p = 0.044) on the perceived temperature of the centre bar. The mean coolness/warmth rating of the middle bar in configuration CCC was -2.96 (SD = 2.36) in the forearm and -2.78 (SD = 2.42) in the calf. The middle bar was perceived as significantly warmer in configuration NCN in the forearm (-0.01, SD = 2.15, p = 0.002) and in the calf (-1.18, SD = 2.86, p = 0.002) and again significantly warmer in configuration WCW in the forearm (1.41, SD = 2.67, p = 0.000) and in the calf (mean = 0.30, SD = 2.34, p = 0.000). Thus, the coolness/warmth of the outer 4 bars (warm, cool, neutral) influenced the perceived temperature of the single cool centre bar at both body sites.

When the centre bar was warm (40°C) (configurations WWW, NWN, and CWC), there was a significant effect of bar configuration (F(2, 20) = 7.282, p = 0.004), but not body site (Figure 3-7; upper graphs). During forearm stimulation, the mean coolness/warmth rating of the middle bar in configuration WWW was 1.76 (SD = 1.69) and did not differ significantly
compared to configuration NWN (mean = 1.21, SD = 1.25, p = 0.410) or configuration CWC (mean = 0.74, SD = 2.29, p = 0.211). In contrast, during calf stimulation, mean coolness/warmth rating of the middle bar in configuration WWW was 2.55 (SD = 1.82) and perceived as significantly cooler in NWN (mean = 1.56, SD = 1.26, p = 0.008) and CWC (mean = -0.33, SD = 3.10, p = 0.001). Thus, the coolness/warmth of the outer 4 bars (warm, cool, neutral) influenced the perceived temperature of the single warm centre bar during calf stimulation only.

Figure 3-7. Perceived coolness/warmth of individual bars during application of configurations where the temperature of the centre bar remained the same (top graphs: 40°C; bottom graphs: 20°C) while the temperatures of the outer bars were warm (40°C), cool (20°C), or neutral (33°C). The line graphs represent the mean coolness/warmth rating (20 cm VAS; -10 to +10) for all subjects for both forearm (left graphs) and calf stimulation (right graphs). Negative values represent perceived coolness; positive values represent perceived warmth.

The same data was also evaluated to compare the effect on the perceived overall coolness/warmth when the outer four bars were constant and the centre bar was either warm or cool is illustrated in Figure 3-8. The coolness/warmth rating of all 5 bars was averaged in each configuration and compared. A repeated-measures ANOVA comparing configurations WWW
and WCW revealed a significant effect of configuration (F(1, 25) = 7.033, p = 0.014), but not body site (F(1, 25) = 0.924, p = 0.346). Configuration WWW elicited significantly higher (warmer) average bar temperature ratings than configuration WCW (F(1, 25) = 7.033, p = 0.014). A separate RM-ANOVA comparing configurations CCC and CWC revealed a significant effect of configuration (F(1, 25) = 43.791, p = 0.000) only. Configuration CWC elicited significantly higher (warmer) average bar temperature ratings than configuration CCC (F(1, 25) = 43.791, p = 0.000).

Figure 3-8. Perceived individual bar coolness/warmth when the outer four bars were constant (top graphs: 40ºC; bottom graphs: 20ºC) and the centre bar was either warm or cool. The line graphs represent the mean coolness/warmth rating (20 cm VAS; -10 to +10) for all subjects for both forearm (left graphs) and calf stimulation (right graphs). Negative values represent perceived coolness; positive values represent perceived warmth. Coolness/warmth rating values for each configuration match those from Figure 3-7, although different comparisons are illustrated.
4 Discussion

