PSYCHOPHYSICAL EXAMINATION OF THE THERMAL GRILL ILLUSION

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ABSTRACT

Daniel Elliott Harper: Psychophysical Examination of the Thermal Grill Illusion
(Under the direction of Mark Hollins)

The thermal grill illusion (TGI) is a phenomenon in which interlaced warm and cool bars elicit sensations of burning heat, and in many cases pain. Although it was discovered in the late 19th Century, there is still no scientific consensus on its underlying mechanism. The primary goal of this dissertation was to test the validity of TGI theories by manipulating the illusion in ways that put the predictions of the theories at odds with one another. In Experiment 1, the TGI was subjected to conditioned pain modulation (CPM), a phenomenon in which one painful stimulus reduces the painfulness of another. CPM equally reduced the painfulness of the TGI and noxious heat, suggesting that the important signals for the TGI ascend to the brain in nociceptive spinal neurons (i.e. those known to be inhibited by CPM). This result is at odds with a cognitive addition theory, but it is compatible with two others. Therefore, Experiment 2 assessed whether the TGI is the product of a simple addition of warm and cool signals, as suggested by one theory, or if it is rather a result of more complicated interactions, as suggested by another. Subjects were selectively adapted to either the warm or the cool bars of the grill before being exposed to both the warm and cool temperatures simultaneously. Cool adaptation significantly attenuated the TGI while warm adaptation was without effect, indicating that the TGI is not due to simple addition. In Experiment 3, I tested the accuracy of anecdotal evidence that the TGI is more robust when

warm precedes cool. The issue is an important one, because any temporal effect could undermine the conclusions of Experiment 2. The protocol of Experiment 2 was altered so as to minimize adaptation while preserving stimulus offset. The results showed no evidence of a temporal order effect, suggesting that the results of Experiment 2 were due to adaptation. Taken together, these experiments suggest that the TGI is a complex product of activity in peripheral afferents that project to nociceptive spinal cord neurons, and that the grill's cool stimulus is particularly important in eliciting their activity.

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LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
COOL	Spinal cord neurons that convey cool sensation
CPM	Conditioned pain modulation
DNIC	Diffuse noxious inhibitory controls
HPC	Heat, pinch, cold
PAG	Periaqueductal gray
TGI	Thermal grill illusion
TRP	Transient receptor potential
VAS	Visual analogue scale
WARM	Spinal cord neurons that convey warm sensation
WDR	Wide dynamic range

CHAPTER 1: GENERAL INTRODUCTION

The thermal grill illusion (TGI) was discovered over a century ago (Thunberg, 1896), but its underlying mechanisms are still unclear. The illusion is created when interlacing warm and cool, but not painful, stimuli are applied simultaneously to the skin. The perceptual result of this stimulation is *synthetic heat*, meaning the grill feels hotter than the warm temperature alone. The TGI additionally includes an experience of pain when generated using more extreme, but still innocuous, temperatures (Craig & Bushnell, 1994). The original grill utilized warm and cool copper coils that were interlaced concentrically (Thunberg, 1896), an arrangement that was reconstructed in a recent study (Bach et al., 2011). However, most designs elicit the TGI using alternated parallel bars, heated and cooled by water flowing through them, that form a grill-like surface (Averbeck et al., 2012; Bishop, 1927; Boettger et al., 2011; Bouhassira et al., 2005; Craig & Bushnell, 1994; Craig et al., 1996; Fruhstorfer et al., 2003; Heavner et al. 1997, Kern et al., 2008a, 2008b; Leung et al., 2005; Li et al., 2009; Lindstedt et al., 2011a, 2011b; Morin et al., 2002; Twitmyer & Fernberger, 1927). This is also the mode of stimulation employed with the thermal grill used in the studies reported here (*See Figure 1.1*). However, the TGI can also be created using fairly unique designs, including interlaced sets of pre-heated or cooled nails that are attached to wooden blocks (Schlosberg & Carmichael, 1931), variable condensors obtained at a radio salvage company (Freeman, 1933), warm and cold air currents applied to the skin (Sullivan

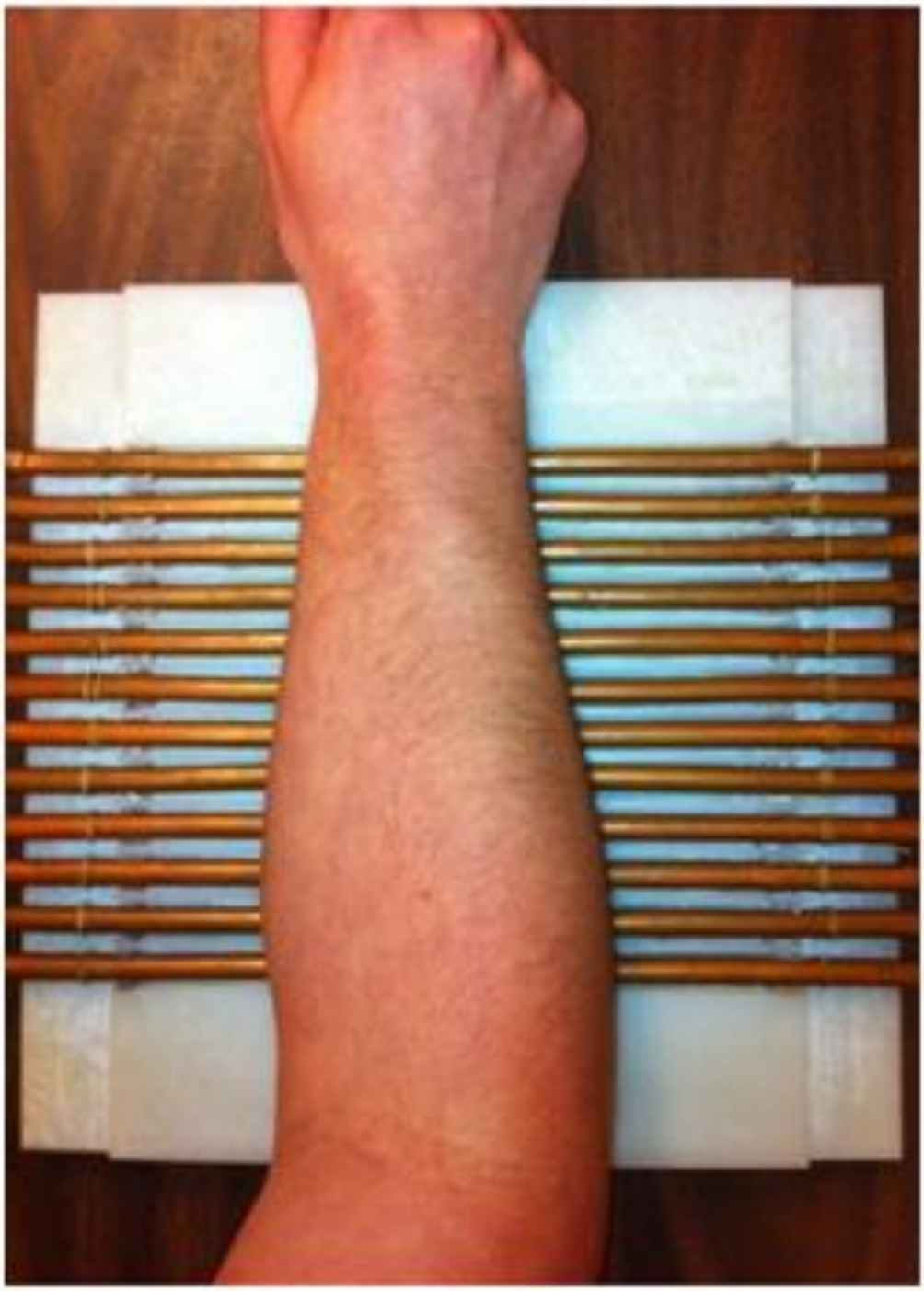

Figure 1.1 The thermal grill. Twelve cylindrical copper bars are arranged parallel to one another and held in place on a plastic base. Water is passed through the hollow centers of the bars from tanks that are located above the apparatus. There are two sets of bars, odd and even (counting from top), and each is connected by plastic tubes to the sides of the apparatus (not shown). To produce the TGI, cool (18°C) water is passed through one set and warm (42°C) is passed through the other set. The subject places his or her volar forearm onto the grill perpendicular to the long axis of the bars, as shown.

& Verda, 1930), two punctate thermodes (Cutolo, 1918; Defrin et al., 2008), or immersion of the index and ring fingers in warm water and the middle finger in cool water (Kammers et al., 2010). Thus, the most important factor in generating the TGI is the simultaneous application of warm and cool stimuli to nearby regions of skin; any apparatus that accomplishes this produces the TGI.

While the TGI is amenable to numerous modes of simultaneous warm and cool stimulation, the magnitude of the difference between the warm and cool temperatures can greatly affect the percept. At small deviations from normal skin temperature, which is approximately 32°C, the simultaneous application of barely perceptible warm (ex. 35°C) and cool (ex. 28°C) can give rise to the perception of synthetic heat without pain (Green, 2002). However, the TGI feels increasingly hot as the difference between the temperatures increases, and eventually feels painfully so to most individuals (Bouhassira et al., 2005; Burnett & Dallenbach, 1927; Leung et al., 2005).

Since Thunberg's (1896) discovery of the TGI, several theories have been espoused in the literature, but none has been conclusively established as valid. The earliest theory stated that the illusion is a high-level (i.e. cognitive) fusion of simultaneous warm and cool sensations (Alrutz, 1898). Two recent theories argue that the important interactions for generating the TGI take place in the spinal cord dorsal horn, where pain and temperature signals from the skin first synapse (Bouhassira et al., 2005; Craig & Bushnell, 1994; Green, 2002; Kern et al., 2008a; 2008b). Each of the three theories has its merits, but none appears capable of explaining every aspect of the TGI.

Understanding the processes that create the TGI is interesting in its own right, but knowledge of how innocuous stimuli transform to become painful could also increase our

understanding of the nervous system's normal code for thermal pain at genuinely noxious temperatures. Furthermore, certain types of neuropathic pain involve the perception of normally innocuous stimuli as painful (Klein et al., 2005), a condition known as allodynia. Unraveling the mysteries of the TGI could therefore provide useful insights into how thermal signals are processed following nerve damage (Craig, 2008).

This dissertation seeks to determine which of the theories of the TGI mentioned above is most probable, in a series of three aims. The theories differ from one another in ways that make one or more of them falsifiable by testing their predictions of how the TGI will fare following various manipulations. Before discussing my experiments at length, I will discuss the framework on which our current knowledge of the TGI is based.

Early research on the TGI

Setting the stage for combining warm and cool. By the late 1880s, several researchers had independently discovered the existence of sensory spots on the skin that are selectively sensitive to particular types of stimulation including pressure, warmth, and cold (Boring, 1942). At the time, Müller's Doctrine of Specific Nerve Energies was the prevailing theoretical paradigm for all of the sensory systems, so the existence of separate end organs and delineated tracts to the brain for the different somatosensory sub-modalities extended this idea to the skin senses. In 1895, several years before the discovery of the TGI, von Frey discovered paradoxical cold, a phenomenon in which cold spots on the skin give rise to a cold sensation, not only when cooled, but also when heated above ~45°C (Boring, 1942). At that time, paradoxical cold was touted as further evidence supporting the specificity of the cutaneous modalities, since stimulation of a cold spot gives rise to a

sensation of coldness no matter how the nerve ending is stimulated. In the years that followed, however, it would serve as the foundation of the first theory of the TGI.

Simultaneous stimulation of warm and cold spots. Alrutz systematically studied the effects of simultaneously stimulating warm and cold spots beginning in 1896 (see Alrutz, 1898), using thermal probes of various sizes (Boring, 1942). Thunberg (1896) designed an apparatus specifically for applying warm and cool temperatures to co-localized areas of skin, in effect stimulating both warm and cold spots simultaneously. This apparatus, which came to be called a *thermal grill*, consisted of two sets of interlaced copper coils (3mm outer diameter), thermally insulated from one another by 5mm, through which warm and cold water could be run.

Thunberg (1896), using himself as a subject, observed that when the apparatus is controlled to warm and cool temperatures and applied to the skin, one initially perceives coolness, then warmth, and finally alternating warmth and cool that can be psychologically distinguished from one another. Depending on the temperatures and the timing, however, he found that this grill apparatus could produce a sensation that was qualitatively different from both cold and warmth. Using extreme but still innocuous temperatures in the grill, which he reported as 44°C and 24°C, he perceived a burning or scorching sensation of heat. Thunberg (1896) still felt that one could individually focus on either of the component sensations, but that the new burning sensation was a blend of cold and warmth. Based on this, he reasoned that the sensation "heat" might, under normal conditions, involve activation of both warm and cold sensibilities, an idea on which Alrutz expounded in the following years.

Alrutz (1898) didn't technically study the TGI, since his experiments never involved the simultaneous application of warm and cool. However, he did supply the first theory of the

TGI and, more generally, speculated on the sensation *hot*, which he considered to be more extreme than and psychologically distinct from warmth, but not necessarily painful. von Frey's specificity theory stated that there were spots on the skin, and thus receptors, for pressure, cold, pain, and warmth; however, no researcher had discovered any "hot" spots, suggesting that the sensation of heat might be due to multiple mechanisms (Boring, 1942). Using punctate stimuli of various sizes and temperatures, Alrutz confirmed von Frey's finding of Paradoxical Cold, that sensations of cold can be elicited by stimulation of a cold sensory spot with both cold (its normal stimulus) *and* heat. He realized that because of the dual nature of this cold response, hot objects, when large enough, cover and stimulate both cold and warm spots on the skin. When Alrutz (1898) stimulated the skin with such objects, he felt an initial sensation of cold followed by a sensation of heat, and then painful heat. He interpreted the initial cold sensation as arising from the paradoxical activation of cold spots, and its initial strength being a result of cool signals' faster transmission to the brain compared to warmth. When he adapted his skin to a warm stimulus before application of a noxious hot stimulus, he first felt cold followed by "… a sharp and pure smarting sensation of pain." (Alrutz, 1898, p. 143) Warmth, and importantly heat, were absent from his perception under these conditions. Based on these findings, he concluded that the perception "hot" is coded by the simultaneous activation of warm and cold spots, and that the perception of burning heat is coded by the simultaneous activation of warm, cold, and pain spots (Alrutz, 1898). Thunberg's (1896) recently published experiments were in complete agreement with the Alrutz theory of heat. In the case of the TGI, the cold channel is activated by the cool bars, rather than paradoxically by extreme heat, and the warm channel is activated by the warm bars. Thus, according to Alrutz (1898), the TGI feels hot because it

mimics the activation that occurs when the skin comes into contact with a noxiously hot object.

An important idea for this early theory of the grill illusion was that the sensation of heat produced during the illusion was a blend of other sensations, specifically "…simultaneous sensations of warmth and cold, localised to the same place" (Alrutz, 1898). *See Figure 1.2*. I will hereafter refer to the Alrutz theory of the TGI as the *cognitive addition theory*, since it involves a blending and reinterpretation of sensations at a high level in the nervous system.

Research on the cognitive addition theory. In the decades that followed, researchers speculated on whether or not heat is a fusion of warmth and cold, and the TGI naturally found a place in these experiments. Many agreed with Alrutz that heat is a fusion of warmth and cold (Alston, 1920; Burnett & Dallenbach, 1927, 1928; Cutolo, 1918; Ferrall & Dallenbach, 1930; Gritman & Dallenbach, 1929; Lowenstein & Dallenbach, 1930).

Cutolo (1918) used two methods to apply simultaneous warmth and cold to the skin, studying them in a few observers trained in introspection. First, he showed, using small punctate stimuli, that heat may be obtained by simultaneously stimulating a warm and a cold spot on the skin. In a second experiment he tested the TGI on the forearm and palm, and again obtained descriptions of "heat" from the subjects. Although these experiments showed that heat can result from the simultaneous stimulation of warm and cold spots, the observers disagreed on whether the heat is painful (Cutolo, 1918).