This is the first study to evaluate the effect of stimulus (i.e. bar) configuration within a TGS on perceived intensities of pain and unpleasantness. Typically, studies on the TGI used a TGS consisting of from 2 to 15 thermal stimulus bars arranged in a pattern of alternating warm and cool bars (Boettger et al., 2011; Bouhassira et al., 2005a; Craig & Bushnell, 1994; Craig et al., 1996; Defrin et al., 2008; Fruhstorfer et al., 2003; Heavner et al., 1997; Kern et al., 2008; Kern et al., 2008; Leung et al., 2005; Li et al., 2009; Lindstedt et al., 2011; Morin et al., 2002; Pinerua-Shuhaibar et al., 2011). The TGS in this study consisted of one central thermal stimulus (either warm or cool) flanked on either side by either warm or cool bars, essentially three alternating warm and cool stimuli in either a WCW or CWC configuration. Configuration WCW evoked significantly higher pain and unpleasantness intensities than the reverse thermal grill configuration (CWC). This was true when stimuli were applied to the forearm or to the calf although these were significantly higher pain and unpleasantness intensity evoked from calf stimulation. The incidence and intensity of pain and unpleasantness in response to WCW was significantly higher than that evoked by any of the control configurations. Although previous studies suggest that the number of alternating bars in a TGS does not affect the occurrence of the TGI (Debrin et al., 2008; Li et al., 2009), our findings show that the arrangement of cold vs. warm thermodes within the thermal grill does affect the incidence and intensity of evoked pain and unpleasantness. The differences in perceived pain and unpleasantness evoked by WCW vs. CWC provide further insight into the mechanisms underlying the thermal perception, pain, and unpleasantness induced by the TGS.

4.1 Response to WCW Versus CWC Stimulation

There were significant differences in the responses to WCW and CWC stimulation for all three quantitative VAS measures (concurrent, post-stimulus, and coolness/warmth ratings). WCW evoked significantly higher intensities of perceived pain and unpleasantness than CWC. Differences were also found in qualitative measures. WCW was most often described as “burning”, whereas CWC was most frequently described as “neutral”. Many previous studies have reported that the descriptor “burning” is commonly used to describe the sensation evoked by an interlaced warm and cool TGS (Bouhassira et al., 2005a; Craig & Bushnell, 1994; Defrin et al., 2008; Green, 2002; Kammers et al., 2010; Kern et al., 2008; Kern, Plantevin et al., 2008;
Leung et al., 2005; Li et al., 2009; Pinerua-Shuhaiar et al., 2011). Also, the highest numbers of reports of those who felt the urge to withdraw from the grill were in response to WCW stimulation.

Additionally, WCW, but not CWC, evoked significantly higher incidence and intensities of perceived pain and unpleasantness than the uniform all warm or cool stimuli of the same surface area. When the thermal grill configurations WCW and CWC were compared to the uniform thermal stimuli (WWW and CCC), only WCW evoked significantly higher intensities of perceived pain and unpleasantness than the uniform warm configuration, as measured by both concurrent and post-stimulus ratings. Subjects rated WCW (post-stimulus) on the forearm to be an average of 10 on a 100 mm VAS and 14 for stimulation on the calf, which was significantly more painful and unpleasant than a uniform 40ºC warm stimulus and 20ºC cool stimulus. Similar to previous thermal grill studies, a TGS composed of interlaced warm (40ºC) and cool (20ºC) stimuli was significantly more painful than uniform warm or cool thermal stimuli to an identical sized surface area. Craig and Bushnell (1994) reported an average pain rating of 12.5 on a 50 mm VAS to a 40/20ºC TGS at the palm, which was significantly hotter than a uniform 40ºC warm stimulus and less cold than a 20ºC cool stimulus. Similarly, Leung et al. (2005) reported an average value of 12 on a 100 mm VAS to a 40/20ºC TGS that was more painful than that to the related component thermal stimuli. Brunello et al. (2010) also reported that the alternating 40ºC and 20ºC TGS was significantly more painful and unpleasant than a uniform 40ºC warm stimulus and 20ºC cool stimulus. In contrast, the other thermal grill configuration, CWC, did not evoke significantly different perceived pain and unpleasantness intensities (concurrent ratings) than the uniform CCC configuration.

4.1.1 Potential Mechanisms

This difference in TGI response evoked by WCW and CWC provides some insight into the potential mechanisms underlying the thermal grill illusion of pain. According to the unmasking hypothesis proposed by Craig et al. (1994), the TGI demonstrated the central integration of ascending temperature and pain sensory channels. Normally, application of a cool stimulus (20ºC) activates both thermoreceptive-specific cells (COLD neurons) and multimodal cells that are responsive to heat, pinch, and cold (HPC neurons) of the lamina 1 STT. With the addition of interlaced warm bars (40ºC) to a cool stimulus, there is a reduction in the activity of
the COLD channel, whereas the activity of the HPC channel remains unchanged. This reduces the COLD pathway inhibition of the nociceptive channel resulting in increased pain (i.e. illusion of pain). Following this hypothesis, the different pain sensations evoked by WCW and CWC could be explained in terms of differing levels of activations of COLD and HPC channels. In the WCW configuration, there is reduced cold inhibition compared to the CWC condition and thus a greater illusion of pain.