Alston (1920) used Cutolo's (1918) method of simultaneously stimulating one warm spot with warmth and one cold spot with cool, using small punctate stimuli on the volar forearm. He also found that this type of stimulation produces a sensation of heat without any

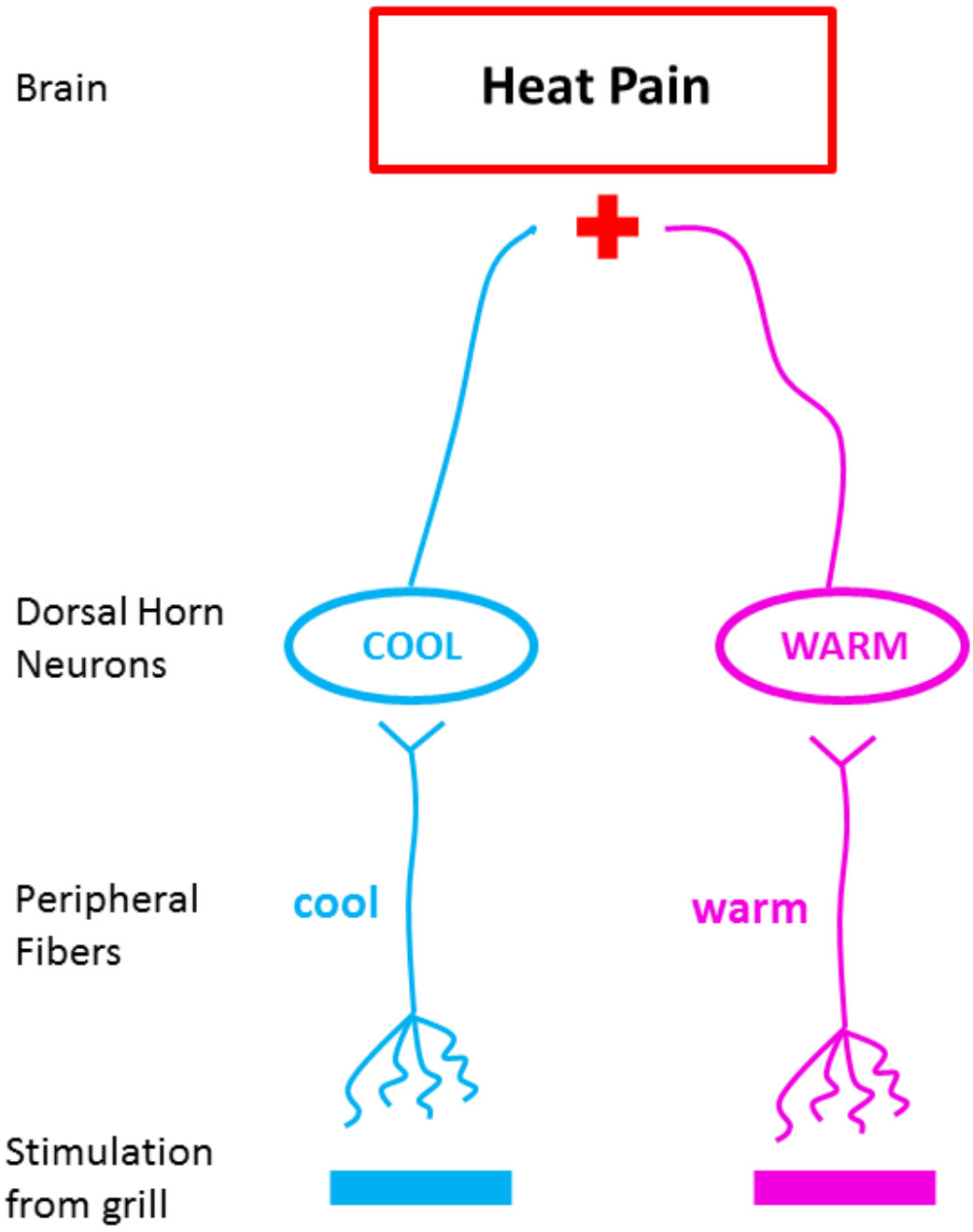

Figure 1.2 Cognitive addition theory. The cool bars (blue) of the grill activate cool fibers and send the signals to the brain. The warm bars (pink) of the grill activate warm fibers and send the signals to the brain. Because of the paradoxical response of cool fibers to noxious heat, the simultaneous sensations of cool and warmth are interpreted as painful heat. The figure depicts the theory using the physiological substrates that are now known to transmit these signals in the periphery and spinal cord dorsal horn, in order to show how the theory fits with the current knowledge of somatosensory transmission.

concomitant sensations of warmth or cold, and thus supported the contention of Alrutz (1898) concerning the nature of hot sensation. By varying the distance between the warm and cold spots being stimulated, he also showed that this fusion of warmth and cold works if the spots are no greater than approximately 10 cm from one another; beyond this distance, the observers more often reported feeling the two component sensations, localized to different areas (Alston, 1920).

Dallenbach and colleagues conducted a series of experiments concerning the nature of heat and the TGI in the 1920s. The illusion was generated on the forearm, using a grill that consisted of two sets of copper tubing, carrying temperature-controlled water, interlaced with one another. First, they systematically varied the cool and warm temperatures, which ranged from 2° to 30°C and 34° to 43°C, respectively, to determine their effect on the TGI (Burnett & Dallenbach, 1927). They found that, in general, the larger the differential between the warm and cool stimuli, the hotter the illusion was perceived to be. Heat appeared without pain at the smaller differentials, but painful heat could be obtained using the largest differentials they employed (Burnett & Dallenbach, 1927).

To quantify how the intensity of the TGI varies with changes in temperature differential, Burnett and Dallenbach (1928) utilized two identical thermal grills, each applied to a different arm of an observer. The authors tested 16 different temperature differentials, pairing them each with the same 16 differentials on the contralateral arm. This yielded a total of 256 trials for each observer, whose task was to say which of the two grills, if either, was hotter on each trial. The results indicated that increasing the temperature of the warm stimulus by 1°C increased the chances of it feeling hotter than the other by the same amount as decreasing the temperature of the cold stimulus by 3°C. In other words, increasing the

temperature of the warm bars by a certain amount had more of an effect on the illusion than decreasing the temperature of the cold bars by the same amount. A separate analysis conducted in three naïve observers came to the same conclusion that the temperature of the warm stimulus is more critical for generating the TGI's heat than the temperature of the cold stimulus, by a factor of three (Gritman & Dallenbach, 1929).

Ferrall and Dallenbach (1930) noted that some of the temperatures used in previous studies of the TGI were near or above the commonly accepted threshold for pain (45°C), and therefore thought that the descriptions of burning, painful heat obtained in them could be a fusion of heat (i.e. warm and cool) and pain sensations. By adding painful electric shocks to the skin while the subject was experiencing a non-painful TGI, the pain of the electric shocks fused with the illusion of heat, causing subjects to experience a burning, hot sensation (Ferrall & Dallenbach, 1930). In a control experiment they showed that, while painful, the electric shock was never described as "burning" when the apparatus was at a neutral temperature. Thus, it appeared that the transition from heat to burning heat was the temperature at which heat pain receptors are stimulated.

As a final test of the Alrutz (1898) theory of heat, Lowenstein & Dallenbach (1930) reasoned that if the sensation "hot" is a fusion of warm and cold sensations, its threshold under normal conditions (i.e. with only one temperature present) should equal the threshold for paradoxical cold, which Von Frey had found to be 45°C. To test this idea, they recruited 100 naïve subjects, who were presented with temperatures ranging from 40 to 51°C. They found that the average thresholds for reporting heat and burning heat were 42.9°C and 47.1°C, respectively. This called into question the idea that heat is a fusion of warmth and cold, since the majority of naïve individuals experienced heat at temperatures below those

that elicit paradoxical cold. However, the authors stuck by Alrutz, suggesting that their use of large stimuli, which covered numerous cold spots, might have summated spatially to elicit paradoxical cold at lower temperatures than shown by von Frey (Lowenstein & Dallenbach, 1930).

The Alrutz theory of heat was well accepted, until a series of studies was published by Jenkins (Boring, 1942). Jenkins (1938a) took issue with the fact that the majority of previous studies had employed trained observers to experience the thermal grill, noting that "If [naïve] subjects report 'hot' when stimulated with an appropriate single temperature, they should also report 'hot' with warm + cold stimulation – provided that the synthetic experience is identical with the natural one." He constructed four different grill apparatuses of parallel rectangular bars, varying from one another in terms of bar spacing and method of temperature control. As a pilot experiment, he asked 157 naïve undergraduate students to feel the thermal grill, the most extreme being a pairing of 44°C and 15°C. "Hot" was reported only 16 times (Jenkins, 1938a). In the main experiment, 126 undergraduates were tested using 32 warm + cool temperature combinations, the most extreme being 50°C and 10°C. Again, hot was not often reported with any of the four grill designs. Instead, the compound stimulation (i.e. adding cool) in many cases seemed to mask the perceived intensity of the warm stimulus, exactly contrary to the results of previous research. Jenkins (1938a) concluded that the "heat" experienced by trained observers during warm and cool stimulation is merely an illusion, and not one that is experienced by untrained subjects.

Jenkins' (1938b) next experiment was designed to replicate, in 108 naïve subjects, those of Ferrall and Dallenbach (1930), in which warm, cool, and painful electric shock were presented. Years earlier, Knight (1922) had shown that an innocuous warm stimulus paired

with a painful prick can yield an experience of burning heat. While this did not rule out the participation of paradoxical cold sensations in the hot sensation, it suggested that cold sensation is not a *necessary* precursor for the experience of heat. Again, Jenkins (1938b) applied various combinations of warm and cool, as well as the temperatures in isolation, this time adding electric shock. The results showed that, like those previously mentioned, that coolness often masked the sensation of warmth rather than adding to it. They did, however, agree with those of Ferrall and Dallenbach (1930) and Knight (1922) in that a noxious stimulus was often capable of generating experiences of burning heat when applied during warm stimulation. Based on finding that the experience of heat can be generated without a response in cold receptors, Jenkins (1938b) disagreed with the notion that cold participates in the coding of heat under normal conditions.

After what Jenkins (1938c) described as an "informal presentation" of his first two experiments, objections over his methods were raised, including the use of rectangular tubing and the lack of instruction of subjects to press hard on the grill, which was said to enhance the TGI's effects. Naturally, he constructed two additional grills using circular copper pipes and, while holding the temperatures of the warm (40°C) and cool (20°C) bars constant, tested the effects of light vs. heavy pressure on the illusion. Neither of the changes caused any appreciable change in the frequency of "hot" responses; roughly 2/3 of the subjects never reported "hot" and, for those who did, the responses were more prevalent for the 40°C stimulus alone (Jenkins, 1938c).

Based on the failures of the fairly exhaustive attempts of Jenkins to obtain synthetic heat using the thermal grill, one is left with the question of why so many previous researchers obtained the phenomenon. The answer that Jenkins (1938a,b,c,d) proposed is that the

phenomenon of synthetic heat is only reported by observers who are trained in introspection. However, in the years leading up to Jenkins' series of experiments, multiple other laboratories created their own thermal grills and claimed that their devices produced the illusion of heat or burning heat in themselves and in their untrained colleagues or students (Barry & Bousfield, 1931; Bishop, 1927; Freeman, 1933; Schlosberg & Carmichael, 1931; Twitmeyer & Fernberger, 1927). Alternatively, the difference might lie in the fact that previous studies undoubtedly used some warm stimuli that were above pain threshold, and thus their reports of heat might have been due to the simultaneous stimulation of warm and pain receptors.

Based on my own experience building and testing thermal grills, the problem might not have been "too hot" in the previous studies, but rather the problem of "not hot enough" in Jenkins'. He reported that, for his main grill design, the temperature of the water flowing into the grill was consistently 2°C warmer than its temperature upon exit, and that the water flowed through holes drilled in the rectangular bars (Jenkins, 1938a). The high degree of heat loss from the water during its transmission through the grill suggests that the bar surface temperature might have been substantially lower than the initial water temperature. Jenkins (1938b) also reported that the problem of warm temperature loss was exacerbated in the conditions where the cool bars were the coolest, suggesting that inadequacies in his design might have negatively influenced the conditions best suited to generate synthetic heat. Coupled with the fact that the bar surface temperature of a warm bar is higher than the temperature at the skin-thermode interface (which I demonstrate in the next chapter), this means that what he reported as 40°C might have been closer to 36°C. In my own experience testing the apparatus, the illusion doesn't work well when a robust cool stimulus is paired

with a weak warm stimulus. Under these conditions, I – like many of Jenkins' (1938a,b,c) observers – experienced coolness that dominated and in some cases detracted from feeling warmth. It should be noted that since modern grills are designed differently across laboratories, apparatus issues could still plague TGI research today. For example, Leung et al. (2005) obtained the TGI but found that it lasted less than 10 seconds, a result that is at odds with the findings of many other labs.

Although the inability of Jenkins to obtain synthetic heat might have been due to flaws in his grill apparatus, his final arguments against the Alrutz (1898) theory of heat were based on experiments using punctate thermal probes that were systematically applied to various spots on the ventral forearms of naïve subjects (Jenkins, 1938d). First, he found that heat could still be experienced from a painfully hot (50°C) stimulus when applied to a small region that had been previously adapted to cold. Next, he mapped out several cold spots and several cold-insensitive spots, and showed that heat from a painfully hot probe could be experienced equally well in both. Finally, he marked off a grid of 25 small areas to be tested with both cold and painfully hot stimulation. In the spots where the hot stimulus generated sensations of heat, he found that a cold stimulus applied to that spot was equally likely to cause reports of neutral as it was sensations of cold. Presumably, half of these "heat" regions were co-localized warm and cold spots, whereas those remaining were warm spots without cold spots (Jenkins, 1938d). Taking the results of these three experiments together provides fairly sound evidence that the experience of "cold" is not a *necessary* precursor for the experience of "hot".

Boring, in his 1942 book *Sensation and Perception in the History of Experimental Psychology,* attempted to mend the rift that was created by Jenkins' attack on the Alrutz

theory and the thermal grill illusion. Boring had, after all, served as one of the trained subjects in the experiments of Cutolo (1918) and Alston (1920), communicated their results, and had thus experienced heat from the thermal grill. According to him, the major problem was a difference in vocabulary; he felt that for objects that are hot enough to activate warm spots and generate paradoxical cold, Alrutz and Dallenbach were willing to call the sensation heat, while for Jenkins the word was reserved for hotter objects that also stimulate pain receptors (Boring, 1942).

Despite Boring's support of the Alrutz theory (1898) and the use of the TGI in research, Jenkins' repeated failures to elicit the illusion in hundreds of naïve subjects gave way to a half-century hiatus in TGI research. However, these dark ages [Nice!] for the TGI saw numerous advancements in neurophysiological techniques and a multitude of discoveries follow from them. New research paradigms would open the door for a theoretical reassessment of the TGI, one rooted in the illusion's underlying neurophysiology.

Thermoreceptive and Nociceptive Neurons

In order to discuss some of the most influential modern literature on the TGI, it is important to give an overview of some of the major neurophysiological discoveries on which current theories of the TGI are based, which I will provide in this section.

The skin houses a variety of thermoreceptor molecules, called transient receptor potential (TRP) channels, which depolarize and transmit action potentials along the fibers in which they are embedded. TRP vanilloid-3 (TRPV3) and TRP melastatin-8 (TRPM8) channels respond to slight (<1°C) increases and decreases in skin temperature (normally ~32°C) and are thought to provide signals for warm and cool sensation, respectively (Schepers & Ringkamp, 2009). Other channels, like TRP vannilloid-1 (TRPV1) and TRPA1

have more extreme thresholds of activation, responding when the skin becomes increasingly hot (ex. >43°C) or cold (ex. <15°C), and are presumed to signal the noxious qualities (ex. burning, stinging, etc.) of painful heat and cold, respectively (Schepers & Ringkamp, 2009). The fibers on which these receptors are expressed form nerve bundles that project to the dorsal horn of the spinal cord, where the primary afferents synapse onto second-order neurons.

The use of single-unit recording in the dorsal horn has enabled researchers to delineate the different neuronal populations that transmit thermal and nociceptive information to the brain, beginning with Christensen and Perl (1970). *Figure 1.3* depicts the thermal responsiveness of the major classes of neurons constituting the spinothalamic tract (STT), the information highway from the dorsal horn to the thalamus. STT neurons (i.e. those whose axons project to the thalamus) receive monosynaptic inputs from Aδ and C nociceptors or thermoreceptors (Craig, Krout, & Andrew, 2001), and the types of primary afferents to which they are connected determine their response properties.

Two types of STT neurons, COOL and WARM, respond to slight deviations from normal skin temperature and are thought to provide the neural substrates for innocuous thermal perceptions. COOL neurons have an ongoing discharge at 34°C, approximately normal skin temperature. Cooling the skin increases the firing rates of these neurons linearly down to 15°C, approximately the threshold for cold pain, while with further cooling their response rate plateaus (Craig, Krout, & Andrew, 2001). COOL neurons might also contribute to the perception of warmth, since warming the skin leads to an inhibition of their spontaneous firing (Dostrovsky & Craig, 1996). However, WARM neurons, whose responses match up well with psychophysical ratings of warmth in humans (Marks &

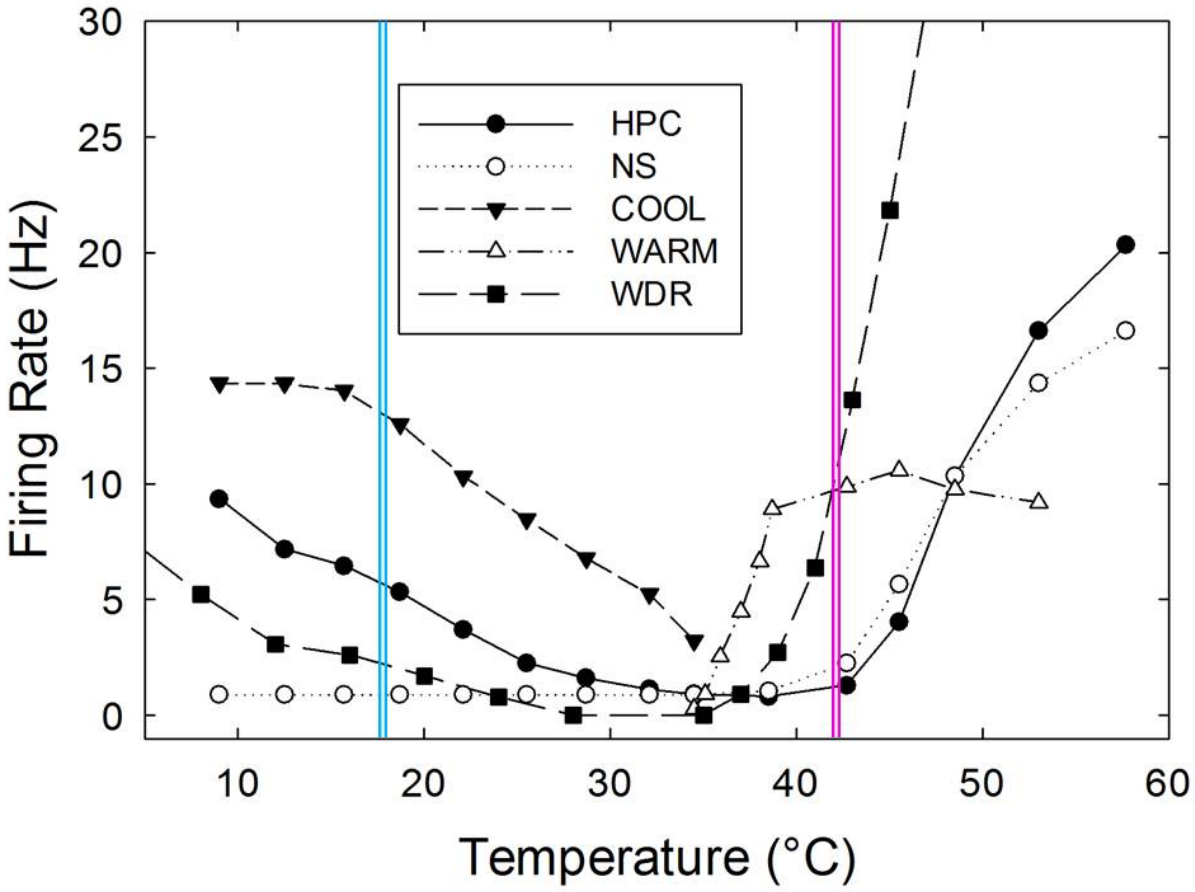

Figure 1.3 Thermal response properties of major classes of dorsal horn spinothalamic tract neurons. HPC = heat / pinch /cold; NS = nociceptive specific; WDR = wide dynamic range. Data on HPC, NS, and COOL neurons were obtained in cats and adapted from Craig, Krout, and Andrew, 2001. Data on WARM neurons were also obtained in cats and were adapted from Andrew and Craig, 2001. Data on WDR neurons were obtained in rats and adapted from Khasabov, Cain, Thong, Mantyh, and Simone, 2001. Data from Khasabov and colleagues (2001) were originally reported as total number of impulses per stimulus. These numbers were divided by the stimulus duration (s) to bring the units in line with those of Craig and colleagues. WDR firing rates continued to increase linearly down to -12°C and up to 51°C (not shown). The blue and pink lines represent the cool and warm temperatures used to produce the TGI in the present experiments.

Stevens, 1968), have also been identified (Andrew & Craig, 2001). Increasing the temperature of the skin leads to a linear increase in their firing rate up to approximately 40°C, at which point their response plateaus.

At more extreme deviations from skin temperature, nociceptive second-order fibers begin to discharge, leading to perceptions of pain for temperatures above and below approximately 43°C and 15°C, respectively. Three distinct populations of nociceptive STT neurons have been discovered and characterized.

Nociceptive specific (NS) neurons, as their name implies, only respond in the extreme ranges of mechanical and heat stimulation (LeBars et al., 1979b). These neurons have a median threshold of 43°C and increase firing roughly linearly up to 53°C (Craig, Krout, & Andrew, 2001). They receive input mainly from Aδ peripheral afferents (those with some myelination) and are thus considered to provide signals for the more rapidly detected first pain (Craig & Andrew, 2002), which is often qualitatively described as "sharp". Some NS cells also respond to mechanical stimulation (ex. pinch), but their thresholds are in the noxious range and are much higher than those of low threshold mechanoreceptors (Craig, Krout, & Andrew, 2001). NS neurons are not activated to any degree by cooling, whether mild or extreme (Craig, Krout, & Andrew, 2001).

In contrast, convergent nociceptive neurons known as wide dynamic range (WDR) neurons and heat, pinch, cold (HPC) neurons respond to both innocuous and noxious stimulation of the skin.

WDR neurons were first characterized by Mendell (1966). He observed a population of neurons that, in contrast to NS cells, responded to stimulation of both low-threshold mechanoreceptive (Aβ) and high threshold Aδ, and C fibers (Mendell, 1966). WDR neurons were studied thoroughly in the decade that followed and became a central figure in theories concerning the neural substrates of pain perception (For an early review, see Price & Dubner, 1977). For example, they have long been known to be recipients of descending inhibition

from the brainstem (LeBars et al., 1979a). Their mean threshold for cooling is 14.6°C, approximately cold pain threshold, but some have thresholds as high as 28°C (Khasabov et al., 2001). WDR neurons also respond to heating of the skin, but only negligibly below 40°C, and have a mean heat threshold of 43.6°C (Khasabov et al., 2001). Their responses correlate well with psychophysical ratings of heat pain intensity throughout the noxious range of heat (Dubner et al., 1989; Maixner et al.,1986), and they are still considered highly important, if not indispensable, for discriminative pain perception (Price, Greenspan, & Dubner, 2003). In the case of cooling, heating and mechanical stimulation, firing rates increase linearly with stimulus intensity. The majority of WDR neurons are located deep (in lamina V) of the dorsal horn, although some are positioned in the superficial layer (LeBars et al., 1979a) alongside COOL, WARM, NS, and HPC neurons.

Like WDR neurons, HPC neurons are also polymodal, but their sole input is from C fibers (Craig, Krout, & Andrew, 2001). Because of their specificity for unmyelinated fiber input, they have been purported to be responsible for the experience of second pain (Craig & Andrew, 2002; Craig, 2004), which can be qualitatively described as dull, achy, or, in the case of thermal perception, burning. HPC neurons respond in a linear, graded fashion to noxious heat above 43°C, but, in contrast to WDR neurons, they do not respond to innocuous mechanical stimulation (Craig, Krout, & Andrew, 2001). They do, however, respond in a graded fashion to noxious pinch. Their threshold of activation for cooling appears to be less extreme (median = 24°C) than that of WDR neurons, but they too respond with linearly increasing activity across progressively colder temperatures, including those well beyond the threshold for cold pain (Craig, Krout, & Andrew, 2001).

All of these neuronal populations have been hypothesized to play a role in the creation of the illusory pain of the TGI with the exception of NS cells, which are not capable of responding to either of the grill's component temperatures (Craig, Krout, & Andrew, 2001) and are thus unlikely candidates for explaining the TGI. The following section describes the field of modern research on the TGI, with a focus on studies that have made substantial theoretical contributions.

Modern Research on the TGI

New techniques give rise to a new theory. Interest in the TGI was rekindled by Craig and Bushnell (1994), who used a combination of neurophysiological and psychophysical techniques to propose what I will call the *spinal disinhibition theory*, which rests on the basic premise that cool signals inhibit pain. This inhibitory ability of cool was illustrated years earlier by researchers who used ischemia (Fruhstorfer et al., 1984) and nerve compression (Wahren et al., 1989) to selectively block myelinated (i.e. Aβ and Aδ) afferents, leaving the responses of small, unmyelinated C fiber afferents intact. As the A fibers ceased to conduct signals, cool thresholds significantly decreased (i.e. to lower, more extreme temperatures), because the sensation of cool is mediated by Aδ fibers. Along with this alteration, cold pain threshold was reached at significantly higher (i.e. less extreme) temperatures and the painful sensation acquired a burning quality so that it became more unpleasant than before the nerve block. The results suggested that a population of Aδ fibers, specifically those that transmit cool sensations, have an inhibitory effect on cold pain under normal conditions.

In line with the hypothesis that cool is inhibitory on pain, Craig and Bushnell (1994) reasoned that illusory pain of the TGI could be explained if the cool signals were somehow

inhibited by the grill, an effect that would unmask the underlying pain signals. To determine whether this is the case, the authors recorded from COOL and HPC neurons in the cat dorsal horn in response to warm (40°C), cool (20°C), and combined (40+20°C) stimuli. The 20°C stimulus elicited robust responses in both HPC and COOL neurons when presented in isolation to the neurons' receptive fields. The grill's warm temperature (40°C) was incapable of causing activity in either of these cell types when presented in isolation. However, when the warm and the cool temperatures were applied simultaneously, warm had an interesting effect on neuronal firing rates. In this case, the firing of COOL neurons was reduced by ~50% but the firing of HPCs was reduced only slightly (~18%), compared with the activity elicited by the cool stimulus in isolation.

According to Craig & Bushnell (1994), burning pain is represented by activation of a population of comparator neurons in the brain (e.g. the thalamus; see Craig et al., 1996) that receives excitatory input from HPC neurons and inhibitory input from COOL neurons. Thus, the larger the HPC - COOL activity difference, the stronger the perception of burning pain will be.

This hypothesis can explain several phenomena, including the TGI. First it provides an explanation for why cool temperatures are not normally perceived as painful, despite their ability to activate HPC neurons – the COOL activity keeps the HPC - COOL difference minimized, since both types of neurons gradually increase their firing throughout the range of innocuous cool temperatures (as illustrated back in *Figure 1.3*). However, at temperatures below 15°C, the response of COOL neurons plateaus, that of HPCs continues to increase, and the HPC - COOL difference increases. Thus, excitation on the comparator neurons predominates and pain is perceived. HPC neurons also respond to hot temperatures, which

could contribute to the burning sensation that normally accompanies noxious heat (Craig, 2004). In this case there is no COOL activity to inhibit the pain, so the HPC activity is readily detected. Finally, the theory explains why reducing the firing in COOL neurons, via the warm bars of the thermal grill or by a selective A-fiber block, leads to a disinhibition of pain. According to this theory, the difference between HPC and COOL activity is artificially enhanced, by inhibiting COOL signals with the warm bars of the grill, to levels that would normally occur only with noxious cold or heat. *See Figure 1.4*.

Psychophysical results in humans showed that the sensation of cool is masked during the illusory condition, which can be readily explained by the inhibition of COOL neurons by warm (Craig & Bushnell, 1994). The grill was also perceived to be painful, whereas the component temperatures were not when presented in isolation. These psychophysical observations, combined with the neurophysiological results, provide strong evidence that the pain of the TGI is unmasked by the inhibition of COOL and the resulting higher HPC - COOL difference.

As a final test of their theory, Craig and Bushnell (1994) used the neurophysiological results to calculate the noxious cold temperature that elicits the same HPC/COOL ratio that is produced by the (20+40°C) grill, and found this value to be 10°C. They then had a subset of subjects rated the painfulness of noxious cold (in isolation) during one run and the painfulness of the (20+40°C) grill during another. The pain of the TGI was not statistically different from that produced by 10°C. This result lends further evidence to the idea that the HPC - COOL difference is the normal code for cold pain and the explanation of the TGI.

Craig and colleagues (1996) reasoned that if the pain from the TGI results from the stimulation of nociceptors and an unmasking of HPC activity, the brain should process the

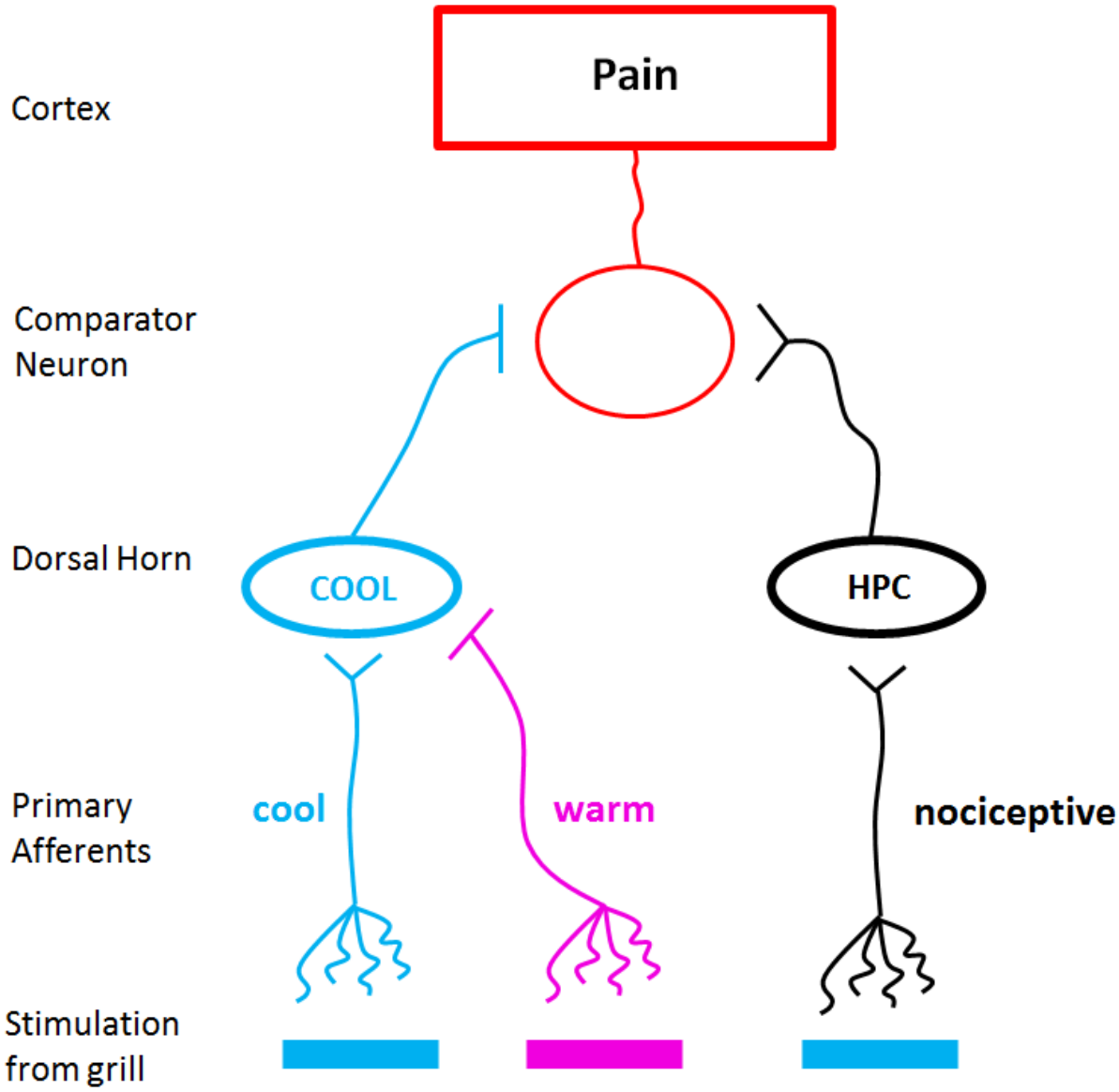

Figure 1.4 Disinhibition theory. Straight lines (–) indicate inhibitory synapses while V-shaped endings indicate excitatory synapses. See the text for detailed explanations of these hypothesized circuits.

TGI and noxious temperatures similarly. Using positron emission technology (PET), they tested the brain activity elicited by the thermal grill (20+40°C), innocuous cool and warm (20°C and 40°C, respectively), and noxious cold and heat (5°C and 47°C, respectively). The main finding was that the thermal grill caused significant activation in the anterior cingulate cortex (ACC), similar to that evoked by noxious cold and heat, while the warm and cool

temperatures alone did not (Craig et al., 1996). The authors took this of confirmation of their theory that the pain of the TGI is conveyed by HPC neurons, whose activity is unmasked by the grill's warm stimulus. However, a more recent neuroimaging experiment using functional magnetic resonance imaging (fMRI) was unable to replicate this result – instead of significant ACC activation compared to the constituent temperatures of their grill, this study found significantly more thalamic activity for the grill vs. individual temperature comparisons (Lindstedt et al., 2011). The authors noted that while this result is not at odds with the disinhibition theory of the TGI, activation of the ACC is not a defining characteristic of the illusion. In any case, these two studies indicate that something about the TGI activates regions of the brain that are not normally activated by innocuous temperatures.

The TGI as an illusion of pain. Craig and Bushnell's (1994) study renewed interest in the TGI and also shifted the paradigm under which it is studied. Whereas researchers in the early 1900s concerned themselves with the TGI as a way of determining the code for heat sensation (Alrutz, 1898), modern researchers have focused mainly on the painfulness, unpleasantness, and dysesthetic qualities of the illusion, with few exceptions (Averbeck et al., 2012; Bach et al., 2011; Fruhstorfer et al., 2003; Green, 2002). Along these lines, most studies have sought to determine the factors that are important for generating a painful TGI and those affecting its magnitude.

Stimulus factors affecting the illusion. Since there is no commercially available grill apparatus, research on the TGI has been subject to a high degree of variability in the apparatus used to produce the illusion. However, with the exception of stimulus temperature (Bouhassira et al., 2005; Leung et al., 2005), other systematic variations of the stimulus characteristics have showed that the TGI is unaffected by a variety of stimulus factors. Li et

al. (2009) varied the number of bars and the distance between bars of the grill, testing 11 different variations on the thermal grill apparatus in two locations. Their results revealed no difference in the incidence of the TGI across grills composed of 2, 3, or 4 bars, bars spaced from 2-10mm; and there was no difference in the illusion on the fingers vs. the palm (Li et al., 2009). Although Defrin and colleagues (2008) produced the TGI using two small thermal probes, they found that the intensity of the burning illusion generated by simultaneous warm and cool is reliable and consistent across distances of up to 30cm between the probes. It therefore seems that the most important factor for eliciting the TGI is the pairing of warm and cool stimuli. Slight variations in grill designs are unlikely to produce differential results across studies.

Physiological and psychological factors affecting the illusion. With this new focus on the TGI as an illusion of pain, it followed that analgesic drugs might affect the illusion. Kern and colleagues (2008a,b) therefore tested the effects of morphine and ketamine on the TGI, in separate studies. First, they showed that systemic ketamine has a profound effect on the illusion, completely eliminating its pain and unpleasantness in 10 of 12 subjects. In contrast, ketamine was not effective in reducing the pain of noxious heat or cold, nor was it in reducing the perceived thermal intensity of warm or cool (Kern et al., 2008a). Since ketamine is a potent N-methyl-D-aspartate (NMDA) receptor antagonist, this result suggests that the painfulness of the thermal grill is produced by NMDA-mediated pathways that are not necessarily involved in the perception of innocuous thermal sensation or acute thermal pain. In a second study, Kern and colleagues (2008b) showed that systemic morphine also reduces the painfulness of the TGI, though in this case the reduction was not selective – morphine also reduced noxious cold pain intensity and both noxious heat and cold pain

unpleasantness. However, it was without effect on ratings of innocuous warm and cool, which suggests that the TGI involves signals that are treated as pain by analgesics acting on pain-inhibitory circuitry.