Our findings are unable to either support or refute the premise that the TGI depends on the summation of inputs from warm and cold fibres on lamina V WDR neurons (Fruhstorfer, 2003; Green, 2002). There is insufficient characterization of the responses of WDR neurons to innocuous warm and cool stimuli, making it difficult to interpret our findings in terms of this theory. Future studies need to address this by examining the activation of lamina V WDR neurons by warm and cold fibres.

4.2 The Influence of Surrounding Thermal Stimuli on a Single Thermal Stimulus

This is the first study to reveal that the temperature of the surrounding stimuli (warm, cool, or neutral) influences the thermal perception of a single central cool or warm bar upon thermal application to a continuous area of the skin (e.g. forearm and calf). In the WCW configuration, the single cool central bar was reported to feel significantly warmer than the single cool central bar in configurations CCC and NCN. A similar phenomenon was reported by Green (1977), when alternating thermal stimuli were applied to three contiguous fingers (i.e. non-continuous area of the skin). Green (1977) stimulated the second and fourth fingers with warm thermodes set at 44ºC and the third finger by a thermode set at varying cool temperatures, 9, 17, 21, 25, and 33ºC. The quality of the sensation at the middle finger was dominated by the warmth sensation produced by the outer stimulators. Many subjects reported sensations indicative of heat at the middle finger, commonly “very warm” or “hot”. A heat sensation at the middle finger was more effectively produced when the middle finger was stimulated at colder temperatures. The sensations were sometimes described as “first feeling cold, then turning very warm or hot”, similar to a comment by one subject in the present study given after WCW stimulation, “felt a cold bar in the beginning quite strongly, but then changed to predominantly warm”. In another study, participants immersed their second and fourth finger in 43ºC water and
their third finger in 14°C water (Kammers et al., 2010). Subjects reported that their middle finger felt significantly hotter when their second and fourth fingers were dipped in the warm water than in 29°C water. Subjects overestimated the temperature of the middle finger by an average of 16.4°C. The opposing configuration CWC was not tested.

In the CWC configuration of the present study, the single central warm bar was reported to feel significantly cooler than the single central warm bar in configurations WWW and NWN. However, this effect was only apparent in the calf, but not the forearm. In Green’s (1977) study, when the participant’s outer fingers were stimulated by cold thermodes (9°C) and the third finger stimulated by a warm (44°C) thermode, the perceived magnitude of the warmth sensation at the middle finger was almost unaltered. He concluded that cold appears to be less able to alter the sensations produced by a warm stimulus than does warm to alter the sensations produced by a cold stimulus. In 1929, Dallenbach and Burnett (1929) came to a similar conclusion. They devised a linear equation to quantify the overall resultant temperature sensation when warm and cool stimuli were simultaneously applied to the forearm. In the equation, the warmth term required a weighting by a factor of three. Thus, this indicated that warmth is more effective in changing the temperature sensation than cold. Our results showed a similar effect only in the forearm. Thus, the influence of the surrounding bars on the temperature perception of the single warm centre bar may differ depending on body sites. Only Kammers et al. (2010) reported that participants experienced a painful sensation at the cool middle finger. The current study did not examine where the “pain” sensation, if present, was localized. Thus, it is unclear if the increased warmth perception produced at the cold site was spatially associated with the “pain” sensations reported.

4.3 Body Site Differences to Thermal Stimuli

There were significant differences in the responses to thermal stimulation of the calf and forearm. The concurrent ratings of pain and unpleasantness intensity to all six thermal configurations were significantly higher in the calf than in the forearm. Also, post-stimulus ratings revealed that all six thermal stimuli elicited significantly higher ratings of unpleasantness, but not pain, in the calf than the forearm. Indeed, the highest unpleasantness ratings were in response to WCW stimulation in the calf. Because no significant interaction between body site and configuration was found in both concurrent and post-stimulus ratings, the effect of body site
was not exclusive to the thermal grill configurations (WCW and CWC), but to all thermal configurations.