While these pharmacological studies show that the signals of the TGI are pain-like since they respond to analgesics, the systemic administration of the drugs did not enable an assessment of whether the TGI's introduction into the nociceptive system occurs at a high or a low level in the nervous system, if at all. This issue is still unresolved and is therefore the focus of the first aim of this dissertation, but there is some evidence that the TGI is affected by both low-level and high-level processes. Averbeck et al. (2012) applied either menthol (a Trp-M8 agonist) or cinnamaldehyde (a Trp-A1 agonist) to the skin of human subjects before application of the grill. The results showed that both chemicals had the effect of enhancing the illusion of heat generated by the interlaced warm and cool stimulation. These results suggest that TRPM8 and TRPA1 might be involved in the peripheral generation of the illusion (Averbeck et al., 2012). A better test of the receptors that transduce the illusion would be to apply agonists of cool, warm, and nociceptive fibers in different combinations, importantly *without* changing the temperature of the skin.

In terms of how the signals are modulated at a higher level, Boettger et al. (2011) showed that the TGI is exacerbated by induction of a sad mood in healthy subjects. Furthermore, the perceived magnitude of the TGI has been shown to have a genetic component related to mood states, specifically whether one has the short or the long form of the serotonin transporter (5-HTT) gene (Lindstedt et al., 2011). There is some evidence that the TGI can be modified by higher-level interactions with tactile signals. Kammers et al. (2010) showed that the illusion can be reduced if a participant touches his or her TGI-

exposed fingers together, while Seckel and colleagues (2012) found that the illusion can spread to a thermally neutral finger that is touching a piece of cardboard placed over the grill.

The TGI in clinical populations. Disturbed processing of acute pain is often observed in a variety of clinical pain populations, and quantitative sensory testing (e.g. heat, cold, and pressure pain thresholds) can provide insight into the way(s) in which the pain system is altered in these patients compared to healthy individuals. The TGI is a striking example of how the central nervous system can create pain signals from inputs that are normally innocuous. In this respect the TGI is similar to allodynia, an alteration in somatosensory processing that is a hallmark of some chronic pain conditions, in that both involve the perception of pain following mild stimulation. The TGI's relation to a symptom of chronic pain could help us better understand how pain is created under normal and abnormal conditions. For example, classic thalamic pain syndrome is a central neuropathic pain condition in which damage to part of the thalamus can lead to an absence of innocuous thermal sensation (i.e. cool and warm) paired with the perception of ongoing burning pain and allodynia to cold in that same area (Craig, 2008). This is exactly what is predicted by the spinal disinhibition theory, wherein an absence of COOL input to higher brain centers would lead to a disinhibition of HPC cell activity in response to mildly cool stimuli.

The TGI has only occasionally been tested in chronic pain patients despite its similarities with a common symptom of chronic neuropathic pain. Craig's (2008) hypothesis that central neuropathic pain is caused by disinhibition, much like the TGI, has received partial support from TGI research in chronic pain patients. Heavner et al. (1997) focused on a woman with neuropathic pain, specifically reflex sympathetic dystrophy in her right hand. The woman's experience of warm, cool, and the TGI were found to be unaltered in her

unaffected hand. In her affected hand, the patient had no change in warmth sensitivity, but had allodynia to cool and a significantly lower tolerance for the grill, removing her hand after 4 sec due to intense burning pain. This high sensitivity to the TGI along with allodynia to cool could be explained by a reduction in COOL activity due to the neuropathy and a larger disinhibition of HPC-mediated pain. However, this case is contrasted by the experience of the TGI in a woman with multiple sclerosis (MS), reported by Morin et al. (2002). The patient had hypoalgesia to heat and allodynia to cool in her limb with chronic pain, but remarkably she reported the TGI to be only slightly painful, less so than the grill's constituent cool temperature (20°C) presented alone. The disinhibition theory has a difficult time explaining this result, though the authors suggested a ceiling effect, in which the woman had so much disinhibition due to her condition that the TGI couldn't add more (Morin et al., 2002). Recently, the TGI was tested in a heterogeneous chronic pain population, whose sensitivity to the synthetic heat of the TGI was found to be less than that of control subjects (Sumracki et al., 2014). Clearly, more research on the TGI in chronic pain patients is needed.

Might the TGI involve addition after all? Only a single pair of temperatures (20+40°C) was implemented in Craig and colleagues' (1994, 1996) experiments, and it was not known whether the illusion also occurs at less extreme temperatures. Green (2002) put this question to the test psychophysically, using an apparatus that consisted of 16 small ($0.64cm^2$) square Peltier thermodes arranged in a grid. To obtain baseline ratings of warm and cool, two of the four rows (ex. 1 and 3) were warmed (35-40°C) or cooled (31-26°C) from a baseline of 33°C while the other rows remained at the baseline temperature. The grill conditions consisted of concurrently warming two rows and cooling the other two, in various

temperature combinations. With warming alone, the sensation "hot" was only reported in 15.6% of trials, and in the majority of cases (75%) "warm" was reported. Cooling the skin by just 2°C (to 31°C) along with simultaneous presentation of the warm stimulus significantly increased the frequency of "hot" reports (40.6%), and an additional 2°C drop increased the frequency further (64.0%).

A separate group of subjects rated the intensity of the sensation produced by these mild temperature differentials, to gauge the magnitude of change in sensation during stimulus combination. Across the range of temperature combinations tested, simultaneous warm and cool were generally perceived to be roughly twice as intense as either stimulus on its own. For a warm (ex. 36°C) and cool (ex. 30°C) stimulus that were rated as equally intense when each was presented in isolation, the perceived intensity doubled when the two were presented concurrently. This suggested to Green (2002) that the perceptual result of pairing warm simultaneously with cool might be additive.

Based on these results, Green (2002) proposed what I will call the *spinal addition theory* of the TGI, which states that warm and cool signals elicited by the grill converge onto WDR neurons in the dorsal horn, adding their excitatory effects. As explained in an earlier section, WDR neurons are involved in integrating information from multiple modalities, and they respond in a graded fashion spanning the transition from innocuous to noxious heat and cold (Khasabov et al., 2001). Thus, they appear to be capable of adding inputs (presumably from warm and cool afferents), which would, according to the spinal addition theory, heighten the firing rate to a level that is normally reserved for noxious stimuli. See *Figure 1.5* for a depiction of this theory.

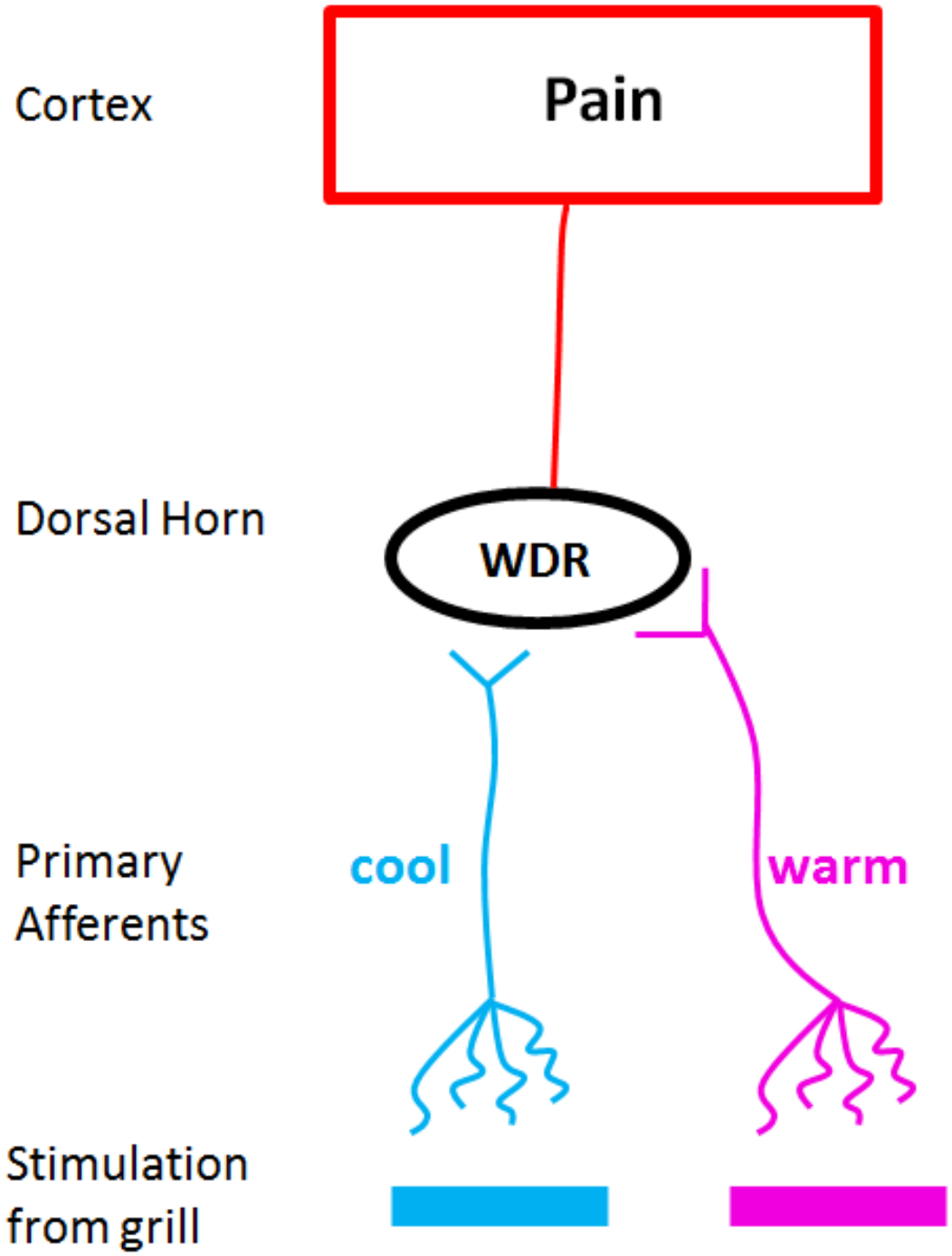

Figure 1.5 Spinal addition theory. Straight lines (–) indicate inhibitory synapses while V-shaped endings indicate excitatory synapses. See the text for detailed explanations of these hypothesized circuits.

The spinal disinhibition theory has also been called into question by Bouhassira and colleagues (2005). The authors tested the occurrence of the TGI over a range of mild to moderate warm and cool temperatures, varying both the difference of each temperature from each individual's thermal pain thresholds and the difference between the warm and the cool temperatures themselves. The main finding was that the perceived intensity of pain from the TGI increased significantly as the difference between warm and cool bar temperatures increased. Pain was reported reliably (~75% of trials) at high temperature differences (21-25°C) and infrequently (~20%) at low (5-10°C) differences, though the latter often produced synthetic heat without pain.

To determine whether the results are compatible with the spinal disinhibition theory, the results were analyzed in terms of whether the illusion increased when one of the two temperatures was made more extreme while holding the other constant (Bouhassira et al., 2005). The disinhibition theory predicts that increasing the warm temperature while holding the cool temperature constant will increase the illusion because the greater inhibition exerted on COOL cells should increase the HPC - COOL difference. In contrast, the disinhibition theory predicts that decreasing the temperature of the cool bars will not affect the intensity of the TGI, because doing so should increase signals in both HPC and COOL neurons roughly equally. The authors found an increase in the TGI when the warm bar temperature was increased, as predicted by disinhibition theory, but they also found a significant increase when the temperature of the cool bars was decreased, a result incompatible with the theory (Bouhassira et al., 2005). Instead, Green's (2002) spinal addition theory fits nicely with these results, since a larger signal in either the warm or the cool channel by making either temperature more extreme would be expected to increase the firing of WDR neurons.

Although this introduction has long since shifted to a discussion of modern findings, it should be noted that the original theory of the TGI has not yet been falsified. In fact, it is compatible with modern psychophysical and neurophysiological research. For example, the cognitive addition theory (Alrutz, 1898) is compatible with Green's (2002) finding that the perceived intensity of the TGI was roughly equal to the sum of the individual sensations. Also, the cognitive addition theory would predict similar increases in the TGI percept following an increase in the departure of either grill component from skin temperature, a result obtained by Bouhassira et al. (2005). Moreover, as it turns out, Alrutz's theory is compatible with the current understanding of somatosensory neurophysiology. Peripheral recordings have confirmed that a subset of cold fibers responds to noxious heat (Kenshalo & Duclaux, 1977; Long, 1977) and COOL STT neurons do fire paradoxically to noxious heat (Dostrovsky & Craig, 1996). *Figure 1.6* illustrates that extreme hot temperatures (ex. 46°C) evoke activity in both WARM and COOL neurons, a situation that contrasts the selective activation of WARM neurons by warmth (ex. 41°C).

The spinal disinhibition theory is certainly an attractive explanation of the TGI. However, the theory's selective focus on pain pays little deference to the history of research on the illusion. In its current formulation, it makes no attempt to explain the synthetic heat that the grill produces. Craig and Bushnell (1994) viewed the illusion in terms of "Unmasking the burn of cold pain," but even their psychophysical results showed that their grill felt hotter than the warm temperature alone. Numerous studies have found that the grill produces synthetic heat (Alrutz, 1898; Bouhassira et al., 2005; Craig & Bushnell, 1994; Craig et al., 1996; Kammers et al., 2010; Leung et al., 2005) in situations where the TGI is also painful. Moreover, the simultaneous application of very mild warm and cool stimuli can

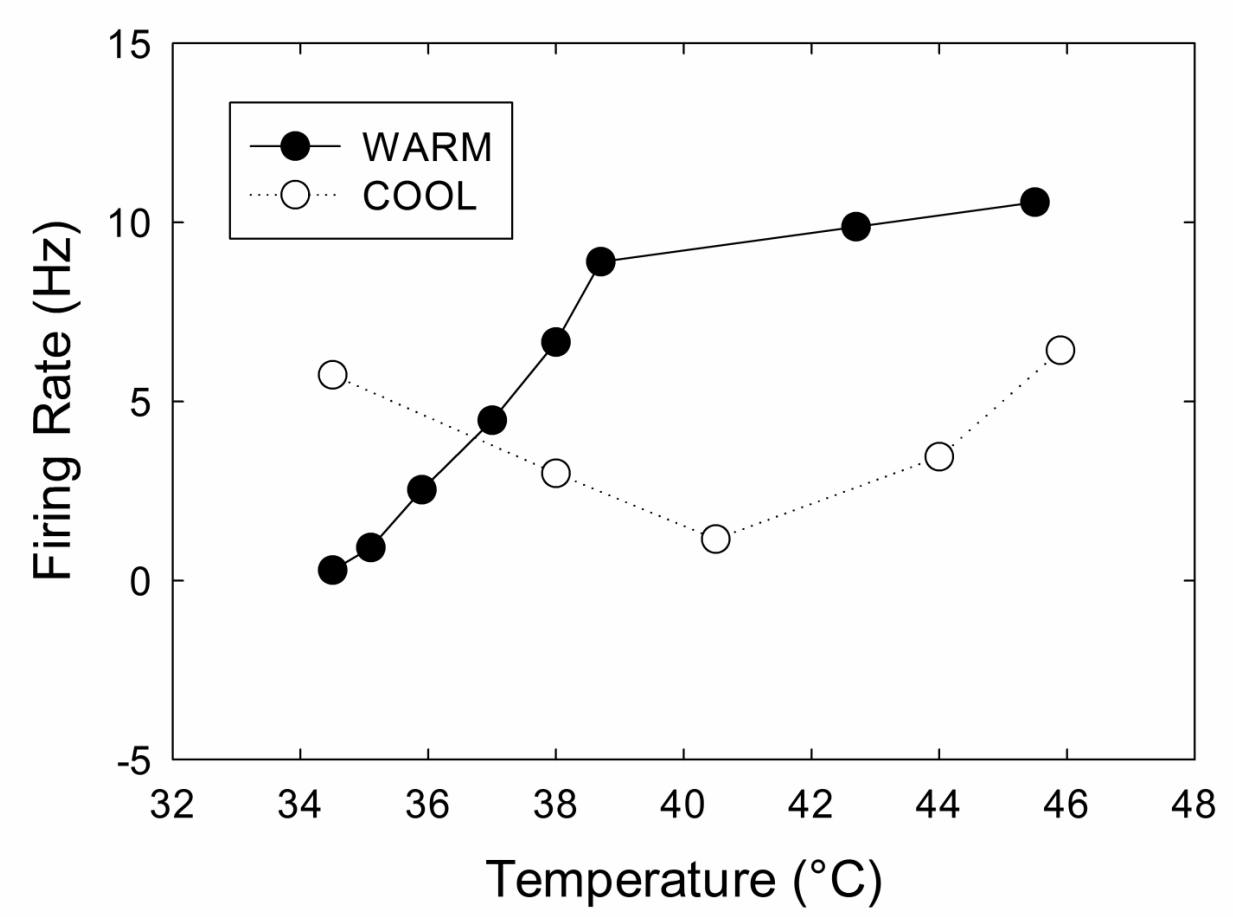

Figure 1.6 Response properties of WARM and COOL neurons to skin heating. This includes the inhibition of COOL firing in response to warming and their paradoxical response to noxious heat. The WARM data are adapted from Andrew & Craig (2001), and are identical to those shown in Figure 2. The COOL data were obtained from monkeys in an experiment by Dostrovsky & Craig (1996). At warm temperatures, COOL neurons are inhibited, but at noxious high temperatures they exhibit a small paradoxical increase in their firing rate. This paradoxical increase means that genuinely hot temperatures involve activity in both WARM and COOL neurons.

produce synthetic heat without the perception of pain (Fruhstorfer et al., 2003; Green, 2002), a phenomenon that the spinal disinhibition theory makes no attempt to explain.

Goals of this Dissertation

While both the early and modern literature have contributed to our knowledge of the factors that are important for the TGI and the means through which it can be manipulated, questions about the illusion remain. Is the TGI genuinely painful, and if so, how and where

does it integrate into the nociceptive system? Are the warm and cool signals equal contributors to the illusion, or does one of the grill's stimuli take precedence in the effect? Are the synthetic heat and the pain of the TGI produced by the same mechanism, or do they rely on separate processes as suggested by Green (2002)? If synthetic heat is not an additive process, what is the underlying mechanism? What are the required temporal parameters for generating the illusion? The subsequent chapters of this dissertation seek to provide answers to these questions.

The primary goal of this dissertation is to enhance our understanding of the way(s) in which the TGI is coded by the nervous system. While the disinhibition theory is an attractive candidate for explaining the TGI and some aspects of neuropathic pain, it and the other theories of the TGI have never been systematically pitted against one another. The three aims of my dissertation are designed to increase our understanding of the TGI by putting its theories at odds with one another. This dissertation will focus on two main differences between the theories, namely: 1) whether the TGI is generated by nociceptive or innocuous ascending signals and 2) whether the TGI's signals interact in an additive manner or through another, more complex, mechanism. The first aim is devoted to testing the former while the second and third aims shed light on the latter.

Specifically, Experiment 1 is designed to determine whether the TGI is the product of nociceptive signals that are present at the level of the spinal cord, or if it is instead a product of interactions at higher levels in the nervous system. Conditioned pain modulation (CPM), in which a strong noxious conditioning stimulus inhibits the perceived pain of a test stimulus applied elsewhere on the body, is known to be mediated by descending inhibition from the brainstem (LeBars et al., 1979a). Descending inhibition attenuates the transmission of

noxious but not innocuous thermal signals at the level of the spinal cord (Heinricher et al, 2009; Leith et al., 2010; Waters & Lumb, 1997). This experiment tests whether CPM similarly reduces the pain of the TGI and of noxious heat. Equal reductions in pain in the two conditions would that the grill's integration into the nociceptive system occurs in the periphery or in the spinal cord, since ascending innocuous warm and cool signals are not targeted by CPM. In contrast, if CPM did not attenuate the TGI, this would suggest that it is carried in innocuous ascending channels. While the experiment helps localize the important interactions that produce the TGI to the spinal cord, it is not capable of distinguishing between theories of the illusion that posit a similar level of nociceptive integration (e.g. the spinal cord).

Experiment 2 therefore tests (based on the results of Experiment 1) which spinal theory of the TGI is more plausible based on how the illusion fares following adaptation to one of the grill's component temperatures. Subjects were adapted to either the warm or the cool bars of the grill before the grill was supplied with the TGI's missing temperature (i.e. cool or warm, respectively). I hypothesized that if adapting to warm reduces the TGI, and adapting to cool does the same, this would imply that the TGI results from addition of warm and cool signals in the spinal cord; any other result would suggest that the TGI involves interactions between the signals (to be discussed later) that are more complicated than a simple addition (e.g. disinhibition). The result of this experiment was not completely compatible with any of the theories being tested, which caused me to consider other possible explanations.

One difference between the conditions employed in Experiment 2, in addition to selective adaptation, was temporal offset (e.g. warm preceding cool) between the stimuli.

Some have claimed that the TGI is affected by this variable (Craig & Bushnell, 1994; Thunberg, 1896) the observed effects could have been due to the fact that the warm and cool were offset, rather than adaptation. Thus, in Experiment 3 I tested whether temporal offset between the warm and cool stimuli, at a duration that minimized adaptation, can affect the illusion. A lack of an effect on the TGI would suggest that 1) the TGI is robust no matter the temporal order of stimulation and 2) the results of the previous experiment were due to adaptation.

CHAPTER 2: THE TGI IS DISRUPTED BY DESCENDING INHIBITION

Abstract

Several theories have been proposed to explain the TGI. Two suggest that the nociceptive system is involved early, in the spinal cord dorsal horn, while another states that the illusion results from a high-level fusion of innocuous signals. To determine whether the TGI is generated by nociceptive dorsal horn neurons, we studied the effect of conditioned pain modulation (CPM: the inhibition of one pain by another) on the illusion. To trigger CPM, subjects placed the left hand in a painfully cold water bath (conditioning stimulus) before placing the right forearm onto a thermal grill (test stimulus). Lower test stimulus pain ratings in this CPM run (6°C bath) compared to those in a Baseline run (33°C bath) were taken as evidence of CPM. To determine whether CPM reduces nociceptive heat pain and illusory heat pain equally, a control group of subjects rated pain and unpleasantness of the grill while all bars were controlled to a noxious temperature (45°C), while a Thermal Grill group rated an equally painful grill consisting of alternating innocuous warm (42°C) and cool (18°C) bars. CPM produced significant, and comparable, reductions in pain, unpleasantness, and perceived heat of both grill stimuli. This result implies that the painfulness and the synthetic heat of the TGI involve signals that are treated as pain at the level of the spinal cord.

Introduction

An early theory of the TGI by Alrutz (1898) was based on the then-recent discovery that noxious heat not only activates warm spots on the skin, but paradoxically activates cold spots as well. The thermal grill, which activates warm spots with warmth and cold spots with cool, was seen by Alrutz as mimicking the dual activity produced by noxious heat. Thus, the TGI, and more generally the perception of heat, was thought "to be the result of different simultaneous sensations" (Alrutz, 1898), or in other words a *cognitive addition* of warm and cool signals.

Alternatively, warm and cool signals might integrate into the nociceptive system, and do so at a low level, as is suggested by other theories.

Craig and Bushnell's (1994) spinal disinhibition theory posits that cool bars of the thermal grill activate convergent heat/pinch/cold (HPC) neurons that code for burning pain, as well as COOL neurons that contribute to innocuous thermosensation; warm bars do not affect HPCs but inhibit COOL neurons. The resulting high difference between HPC and COOL cell activity resembles that which occurs when a thermal stimulus enters the noxious cold range (Craig & Bushnell, 1994).

The spinal addition theory, in contrast, states that warm and cool signals from the grill converge and add their excitatory effects onto wide dynamic range (WDR) neurons in the dorsal horn (Bouhassira et al., 2005; Green, 2002), causing high firing rates that normally occur only in the presence of a genuinely noxious thermal stimulus. Importantly, both the disinhibition and addition theories suggest that the TGI's signal for pain (i.e. in HPC or WDR activation) is transmitted in nociceptive neurons in the spinal cord.

As a first step toward deciding among these theories, I used another perceptual phenomenon, conditioned pain modulation (CPM), as an analytical tool. CPM involves measuring reductions in the pain of a noxious test stimulus (ex. TGI induced on right forearm) during application of a noxious conditioning stimulus, which is applied to a remote body location (ex. cold pressor applied to contralateral hand). CPM is thought to be a product of activating diffuse noxious inhibitory controls (DNIC; a spinal-bulbar-spinal loop), which selectively inhibits WDR neurons (LeBars et al., 1979a) but neither NS (LeBars et al., 1979b) nor COOL (Dickenson et al., 1980) neurons in the dorsal horn. CPM may also recruit additional descending inhibitory projections from other regions, including the anterior cingulate cortex and the periaqueductal grey (Sprenger et al., 2011); however, it is generally accepted that the inhibitory effects of CPM descend to the spinal cord to dampen incoming pain signals. Descending inhibition from the brainstem has been shown to affect spinal cord responses to noxious stimuli while leaving the responses to innocuous stimuli relatively unaltered (Heinricher et al., 2009; Leith et al., 2010; Waters & Lumb, 1997).

The TGI is produced by combining stimuli that are individually described as innocuous. The question of where in the nervous system the signals triggered by these innocuous stimuli interact to produce a pattern of activity that achieves noxious status can be answered with CPM, because CPM (1) acts at the level of the spinal cord, and (2) reduces nociceptive but not innocuous signals. If CPM is found to reduce nociceptive heat pain and TGI pain to the same extent, this would suggest that the thermal grill produces nociceptive signals that are present at the level of the spinal cord.

Methods

Participants. Subjects were recruited from a posting on the UNC-CH Psychology Department's participant pool website. Written informed consent was obtained prior to the start of the experimental session. Upon completion of the experiments, participants were compensated with credit towards the Introductory Psychology research participation requirement. All procedures were approved by the University's Institutional Review Board.

Thirty-seven healthy undergraduate students participated in the main experiment, which tested the effects of CPM on noxious heat and the TGI. Subjects were randomly assigned to one of two groups. Nineteen subjects (7 male) were in the Noxious Heat (NH) group and 18 subjects (7 male) were in the Thermal Grill (TG) group. The mean age was 19.1 years in both groups.

Twenty-four additional subjects (7 male) were enrolled in a separate experiment, in which the warm and cool temperatures used to produce the TGI in the main experiment were applied individually (and without CPM). Their mean age was 18.6 years. None had participated in the main experiment.

Study design. Subjects assigned to the TG group were exposed to a grill consisting of interlacing warm (42°C) and cool (18°C) bars to produce the TGI. Subjects in the NH group were exposed to bars that were all heated to a noxious temperature (45°C), to elicit nociceptive heat pain. Participants in both groups were exposed to their respective grill stimulus on the right volar forearm twice, in separate runs. One exposure (Control run) took place while the left hand was immersed in a neutral (33°C) water bath and the other (CPM run) while it was positioned in a painfully cold (6°C) bath. The order of the two runs was counterbalanced within each group.

Thus, the between-subjects factor was the type of pain that subjects experienced (NH vs. TG), and the within-subjects factor was whether the left hand was in painfully cold or neutral water (CPM vs. Control) while the grill was presented. This design permitted analysis of three effects: 1) Pain experience of noxious heat vs. the thermal grill; 2) Amount of pain reduction by CPM; and 3) Assessment of CPM's relative ability to reduce the two types of pain.

The experiment using separate component temperatures was implemented to measure the sensations associated with separate presentations of the warm and cool temperatures that were used to induce TGI. Subjects participated in two runs, one with all of the bars at the warm temperature and a second with all of the bars at the cool temperature. The order of these two runs was counterbalanced. No water bath was used in this experiment.

Apparatus.

Thermal grill. The thermal grill apparatus consisted of 12 copper tubes (length 33cm; diameter 1cm; thickness 0.4mm) that were secured with twine onto the top of a plastic holder. For reference, refer back to *Figure 1.1*. Each bar rested in a trough (1.25 cm wide and 0.5 cm deep), and thus was separated from its neighbor(s) by 0.5 cm. In order to gain thermal control over the bars, two sets of plastic tubing through which water could be circulated were connected to the ends of the bars. Set 1 was comprised of the odd-numbered bars (1, 3, 5, etc.) and Set 2 the even-numbered bars. The two sets of bars were also delineated by separate intake tubes that were connected to bars 1 and 2. Each intake was connected to a thermally insulated 19-L tank that was positioned on a shelf 0.65 m above the tabletop. Before an experimental run, the experimenter filled each tank with 15 L of water, controlled to the desired temperature. One minute before each run began, the experimenter opened valves on

the tanks to allow gravity to carry the water through the grill apparatus and control it to the temperature (or temperatures) desired for that run. Each set of tubes had an outtake that passed water into a receptacle on the floor, which was emptied after each run.

Two thermistor probes (YSI 400 series) were attached to small sections of copper tubing that were inserted into the flow path between the tanks and the first two bars of the grill, to record bar-surface temperatures during experiments. Temperatures were monitored on a digital display visible only to the experimenters.

Measurement of CPM. To measure the effects of CPM in the main experiment, the left hand was positioned in a water bath before application of the grill. The conditioning stimulus for inducing CPM consisted of an 11-L plastic cooler (23.5cm^3), which was filled with 10 L of water before the start of each run. An aquarium pump was used to circulate water and ensure stable temperatures surrounding the hand. A plastic grate divided the interior of the cooler into two chambers, in order to separate the subject's hand from the pump and any ice cubes. A thermometer was used to measure the temperature of the water and control it (by adding ice or warm water) to the desired temperature before a given run. The temperature of the water was painfully cold (M = 6.1°C; SD = 0.08) during the CPM run and was neutral (M = 32.7°C; SD = 0.26) during the Control run.

Questionnaires. Before participating in the experimental runs, all subjects filled out a demographics questionnaire (age, sex, race, and handedness).

Immediately following each experimental run, NH and TG subjects were provided with a Sensation Questionnaire, devised by our lab, containing several descriptors used in the McGill Pain Questionnaire and some additional innocuous thermal descriptors. *See Appendix A.* This questionnaire asked subjects to characterize any sensations associated with the grill

by circling words from a list of descriptors. This questionnaire was used to assess qualitative differences in the pain and thermal sensations experienced under the different experimental conditions.

Subjects in the component temperatures experiment also filled out sensation questionnaires following each exposure to the grill, which was in their case either warm or cool. Descriptors for this questionnaire were: neutral, cool, cold, warm, hot, painful, comfortable, pleasant, and unpleasant. Subjects were instructed to indicate any that applied to their experience.

Procedure.

Main experiment. The experimenters filled the tanks supplying the grill and the water bath with water and controlled them to the desired temperatures prior to the subject's arrival to the lab.

After giving informed consent and filling out the demographics questionnaire, the subject was trained to use a 0-100 scale to rate the intensity of his or her sensations. Here, the experimenter presented pictures of different food items (ex. steak, broccoli, etc.) to the subject in succession, the subject's task being to verbally rate the deliciousness of each item using a 0-100 scale, where 0 meant "not at all delicious" and 100 meant "the most delicious food imaginable". Responses were not recorded.

Following training, the subject was seated at a table on which the thermal grill apparatus rested, and the procedures were explained in detail. The water bath, for left hand immersion, was positioned on a chair 0.5 m to the left of the subject. The valves on the tanks supplying the thermal grill were opened 1 min before the start of a run, allowing the apparatus to stabilize at the desired temperature prior to the run. The run began when the

subject placed his or her left hand into the water bath up to the wrist. The subject was prompted for verbal ratings of pain intensity of the water bath on a 0-100 scale, where 0 meant "no pain" and 100 meant "the most intense pain imaginable", every 15 s for the first 45 s of the run. One minute into the run, the subject was told to place the volar surface of his or her right forearm onto the grill apparatus, and 5 s later was told to remove it. The subject was then prompted for verbal ratings of grill pain intensity (0-100 scale) and unpleasantness (0-100 scale from "not at all unpleasant" to "the most unpleasant sensation imaginable"). After grill ratings were obtained, the subject removed his or her hand from the water bath and dried it off with paper towels. The sensation questionnaire for that run was administered immediately afterward.

The subject took a 25-min break between runs to minimize any lingering effects (e.g. sensitization or habituation) of exposure to the thermal stimuli during the first run, and to allow the experimenters to prepare the stimuli for the second run. The grill temperatures for the second run were the same as for the first (i.e. 42°C/18°C for subjects in the TG Group or 45°C/45°C for those in the NH Group). The temperature of the conditioning water bath was adjusted to either 6°C, if the subject underwent the Control run first, or 33°C, if the first was a CPM run. Procedures were otherwise identical in the two runs. Following administration of the sensation questionnaire for the second run, the subject was debriefed and awarded credit for his or her participation.

Bar-surface temperature was recorded in the seconds before the subject placed his or her forearm on the grill to ensure that the applied temperatures were within the desired range. For the NH group, the average bar temperatures of sets 1 and 2 were 44.75 (SD = .39) and 44.87 (.41) during the Control run and 44.71 (.48) and 44.87 (.41) during the CPM run,

respectively. For the TG group, the average bar temperatures of the warm and cool sets were 41.96 (.37) and 18.18 (.15) during the Control run and 42.01 (.39) and 18.22 (.17) during the CPM run, respectively.

Component temperatures experiment. In order to determine whether the warm and cool bars were perceived to be innocuous on their own in naïve subjects, 24 additional participants were enrolled in an experiment in which they felt the grill with all bars controlled to either 18°C or 42°C. Prior to each run, both tanks supplying the grill were filled with water that was either warm (for the warm run) or cool (for the cool run).

After giving informed consent and filling out the demographics form, the subject was seated at the experimental table on which the grill rested. The valves for the water were opened and water was allowed to flow through the bars for at least 1 min to allow ample time for bar temperature stabilization. The experimenter then told the subject to put his or her right forearm onto the grill and indicated (after 5s) when to remove it. The sensation questionnaire was administered immediately thereafter.

The subject took a 20-min break before participating in a second run, in which he or she was exposed to the temperature (warm or cool) not used during the first run. The procedures were identical. Run order was randomized prior to the beginning of experimentation, subject to the constraint that half of the subjects underwent the warm, and the other half the cool, run first.

Data from four runs (one cold and three warm) in which the temperatures of one or both sets of bars differed from the target temperature by > +/- 0.5°C were discarded. The data from the remaining 44 runs were analyzed. The temperatures of bar sets 1 and 2 during the

warm run were 42.00°C (SD = .30) and 41.94°C (.24), respectively. During the cool run, the temperatures of bar sets 1 and 2 were 18.09°C (.15) and 18.17°C (.11).