Only a couple of studies have reported differences in the TGI response at varying body sites. One reported slight differences in TGI incidence upon TGS application to the palm (49.3%) versus the fingers (44.9%), although the significance of this difference was not analyzed (Li et al., 2009). Another previous study by Brunello et al. (2010) reported differences in the TGI response at four body sites: palm, back, calf, and foot. Higher pain and unpleasantness ratings to the TGS were evoked in response to stimulation of the palm and back. Fewer participants reported pain and unpleasantness when the TGS was applied to the calf and foot. The results of the current study clearly reveal body site differences to both thermal grill stimuli and uniform thermal stimuli, suggesting a common underlying mechanism.

4.4 Using a Concurrent VAS to Measure Pain and Unpleasantness

Previous thermal grill studies quantified the intensities of sensations, notably pain, using a post-stimulus VAS and yielded similar results to this study (discussed in section 4.1) (Boettger et al., 2011; Bouhassira et al., 2005a; Craig & Bushnell, 1994; Kern et al., 2008; Lindstedt et al., 2011). Only Brunello et al. (2010) used a concurrent VAS to record changes in intensities of pain and unpleasantness to a TGS over a fixed time period. In the current study, the concurrent VAS recordings revealed that the perceived intensities of pain and unpleasantness rapidly increased during approximately the first five seconds of stimulation, and then remained relatively steady. However, Brunello et al. (2010) reported progressive increases or decreases in intensities after about 20 seconds depending on body region stimulated. Upon application of the TGS to the palm and back, the pain intensity ratings steadily increased, whereas to the calf and foot they steadily decreased. Another study reported a slow, but significant, increase in the perception of pain during thermal grill stimulation over 8 minutes, measured at three time points (0, 4, and 8 minutes) (Pinerua-Shuhaibar et al., 2011). In the current study, distinct patterns of increase or decrease of VAS ratings after the initial rapid increase in ratings were not apparent. This could possibly be due to the shorter time period of stimulation (30 seconds).
4.5 Presence of Painful Responses to the Uniform Innocuous Thermal Stimulation

Typically, pain VAS ratings to warm and cool thermal stimuli (i.e. 40°C and 20°C) below pain thresholds are zero or very low. For example, Craig and Bushnell (1994) reported a low average pain rating (<5 on a 50 mm VAS) to uniform cool (20°C) and warm (40°C) stimulation. Brunello et al. (2010) also reported low pain ratings to both uniform cool (20°C) and warm (40°C), which were similar across all body sites (palm, back, calf, and foot). Stimulation with a uniform 40°C stimulus to the calf elicited an average pain rating of around 0.5 (0-10 numeric rating scale), which was similar to that evoked by a uniform 20°C stimulus. Similar average pain VAS ratings were reported in this study upon WWW (3 on 100 mm VAS, post-stimulus) and CCC (4 on 100 mm VAS, post-stimulus) stimulation to the calf.

On the other hand, a couple of TGI studies have not reported the presence of painful sensations to uniform warm and cool stimulation. None of the subjects in the study by Leung et al. (2005) perceived pain to the uniform 20°C and 40°C thermal stimuli. Another study reported that none of the 42 participants in the study chose the descriptor “pain” to describe a 15°C uniform thermal stimulus during a 30-minute stimulation to the hand (Fruhstorfer et al., 2003). Instead, it was predominantly described as “cold” or “icy”.

These variations in pain reports may be explained from differences in methodology. For example, the use of different measures (concurrent versus post-stimulus VAS) may yield slight differences in results. Because the intensity of pain and unpleasantness ratings may fluctuate over time, using a concurrent VAS may be more accurate in assessing perceived sensations than a single rating VAS. In addition, presenting pain descriptors in a questionnaire could predispose subjects into believing a painful sensation would be perceived.

Interestingly, pain, albeit low, was evoked by thermal stimulation below HPT and CPT (generally accepted values of 45°C and 15°C, respectively). Green (2008) suggests that the perception of pain upon innocuous thermal stimulation may be indicative of “low-threshold nociception” (Green et al., 2008). He reported that nociceptive sensations (stinging, pricking, or burning) could be evoked by cooling or heating the skin within the supposedly innocuous temperature range of 28°C to 36°C. In this study, reports of pain to uniform innocuous 20°C and 40°C stimuli could be cases of low-threshold nociception. This finding provides further evidence
that primary afferents sensitive to innocuous warming and cooling may relay to the nociceptive system.