Results

Conditioning stimulus pain ratings. Subjects gave verbal pain intensity ratings of the water bath at 15, 30, and 45 s during the conditioning procedure. Pain ratings for the neutral water bath, during the Control run, were negligible. During the 6°C conditioning stimulation (i.e. CPM run), pain ratings increased from 38.8 (SD = 24.6) to 52.1 (27.0) to 62.5 (26.5) at the three time points in the NH group and from 37.2 (23.5) to 49.4 (26.5) to 58.1 (27.7) in the TG group. A 3 x 2 repeated-measures ANOVA with *Rating Number* as the within-subjects factor and *Group* (TG or NH) as the between-subjects factor showed that the increase in pain was significant across ratings [$F(2,70) = 58.7$; $p < .001$]. The lack of a significant main effect of *Group* [$F(1,35) = 0.1$; $p = .73$] and the absence of an interaction between the factors [$F(2,70) = 0.3$; $p = .78$] show that sensitivity to and summation of nociceptive cold pain were not different between groups.

Grill pain intensity and unpleasantness. During the Control run (i.e. contralateral hand in neutral water), the 45°C bars and the 42°C/18°C bars produced pain intensity ratings of 35.7 (SD = 25.1) and 27.1 (23.9), respectively. During the CPM run (i.e. contralateral hand in cold water), pain intensity of the NH and TG grills was reduced by 48.2% (M = 18.5; SD = 20.1) and 47.0% (M = 14.3; SD = 13.5), respectively. See *Figure 2.1A*. A 2 x 2 repeated-measures ANOVA was carried out. The between-subjects factor was *Group* (TG or NH) and the within-subjects factor was *Run Type* (CPM or Control). The main effect of *Run Type* was significant [$F(1,35) = 19.8$; $p < .001$], indicating a robust reduction in grill pain intensity by CPM. The main effect of *Group* was not significant [$F(1,35) = 1.1$; $p = .30$],

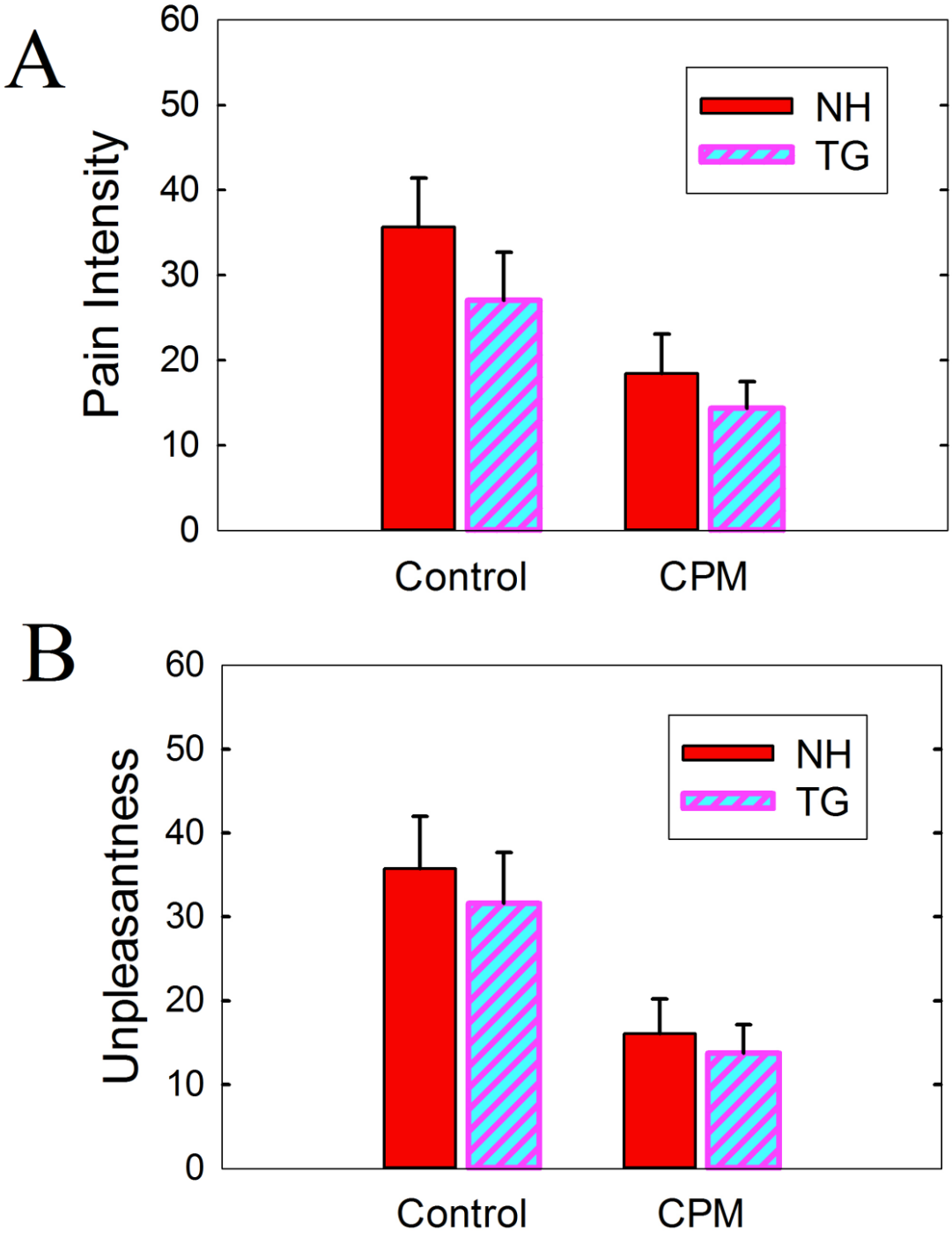

Figure 2.1 Effects of CPM on pain and unpleasantness. The red bars show the results for the noxious heat (NH) group and the striped bars show the thermal grill (TG) group. CPM significantly reduced pain (A) and unpleasantness (B) in both groups, and there was no significant interaction in either case. Error bars = +/- 1 SEM.

meaning there was no difference in pain intensity between the hot and warm/cool grills. The interaction between the two factors was not significant [$F(1,35) = 0.4$; $p = .51$], showing that CPM reduced pain intensity similarly in both groups.

NH unpleasantness ($M = 35.8$; $SD = 27.1$) and TG unpleasantness ($M = 31.6$; $SD = 25.7$) during the control runs was reduced during CPM by 55.2% ($M = 16.1$; $SD = 18.1$) and 56.6% ($M = 13.7$; $SD = 14.6$), respectively. See *Figure 2.1B.* A 2 x 2 repeated-measures ANOVA revealed a pattern of significance similar to that for pain intensity. The main effect of *Run Type* was highly significant [$F(1,35) = 26.5$; $p < .001$], while the main effect of *Group* [$F(1,35) = 0.3$; $p = .61$] and the interaction between the factors [$F(1,35) = 0.1$; $p = .80$] were not.

Grill descriptors. In addition to determining the amount of pain intensity and unpleasantness produced by the grills and the amount of CPM-induced reduction in them, we also wished to determine 1) how the grills were perceived qualitatively and 2) how CPM changed the way that the grills were described. Subjects indicated after each run which of 9 sensations were experienced from the grill.

The percentages of subjects attributing each of the descriptors to his or her grill experience during the control run are plotted in *Figure 2.2A.* NH subjects most often described their grill as "hot", followed by "burning", then "stinging". TG subjects most frequently described their grill as "hot", followed by "burning" and "sharp". Mann-Whitney U tests (one for each descriptor) were conducted to determine whether NH produced different sensations from TG. These tests revealed that TG subjects significantly more often reported a sensation of "sharp" than NH subjects [$Z = -2.43$; $p = .015$]. The frequencies of

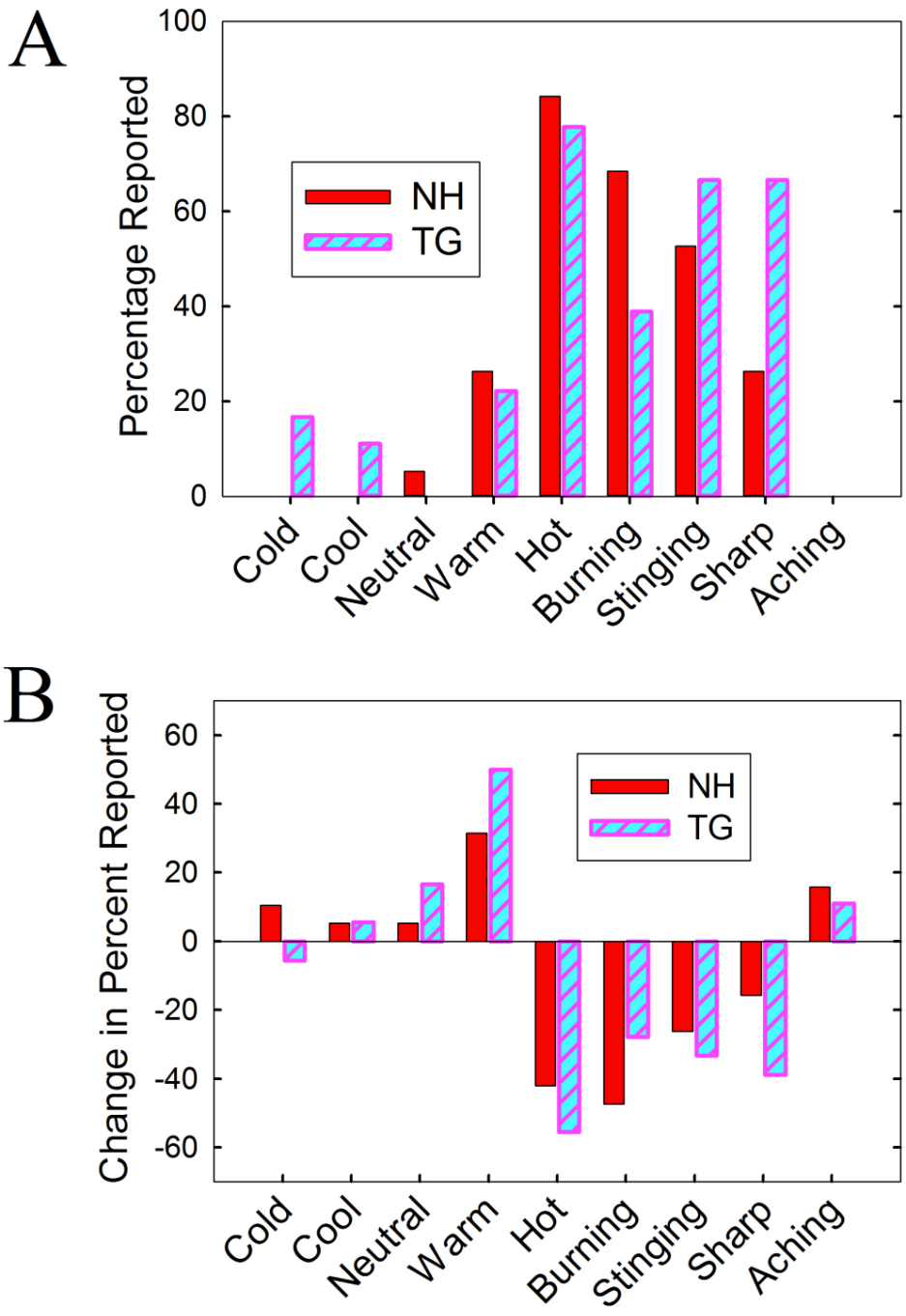

Figure 2.2 Sensations reported and the effects of CPM on them. (A) Percentage of subjects in the control (neutral water bath) condition who reported the indicated sensations. The only significant difference between the noxious heat (NH) and the thermal grill (TG) groups' reported sensations in the control condition was significantly more "Sharp" responses in the TG group. (B) CPM significantly reduced the frequencies of burning and hot reported in both groups and the frequency of "Sharp" in the TG group. CPM also significantly increased the frequencies of "Warm" reported in both groups. No other changes were significant.

responses for all other descriptors were not significantly different for the different grill types [$p > .05$ for all].

Figure 2.2B shows the change in response frequency for the two grills from the Control run to the CPM run. Whether the frequencies of responses changed significantly between runs was tested using Wilcoxon Signed Ranks tests. Each of these tests compared the frequency of responses for one descriptor and one grill type during the Control run to that of the CPM run. CPM significantly reduced the frequencies of describing NH as "burning" [$Z = -2.71$; $p = .007$] and "hot" [$Z = -2.53$; $p = .011$], while it significantly increased the amount of "warm" [$Z = -2.45$; $p = .014$] responses. For TG, CPM significantly reduced the number of "burning" [$Z = -2.24$; $p = .025$], "hot" [$Z = -3.16$; $p = .002$], and "sharp" [$Z = -2.33$; $p = .02$] responses; conversely, CPM significantly increased the frequency of "warm" [$Z = -2.71$; $p = .007$] responses.

The pattern of results from the grill descriptors is in line with the results of the effects of CPM on pain intensity and unpleasantness. With the exception of the different proportion of "sharp" responses for the two grills, they were very similarly described. Furthermore, CPM changed the way that the two grills felt in the same manner; both grills were less often described as "burning" and "hot", and more often indicated as being "warm", during the CPM compared to the Control run.

Component temperatures experiment. The two temperatures comprising the TG in the main experiment were generally experienced as innocuous when presented individually in the control experiment. No subject reported pain from the 18°C bars, and only three described the 42°C bars as painful. See *Figure 2.3.*

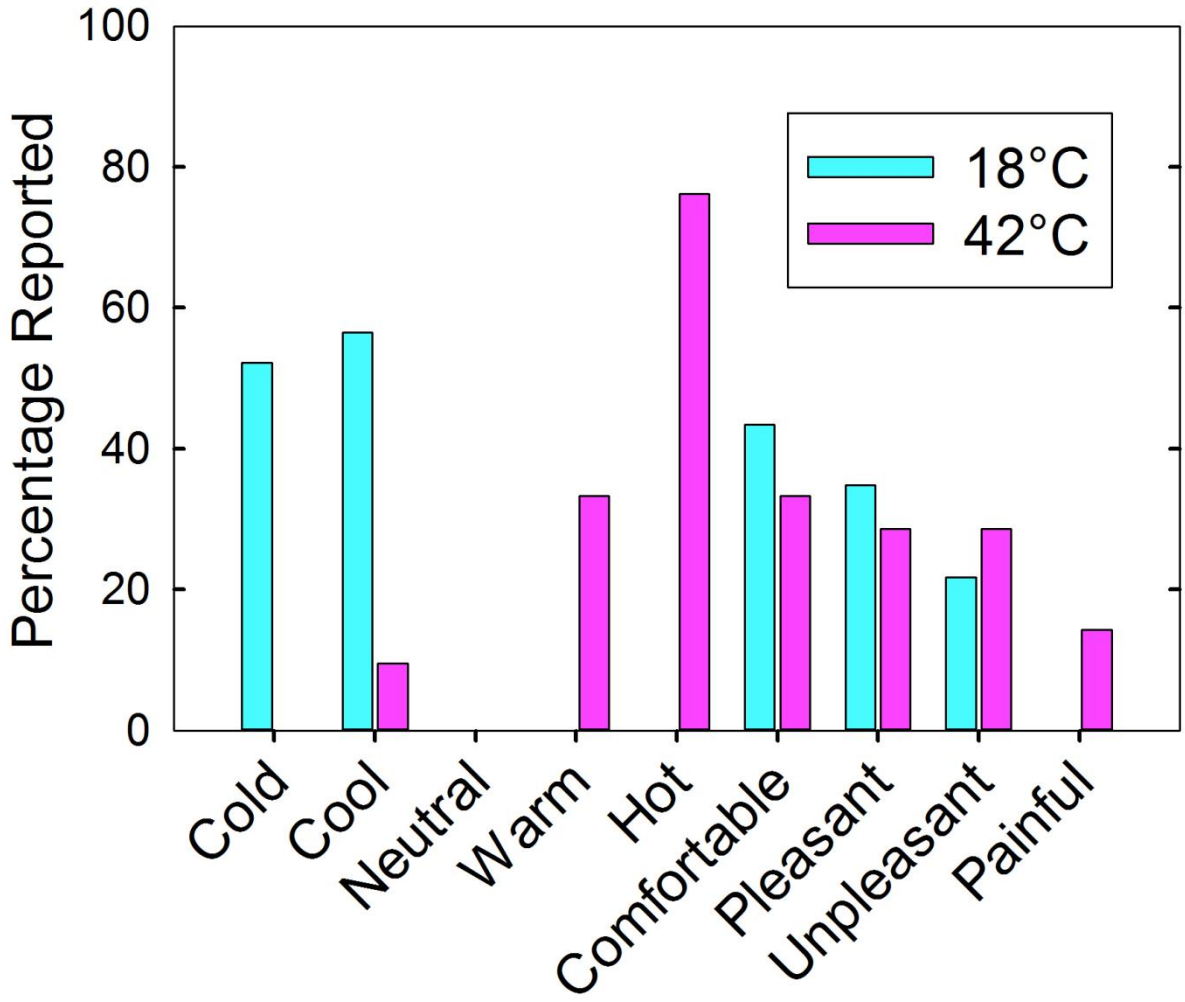

Figure 2.3 Sensations reported in the component temperatures experiment. The 18°C bars were significantly more often reported to be "Cool" and "Cold" than the 42°C bars, while the 42°C bars were significantly more often called "Warm" and "Hot". There were no other differences between the stimuli. Importantly, each component temperature used to produce the TGI in the main experiment was generally perceived to be innocuous on its own.

Wilcoxon Signed Ranks tests showed that the 18°C bars were significantly more often described as "cool" [$Z = -2.31$, $p = .02$] and "cold" [$Z = 3.46$; $p = .001$] than the 42°C bars, which were more often described as "warm" [$Z = -2.65$; $p = .008$] and "hot" [$Z = -3.87$; $p < .001$]. There was no significant difference in the amount of attribution of the remaining descriptors to the cool vs. the warm grill [$p > .05$ for all].

It is important to note that *all* bars were either warm or cool in this control experiment, as opposed to just half warm and half cool in the TG stimulus in the main experiment. This likely introduced greater spatial summation within the warm and cool pathways than was present during the main experiment (Defrin et al., 2009; Hardy & Oppel, 1938; Stevens & Marks, 1971). Therefore, the frequencies of cold and hot reported in the control experiment are probably overestimates of the sensations associated with the TG component temperatures.

Discussion

The results of Aim 1 shed new light on the TGI. They show that CPM is capable of reducing TGI pain to the same extent as pain from a nociceptive heat stimulus, which implies that the signals triggered by warm/cool grill exposure are treated as equivalent to heat pain signals, at the level of the spinal cord. Furthermore, CPM produced similar changes in the thermosensory and dysesthetic qualities assigned to the grill during both "real" (i.e. nociceptive) and illusory pain. The implications of these findings are discussed in the following sections.

CPM reductions in pain intensity and unpleasantness. CPM was found to significantly reduce pain intensity and unpleasantness of both noxious heat and TGI. There were no differences in the magnitude of CPM-induced reduction for nociceptive and illusory pain, suggesting that the inhibition treats signals associated with these two types of pain as equivalent.

CPM is thought to be mediated primarily by DNIC, a spinal-bulbar-spinal loop through which ascending nociceptive signals activate descending projections in the subnucleus reticularis dorsalis (SRD) (Bouhassira et al., 1992a) to inhibit the processing

other remote noxious stimuli in the dorsal horn (LeBars et al., 1979a). Electrophysiological studies in animals show that another important region for descending inhibition, the PAG, is not necessary for DNIC but it can play a modulatory role (Bouhassira et al., 1992b). Recent neuroimaging evidence suggests that the PAG may also be activated in human subjects during CPM (Sprenger et al., 2011). Whether the pain-inhibiting effects of CPM are a result of DNIC, PAG-induced inhibition, or both, numerous studies have found that descending inhibition operates predominately, and in many cases exclusively, on nociceptive dorsal horn neurons (Heinricher et al., 2009; Leith et al., 2010; Waters & Lumb, 1997).

In the case of innocuous and noxious thermal sensation, DNIC reduces WDR responses to noxious heat and cold but it does not reduce activity in thermospecific COOL dorsal horn neurons (Dickenson et al., 1980). Electrical stimulation of various brainstem regions with descending projections produces results with similar inhibitory specificity (Davies, 1984; Dawson et al., 1981). Furthermore, PAG stimulation has been shown to inhibit the WDR response to noxious, but not innocuous, cold (Leith et al., 2010). Taken together, these results suggest that descending inhibition does not reduce activity in neurons that convey innocuous thermal information.

The present results are therefore incompatible with the idea that the TG produces warm and cool sensations that are together interpreted as heat, as suggested by Alrutz (1898). Instead, it seems that the TGI involves signals that activate pain-transmitting neurons at the level of the spinal cord, where they are then subject to descending inhibition.

Possibilities for TG integration into the nociceptive system. Craig and Bushnell's (1994) thermal disinhibition theory posits that TGI pain results from an abnormally high (for innocuous temperatures) ratio of HPC to COOL cell firing in lamina I dorsal horn neurons in

response to simultaneous warming and cooling of the skin. Electrophysiological recordings in cats showed that a cool stimulus caused robust firing in both COOL and HPC cells. Addition of a warm stimulus caused drastically reduced firing in COOL but firing in HPC neurons remained relatively unchanged, leading to a higher ratio of HPC/COOL neuron signaling. The median HPC threshold for cool stimulation is 24°C and their rates systematically increase as temperature is lowered further, down to at least 9°C (Craig, Krout, & Andrew, 2001). COOL fibers, in contrast, have thresholds just below normal skin temperature and they increase firing fairly linearly down to about 15°C, at which point the response plateaus. Therefore, the HPC/COOL signal ratio starts to increase substantially below 15°C, and is considered by some to signal the burn of cold pain. Thus, the disinhibition theory is based on the idea that the thermal grill produces illusory pain by mimicking the across-fiber pattern of activity in dorsal horn neurons that is produced by nociceptive cold (Craig & Bushnell, 1994). The possibility that cool temperatures convey signals to the nociceptive system is supported by psychophysical evidence showing that, under certain conditions, mild cooling (ex. 27°C) of the skin can produce sensations of burning and stinging in a majority of subjects (Green, 2002; Green & Pope, 2003).

The interpretation of a high HPC/COOL signal ratio as pain is thought to occur in the insular cortex or the thalamus, as suggested by neuroimaging evidence (Craig et al., 1996; Davis et al., 2004; Lindstedt et al., 2011a). However, it is important to note that the abnormal patterns of responses in these channels are present at the level of the spinal cord. With respect to this theory of TGI, CPM could have reduced TGI pain and unpleasantness by 1) increasing COOL cell activity or 2) decreasing HPC activity; either of which would have lowered the ratio of HPC/COOL firing to a level that occurs at less extreme temperatures.

The first possibility is unlikely, since DNIC does not affect the firing rates of COOL neurons (Dickenson et al., 1980). In terms of the latter, no study to date has systematically studied the effects of DNIC on HPC neurons, but DNIC is known to inhibit activity in dorsal horn lamina I (Morgan et al., 1994), the location of many HPC neurons (Craig, Krout, & Andrew, 2001).

Alternatively, TGI pain could be produced by additive, or perhaps even synergistic, responses of warm and cool afferents onto WDR neurons, as posited by Green (2002) and further supported by the results of Bouhassira and colleagues (2005). WDR firing rates in animals across a range of noxious heat intensities correspond very well with psychophysical ratings of perceived pain (Coghill et al., 1993; Maixner et al., 1986). If the grill elicits firing rates in WDR neurons that resemble those produced by noxious heat or cold, this could explain the pain of TGI.

The reductions in TGI pain by CPM observed in the present study are compatible with this theory. The classic study of DNIC by Le Bars and colleagues (1979a) showed that heterotopic noxious stimuli produce profound reductions in WDR firing rates in response to painful test stimuli. Thus, the reduction of TGI pain and unpleasantness by CPM could be explained by CPM-induced reductions WDR firing rates to those that are produced by less extreme temperatures.

Sensations associated with the different grill combinations. While some have argued that TGI is not actually painful (Fruhstorfer et al., 2003), our results are in agreement with those of many other researchers showing that interlacing warm and cool bars are capable of producing pain (Boettger et al., 2011; Bouhassira et al., 2005; Craig & Bushnell 1994; Craig et al., 1996; Defrin et al., 2008; Kern et al., 2008a, 2008b; Leung et al., 2005; Li

et al., 2009; Lindstedt et al., 2011a, 2011b). Three subjects (17%) in the TG group did not, however, report any pain from the TGI during the Control run, confirming previous studies suggesting that a small percentage of individuals might not be sensitive to the painful component of the TGI (Bouhassira et al., 2005; Kern et al., 2008a, 2008b). Two of these subjects did report that the grill was "burning" and "stinging" and the third reported that it was "sharp," suggesting that subjects who don't report pain from TGI may still feel its dysesthetic qualities. In addition to reducing the pain intensity and unpleasantness of TGI and nociceptive heat, CPM also reduced the frequency of pain-related adjectives assigned to the two types of pain. "Burning" was significantly less frequently reported in both groups in the CPM run, and "sharp," which was more frequently reported for TG than for NH in the Control run, was less often attributed to the TG during CPM. Thus, the similar reductions in TG and NH pain by CPM are paralleled by similar reductions in the pain-like qualities assigned to them.

Some authors have suggested that the pain experienced in TGI is comparable to that experienced during noxious cold (Craig & Bushnell, 1994; Craig et al., 1996), and TGI pain intensity can be psychophysically matched with cold pain intensity (Craig & Bushnell, 1994); however, TGI and noxious heat pain intensity can be equated in subjects as well (Leung et al., 2005). Furthermore, in many studies subjects experience synthetic heat sensation (Alrutz, 1898; Bouhassira et al., 2005; Craig & Bushnell, 1994; Craig et al., 1996; Fruhstorfer et al., 2003; Green, 2002; Kammers et al., 2010; Leung et al., 2005), meaning the grill feels hotter than either of the component temperatures, along with pain from TGI. In the Control run of the present study, the 42°C/18°C grill was most often called "hot" but hardly ever "cool" or "cold", and this pattern of responses was not significantly different from that

of noxious heat. The convergent neurons (i.e. WDR and HPC) that convey some of the nociceptive qualities of thermal pain are thermally bimodal, responding to both ends of the temperature spectrum, so their activity could indicate the presence of potentially threatening heat or cold. However, it seems that the innocuous thermal backdrop of the TGI experience is more appropriately regarded as heat.

The effect of CPM on the thermal qualities assigned to the grills lends further credence to the idea that TGI involves synthetic heat and a perceptual masking of cool sensation. In both groups, the frequency of "hot" responses was significantly reduced during CPM and the most frequent response became "warm". These results suggest that CPM dampened the perceptual intensities of synthetic heat and actual heat, and again treated the signals resulting from both types of stimulation similarly. Since CPM selectively reduces nociceptive signals, this result also implies that nociceptive signals likely participate in the coding of thermal sensation magnitude.

In summary, the data presented in this chapter show that TGI pain intensity, unpleasantness, and perceived heat are reduced by CPM to the same extent as the sensations resulting from noxious heat. Based on current understanding of CPM mechanisms, this indicates that signals from the warm/cool grill give rise at the level of the spinal cord to signals that are treated as pain signals by descending inhibitory mechanisms. The cognitive addition theory cannot explain the results. Instead, the results are compatible with both of the spinal theories of the TGI. The experiments reported in the following chapter are therefore devoted to determining which of the two spinal theories provides a better explanation of the illusion.

CHAPTER 3: COOLNESS BOTH UNDERLIES AND PROTECTS AGAINST THE PAINFULNESS OF THE THERMAL GRILL ILLUSION

Abstract

In this experiment, I investigated the contributions of warm and cool signals in generating the thermal grill illusion (TGI) by selectively adapting them. Each subject underwent three runs, two of which tested the effects of pre-adapting subjects to the grill's warm or cool bars (while the interlaced bars were thermally neutral) on the subsequent intensity of the illusion. In a control run, all bars were neutral during the adaptation phase. Thermal VAS ratings during the warm and cool adaptation periods revealed significant and equivalent adaptation to the two temperatures. Adaptation to the grill's cool bars significantly reduced pain and perceived thermal intensity of the TGI, compared to the control condition, while adaptation to the grill's warm bars had little effect. These results suggest that the cool stimulus triggers the pain signals that produce the illusion. The inability of warm adaptation to attenuate the TGI is at odds with theories suggesting that the illusion depends upon a simple addition of warm and cool signals. While the grill's cool bars are necessary for the TGI's painfulness, we also observed that the more often a participant reported feeling coolness or coldness, the less pain he or she experienced from the TGI. These results are consistent with research showing that cool temperatures generate activity in both pain-inhibitory COOL neurons and nociceptive dorsal horn neurons.

Introduction

The results of Experiment 1, presented in Chapter 2, showed that CPM attenuates the painfulness of noxious heat and the TGI similarly. This is at odds with the predictions of the cognitive addition theory (Alrutz, 1898), since innocuous signals should not have been affected by CPM's descending inhibition. While the results showed that the TGI involves nociceptive signals at the level of the spinal cord, they are unable to shed light on the nature of the interactions that generate those signals.

Two different theoretical perspectives characterize modern thinking about spinal-cord processes that may contribute to the TGI. According to Craig and Bushnell (1994), the grill's cool bars stimulate both Aδ cool and C nociceptive afferents, which send signals to COOL and heat-pinch-cold (HPC) second-order neurons in the dorsal horn, respectively. Normally, at innocuous cool temperatures, the first of these signals masks the second (Fruhstorfer et al.,1984; Wahren et al., 1989). However, single-cell recordings in cat dorsal horn showed that alternating warm bars with cool reduces the firing of COOL neurons (Craig & Bushnell, 1994). Thus, these authors proposed that the TGI is due to an unmasking of HPC nociceptive signals via warmth's inhibition of pain-inhibitory COOL signals.

In contrast, some psychophysical results suggest that the interaction between the grill's warm and cool stimuli might be additive. Green (2002) showed that the intensity of the TGI is similar to the sum of the perceived intensities of the warm and cool component temperatures. Bouhassira and colleagues (2005) later found that, for a given cool temperature, the illusion increases if the warm bars are made warmer. This could be explained by increased inhibition of COOL neurons at warmer temperatures. However, they also found that, for a given warm temperature, the illusion increases if the cool temperature is

decreased. Since the rates of both COOL and HPC neurons increase similarly throughout the range of temperatures employed (Craig et al., 2001), the disinhibition theory appears to be at odds with this result. Instead, the symmetrical result of increasing the TGI by making either of the two temperatures more extreme suggests that warm and cool signals might add to one another to produce the illusion.

The present study utilized thermal adaptation to disentangle the relative contributions of warm and cool in generating the TGI. The addition theory states that the strengths of the warm and the cool signals are equally important for the illusion (Bouhassira et al., 2005; Green, 2002); thus, it predicts similar reductions in the TGI following adaptation to either temperature. The disinhibition theory predicts different results following warm or cool adaptation. Since warmth is thought to inhibit cool signals and unmask an underlying nociceptive signal (Craig & Bushnell 1994; Craig et al., 1996), adaptation to warmth should reduce the illusion by reducing the inhibition on COOL neurons. Since both COOL and HPC neurons respond to the cool bars of the grill, cool adaptation should fatigue both neuronal populations. Furthermore, the cool adapting stimulus (18°C) will excite COOL neurons more than HPC neurons (Craig et al., 2001) and should therefore adapt them to a greater extent. Thus, based on the disinhibition theory, adaptation to cool could be expected to either have no effect on or to slightly increase the TGI since the pain-inhibitory cool signals should be dampened at least as much as the pain-excitatory nociceptive signals.

Methods

Participants. Twenty-six undergraduate students participated in the study. Recruiting was carried out through the UNC Psychology Department's participant pool website. Age of participants ranged from 18 to 23 years (M=20.5; SD=1.9). The study was

approved by the University's IRB and written informed consent was obtained from all subjects prior to their participation. Subjects were compensated with research credit for an Introductory Psychology course for their participation.

Study Design. Each subject participated in three separate runs. Each run consisted of a 3-min adaptation phase, followed by a test phase. The test phase was identical in all three runs, with interlaced warm (42°C) and cool (18°C) bars, but the adaptation phase differed across runs. During the *cool adaptation run* half of the bars were at the cool temperature (18°C) that was later used to produce the TGI, while the interlaced bars were held at a neutral temperature (32°C). The *warm adaptation run* consisted of warm bars (42°C) interlaced with neutral bars (32°C). Following the adaptation period in each of these runs, the neutral bars were heated or cooled to produce the test stimulus (i.e. 18°C bars interlaced with 42°C bars). Each subject also participated in a neutral adaptation run; in this case all of the bars were held at 32°C during the adapting period. It should be noted that the subject's forearm remained in place throughout the run, so that the adapted regions of skin were still positioned over bars of the adapting temperature during testing of the TGI. The order of runs was counterbalanced across subjects. See *Figure 3.1*.

Apparatus.

Thermal grill. The thermal grill apparatus was the same as that used in the first experiment. It consisted of 12 pieces of copper tubing (length 33cm; diameter 1cm; thickness 0.4mm) that were secured onto the top of a plastic holder. Each bar rested in a trough (1.25 cm wide and 0.5 cm deep) and was separated from its neighbor(s) by 0.5 cm. In order to gain thermal control over the bars, two sets of plastic tubing through which water could be circulated were connected to the ends of the bars. Set 1 was comprised of the odd-numbered

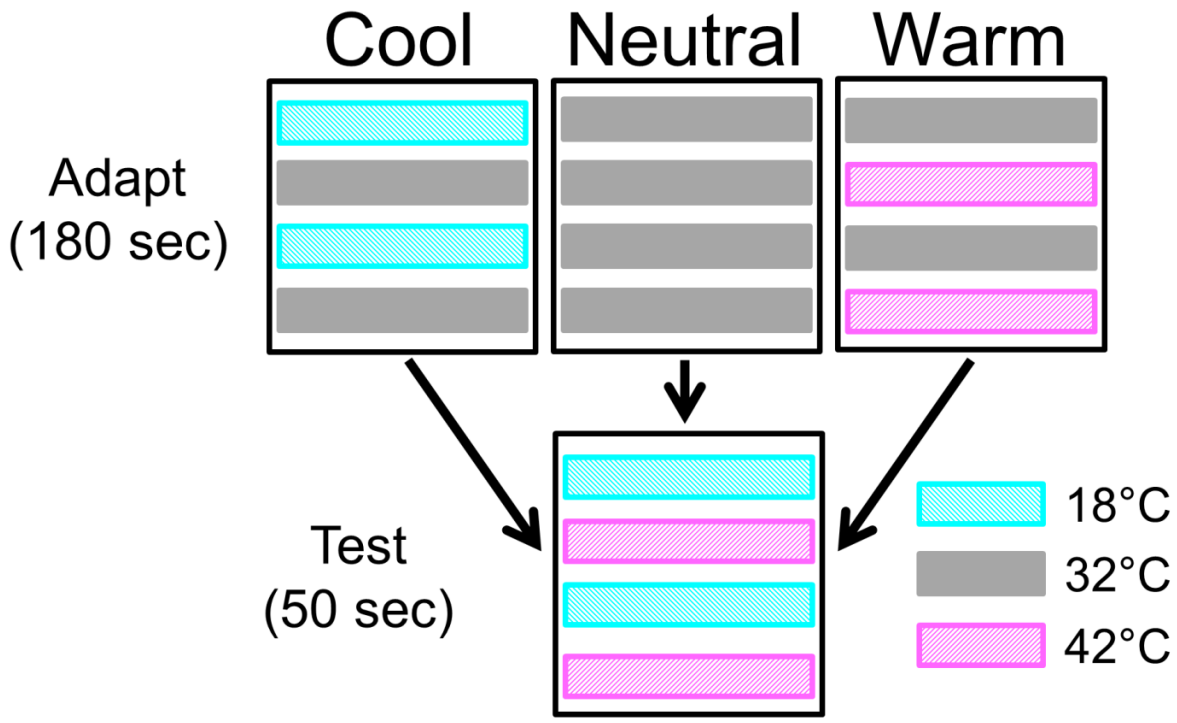

Figure 3.1 Experiment 2 design. Each run, including cool adaptation, warm adaptation, and neutral adaptation, consisted of a 3-min adaptation phase (Adapt) followed by a 50-sec presentation of the TGI (Test) stimulus. The subject rated perceived thermal intensity throughout the adaptation phase and for the first 30 sec of the test phase. Then, following a 5-sec countdown, the subject rated the painfulness of the TGI for 15 sec.

bars and Set 2 the even-numbered bars. The two sets of bars also had separate intake tubes that were connected to bars 1 and 2. Four thermally insulated 19-L tanks were positioned on a shelf 0.65 m above the tabletop for this experiment, since each set of bars in many cases required two temperatures (i.e. adaptation vs. test) during a single run. Rapid switching of the input temperature was accomplished by routing tubes from two tanks to a T connector, whose single output carried water to one of the grill intakes. At the end of the adaptation phase, the experimenter would open the valve(s) for the test temperature water to flow and close the valve(s) for the adaptation temperature water. Each set of bars had an outtake that passed water from the grill into a receptacle on the floor. Two thermistor probes (YSI 400

series) were used to record bar temperatures during experiments. They were attached to small sections of copper tubing that were inserted into the flow lines, near the grill.