4.6 Strengths, Limitations, and Future Directions

This study presented findings that can contribute to understanding thermoreceptive and nociceptive function, and their interactions, within the somatosensory system. An increased warm-to-cool ratio of stimulation area evoked increased intensities of pain and unpleasantness. This finding is consistent with Craig and Bushnell’s proposed mechanism of cold-evoked inhibition of pain (Craig & Bushnell, 1994). One the other hand, our findings are unable to further the validity of the hypothesis that the TGI depends on warm and cold fibre summation on lamina V WDR neurons (Fruhstorfer, 2003; Green, 2002).

The current study presents certain limitations. For example, the pressure of each body site placed onto the thermal grill was not controlled, preventing us from concluding that pressure was not a significant contributing factor to the observable body site differences. Also, the exact location of thermal stimulation within each body site was not considered. This leaves it uncertain whether location (and possibly the effect of placing thermal stimuli within and/or between dermatomes) plays a role in the differences in responses to the thermal stimuli. As well, the natural size difference between the calf and forearm (and thus area of stimulation) of individuals was not considered and could be an additional factor contributing to body site differences. One more drawback of the study was the non-randomization of thermal stimulus presentation to each body site (stimuli always presented to forearm before calf) and recordings of concurrent pain and unpleasantness VAS responses (concurrent pain VAS ratings always recorded before concurrent VAS ratings). This approach may have presented an order effect. Preceding stimuli may have influenced the responses to subsequent stimuli.

This study has certainly presented some intriguing findings. However, there are still many unanswered questions. This study used a constant size of alternating stimulus (1 bar flanked on each side by 2 bars). Thus, it is unclear how the differing ratios of warm-to-cool stimulation area in WCW and CWC affect the thermal grill illusion. To test this, two different thermal configurations possessing the exact same warm to cool ratio (e.g. 1:1 ratio) should be used for stimulation. The two configurations should differ in the arrangement of warm or cool stimuli (e.g. positioning the warm stimuli on the outer edges, WCCW versus positioning the cool
stimuli on the outer edges, CWWC). If the ratio of warm-to-cool stimulation area is found to be of importance, studies could further determine how increasing or decreasing this ratio affects the TGI (comparing WCW [in this case, only 1 outer warm bar] to WWCWW and WWWCWWW).

Future studies should also further investigate the role of warm and cool patterns of stimulation and spatial limitations in eliciting the TGI. Different patterns of warm flanking cool could be evaluated to determine if this effect is only limited to a WCW stimulation pattern produced by parallel grill elements. For example, comparing the response to an array of circular thermal stimuli (e.g. where an outer circular warm stimulus surrounds an inner circular cool stimulus) to the linear WCW pattern of thermal stimulation used in this study may allow us to further understand the integration of sensory inputs from the skin. In addition, since not much is known about the influence of skin warm and cool skin areas on the firing of HPC and COOL neurons, electrophysiological recordings of lamina I dorsal horn HPC and COOL cells in mammalian species would help support or refute our proposed mechanism underlying the differences in response to WCW and CWC.

4.7 Conclusions

This is the first study to reveal that the application of warm stimuli flanking a single cool stimulus produces a significantly greater TGI than the opposite configuration. Important insight was provided towards the hypothesized mechanisms underlying the TGI. This study also delivers important implications concerning the position of warm and cool stimuli that researchers place within the thermal grill and the subsequent interpretation of the results. Continuing studies should be conducted to further elucidate this puzzling sensation elicited by the thermal grill.
References


Towell AD, Purves AM, Boyd SG. CO2 laser activation of nociceptive and nonnociceptive


Appendices
Appendix A  Recruitment Flyer

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

Graduate Student: Jason Lam  
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 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: forearm & calf

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

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

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

Graduate Student: Jason Lam

Supervisors: Judi Hunter, PhD; Jonathan Dostrovsky, PhD

Contact Information: Please contact Jason Lam: 416-978-5289 or thermal.study@gmail.com

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 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: The thermal stimuli will be delivered by a group of give 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. Application of the thermal stimuli will be to the forearm and calf.

**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 participate 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.

Participant Name (Please Print)  Participant Signature  Date
<table>
<thead>
<tr>
<th>Witness Name (Please Print)</th>
<th>Witness’ Signature</th>
<th>Date</th>
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</thead>
<tbody>
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</tbody>
</table>
Appendix C  Response Forms

Questionnaire – 5s Post-Stimulus

What does the stimulus feel like?

Does it have a thermal quality?

Yes  No

Is the thermal sensation uniform?

Yes  No

If it is not uniform, please elaborate on the spatial distribution of the sensation.
Questionnaire – 30s Post-Stimulus

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

Was the sensation ever unpleasant? If so: Unpleasantness (make a tick):
- Yes
- No

Was the sensation ever painful? If so: Rate the pain (make a tick):
- Yes
- No

Choose the words that best describes the sensation you experienced:
- Burning
- Aching
- Heavy
- Tender
- Splitting
- Tiring/Exhausting
- Sickening
- Fearful
- Punishing/Cruel
- Annoying
- Tingling
- Troublesome
- Other ______

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

Comments:
Questionnaire – Individual Bar Ratings of Coolness/Warmth

Please rate your sensation on the temperature spectrum:

<table>
<thead>
<tr>
<th>Thermal Grill</th>
<th>Cold Pain</th>
<th>Cool</th>
<th>Neutral</th>
<th>Warm</th>
<th>Heat Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate in this order:</td>
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<tr>
<td>Bar 1</td>
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<td>Bar 2</td>
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<td>Bar 3</td>
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<td>Bar 4</td>
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<tr>
<td>Bar 5</td>
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</tbody>
</table>

Circle if unsure: Unsure
Appendix D  Non-Responders

Five of twenty-six subjects (19.2%) did not feel any pain or unpleasantness to any of the six configurations and hence, were regarded as “non-responders”. Responders and non-responders were compared in terms of how they described configurations CWC and WCW. Non-responders chose significantly fewer descriptors from the MPQ-SF for all configurations than responders did (Mann-Whitney test; U = 18.5, p = 0.025). Non-responders also chose fewer descriptors for configurations CWC and WCW, but this difference did not reach significance (Mann-Whitney test; U = 35.0, p = 0.222 for configuration CWC; U = 33.0, p = 0.189 for configuration WCW). Non-responders were less likely to describe their sensation as “burning” to configurations WCW than responders ($\chi^2(1) = 3.27, p = 0.070$ in forearm; $\chi^2(1) = 4.54, p = 0.033$ in calf). However, non-responders did not significantly differ from responders in the likeliness to describe their sensation as “burning” in configuration CWC ($\chi^2(1) = 0.033, p = 0.856$ in forearm; $\chi^2(1) = 0.337, p = 0.562$ in calf). In addition, responders were the ones who mainly volunteered additional comments on the valence of the stimulus. Only one non-responder volunteered an additional comment.
Appendix E  Average intensities of concurrent pain and unpleasantness (entire 30s analyzed)

The average perceived intensity of pain (avgPVAS) and unpleasantness (avgUVAS) during stimulus application for all subjects averaged across the 30s concurrent response is illustrated in Figure F-1. There was a main effect of configuration for perceived pain (avgPVAS: F(5, 16) = 5.339, p = 0.004) and unpleasantness (avgUVAS: F(5, 16) = 6.764, p = 0.001). There was only a significant effect of body site for pain (avgPVAS: F(1, 20) = 7.382, p = 0.013; avgUVAS: F(1, 20) = 8.220, p = 0.010).

Post-hoc analysis of the main effect for configuration (Table F-1) revealed that WCW elicited significantly higher pain intensity than all other configurations (Table F-1) including the experimental configuration CWC (avgPVAS; p = 0.005) and the control configurations (except WWW). Perceived intensity of unpleasantness to WCW stimulation was significantly greater than all other configurations with the exception of that evoked by CWC and WWW. In contrast, configuration CWC did not evoke significantly different intensities of pain or unpleasantness than any of the control configurations except NWN.
Figure F-1. Average intensities of perceived pain and unpleasantness for each configuration. The bar graphs display the concurrent intensity (VAS, 0-100) ratings for pain (left graphs) and unpleasantness (right graphs) averaged across the 30 second stimulus period (n = 26); error bars represent SEM. Ratings during forearm stimulation are shown in the top two graphs; ratings during calf stimulation are shown in the bottom two graphs.

Table F-1. P-values from post-hoc analysis of the concurrent intensity (concurrent VAS) averaged across the 30 seconds of stimulus application. P-values from comparison of pain intensity ratings are in the upper table and p-values from comparison of unpleasantness intensity rating are in the lower table. The bolded numbers highlight significant p-values (p<0.05).