Based on preliminary testing, we determined warm and cool temperatures that produced a moderately intense TGI but were not perceived to be painful on their own. These bar temperatures were ~42°C and ~18°C.

In a calibration, we made repeated measurements in one individual of the skin-thermode interface temperatures produced by these bar-surface temperatures. These tests used a K/J Thermometer (421502; Extech Instruments Corp., Nashua, NH), with a K-type bead thermocouple (TP870; Extech Instruments Corp.). The thermocouple was positioned (over a thin layer of epoxy) on a copper bar that was inserted in the apparatus in place of the fourth bar of the grill. The skin of the volar forearm rested on the apparatus and the temperature was allowed to stabilize. These measurements revealed that the skin-thermode interface temperatures were slightly less extreme than the bar-surface temperatures we recorded during runs; the warm (42°C) and cool (18°C) bar temperatures produced skin temperatures of ~41°C and ~20°C, respectively.

Pain and thermal ratings. The main dependent measures were sensation ratings obtained using computerized visual analogue scales (VAS) that were custom-designed using LabView 12.1 (National Instruments Corp, Austin TX USA). The scales appeared on a monitor positioned in front of the subject, who manipulated them by moving a mouse held in the right hand. One VAS was used to record continuous ratings of pain intensity. The left end of the scale was labeled "No Pain" and the right end was labeled "The Most Intense Pain Imaginable". The background of the bar was a dark gray, and the bar could be filled with a lighter gray color by moving the mouse to the right, to indicate increasing pain. See *Figure*

3.2A. The second scale was designed to provide continuous ratings of thermal sensation intensity. The middle of the VAS was labeled "No Thermal Sensation". Subjects had the option of moving the mouse to the left to rate coldness up to "The Most Intense Cold Imaginable" or to the right to rate heat up to "The Most Intense Heat Imaginable". The bar initiated in the middle of the scale, i.e. no thermal sensation, on a gray background. See Figure 3.2B. Movement to the left began to fill the left side of the bar with a blue color and movement to the right caused that side to fill with a red color. The pain and thermal sensation scales were never present on the monitor simultaneously.

Questionnaires. Before the experiments, subjects completed a demographics questionnaire (age, sex, race, ethnicity, and handedness). At the end of each experimental run, they completed a sensation questionnaire, which asked them to indicate any sensations felt by circling any of the following descriptors: cold, cool, neutral, warm, hot, stinging, sharp, burning, aching. Subjects were instructed to choose all that applied. This questionnaire was essentially the same as that used in Aim 1, but specified that we were only concerned with sensations felt in the moments before the run ended (i.e. during the presentation of the thermal grill). This qualification was needed since they also felt sensations during the warm and cool adaptation periods.

Procedure. First, the subject was seated at the experimental table on which the thermal grill apparatus rested. He or she was first trained to use a VAS to rate sensation intensity, by continuously rating the perceived moment-to-moment loudness of a short piece of classical music presented through headphones. The scale ranged from "No Loudness" on the left to "The Loudest Sensation Imaginable" on the right. The scale resembled, in appearance and operation, the pain scale that was used in the main experiment.

Following training, one of the experimenters explained and demonstrated how to place the forearm onto the grill and how to use the thermal and pain scales to rate sensation intensity. The experimenter reiterated that it was very important to keep the arm still and in constant contact with the grill throughout the experimental run. Any questions about the procedure were answered at that time.

The bars of the grill were controlled to the temperatures appropriate for the experimental condition of the first run. The VAS program was initiated by the experimenter and a 5-sec countdown appeared on the monitor. At the end of this countdown the subject placed his or her left volar forearm onto the grill halfway between the wrist and the elbow and began to rate the thermal intensity of the grill. Adapt Initial temperatures (*See Table 3.1*) were recorded at this time. The initial temperatures were held constant for a period of 3 min, to ensure substantial thermal adaptation. Temperature recordings at 30-sec intervals showed that the temperatures remained steady throughout the adaptation period. At the end of the third minute the Adapt Final temperatures were recorded, and the temperatures to produce the thermal grill illusion were allowed to flow through the bars. Bar temperatures gradually changed to the new set-points over the next 30 sec. In the warm and cool adaptation conditions, this meant cooling or warming the 32°C bars to 18°C or 42°C, respectively, while the bars used to adapt remained at their set temperature. In the neutral adaptation condition, all of the bars started at 32°C, so half were warmed and the others were cooled simultaneously. The subject continued to rate thermal intensity, now of the TGI, for the next 30 sec while the temperatures of the grill stabilized at the new setting.

Following the 30-sec temperature stabilization period (i.e. 3.5 min into the run), the Test Initial temperatures were recorded and the thermal VAS was replaced on the screen with

		Adapt Initial	Adapt Final	Test Initial	Test Final	Test Final Difference
Cool Adapt	Cool Set	18.1 (0.2)	18.0 (0.2)	18.2 (0.2)	18.3 (0.2)	23.7 (0.4)
	Warm Set	32.0 (0.2)	32.1 (0.2)	40.8 (2.1)	42.0 (0.3)	
Neutral Adapt	Cool Set	32.0 (0.3)	32.4 (0.2)	19.3 (1.0)	18.5 (0.2)	23.6 (0.3)
	Warm Set	32.1 (0.2)	32.3 (0.2)	41.5 (0.8)	42.1 (0.3)	
Warm Adapt	Cool Set	32.1 (0.5)	32.6 (0.2)	20.1 (2.9)	18.6 (0.2)	23.7 (0.3)
	Warm Set	41.7 (0.5)	42.4 (0.3)	42.3 (0.3)	42.3 (0.2)	

Table 3.1 Bar temperatures at various time points. Depicted above are the temperature of the bars recorded at the beginning and end of the adaptation and test periods and the difference between the temperatures of the warm and cool bars.

the pain VAS. This VAS was activated at the end of a 5-sec countdown, and the subject rated TGI pain intensity during the final 15 sec of the run. After these ratings were obtained, the Test Final temperatures were recorded and the subject removed his or her forearm from the grill. The experimenters then immediately administered the sensation questionnaire for that run. The subject's forearm remained on the grill in the same position for the entirety of the run, a total of 3 min 50 sec.

The subject took a 15 min break between runs. The second and third runs were completed using the same procedures as the first, except using different temperatures during the adaptation phase. After the participant filled out the third sensation questionnaire, he or she was debriefed and awarded credit for his or her participation. The order in which subjects underwent the three conditions was counterbalanced.

Results

Adaptation to warm and cool stimulation. Two subjects rated the cool adapting stimulus as hot during the adaptation period and their data were therefore excluded from further analysis. This left 24 archival subjects (16 female) whose data were analyzed.

For purposes of directly comparing the time courses of warm and cool adaptation, absolute values were utilized. The instantaneous ratings recorded at 15-sec intervals (i.e. T=15, T=30, etc.) were used for statistical analysis. See *Figure 3.2*.

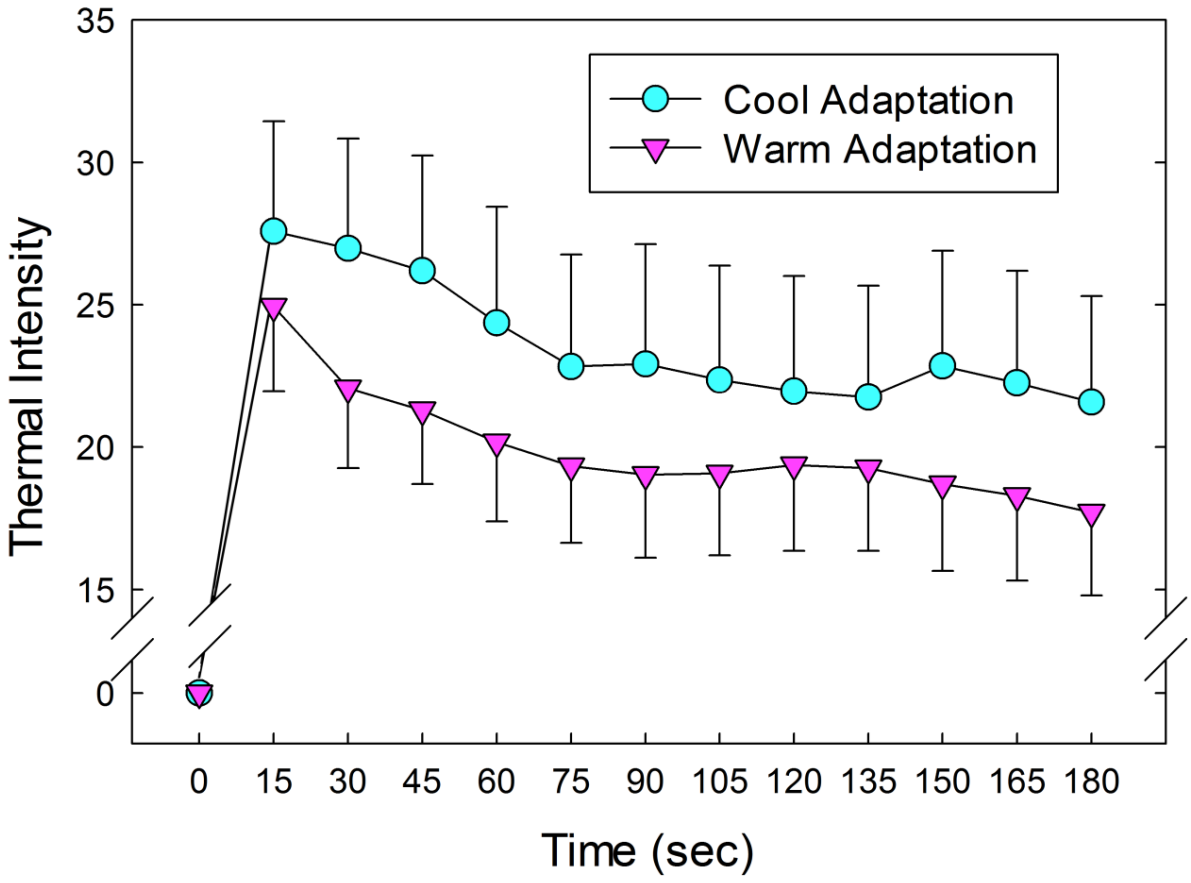

Figure 3.2 Thermal adaptation. Instantaneous thermal ratings at 15-sec intervals are shown. Data from the first time point were omitted from the statistical analysis of adaptation. Error bars represent 1 SEM.

Since ratings started at zero and generally continued to build during the first 15 sec, the T=0 time point shown in Figure 3.2 was excluded from statistical analysis to better capture the period during which adaptation occurred. A 2 x 12 repeated-measures ANOVA analyzed the main effects of *Run Type* (i.e. cool vs. warm adaptation periods) and *Time* (i.e. the 12 bins), and the interaction between these factors. The main effect of *Time* was significant [$F(11,253)= 4.12$, $p<.001$), while the main effect of *Run Type* [$F(1,23)=1.41$, $p=.25$] and the interaction between the factors [$F(11,253)=.27$, $p=.99$] were not. This result indicates that 1) significant adaptation occurred, 2) the warm and the cool stimuli were perceived to be similarly intense, and 3) the magnitude of adaptation did not differ for warm and cool.

Effects of adaptation on the pain of the TGI.

Subjects rated the painfulness of the TGI for a period of 15 sec before the end of each run. See *Figure 3.3*. Since the pain VAS was set initially at zero, most subjects spent the initial five seconds of this period adjusting the VAS to reflect their pain intensity. Therefore, ratings recorded during the final 10 sec of the 15-sec pain rating period were averaged for statistical analysis. A repeated-measures ANOVA showed that the difference in pain across the three run types was significant [$F(2,46)=7.5$, $p=.002$]. Post-hoc paired samples t-tests showed that pain was significantly lower in the cool adaptation condition compared to both the neutral adaptation condition [$t(23)=3.40$, $p=.002$] and the warm adaptation condition [$t(23)=2.64$, $p=.015$]. In contrast, there was no significant difference in pain between the warm and neutral adaptation conditions [$t(23)=.94$, $p=.36$]. Thus, the results show that cool adaptation reduced the pain of the TGI, while warm adaptation was without effect.

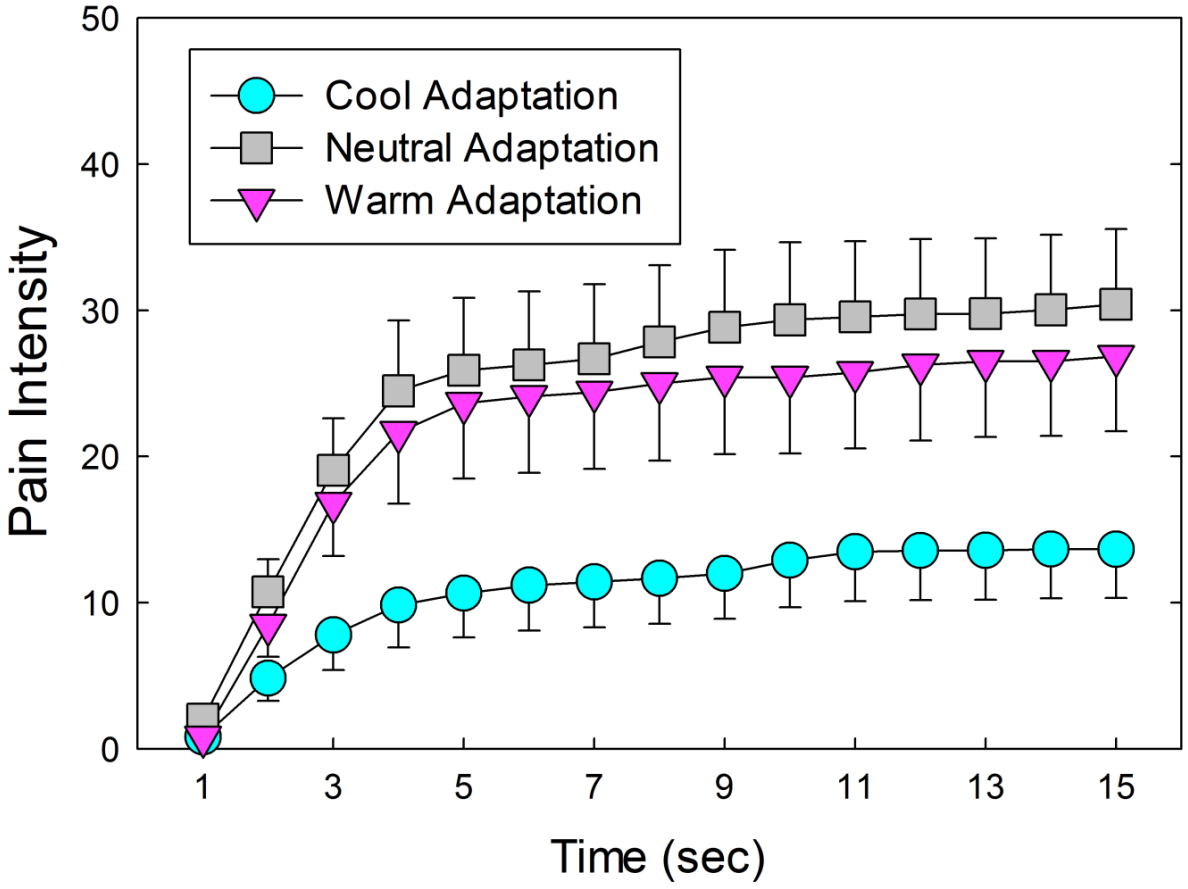

Figure 3.3 Perceived pain of the TGI. Subjects indicated pain using a VAS scale ranging from "No Pain" to "The Most Intense Pain Imaginable", which correspond to values of zero and 100, respectively. Error bars represent 1 SEM.

Sensations attributed to the TGI. In the neutral condition, 20 of the 24 subjects (83.3%) reported the TGI as feeling "hot". Ten subjects reported feeling cool and/or cold during this run, and 8 of them also reported feeling warmth and/or heat. The perception of warmth and/or heat from the thermal grill was absent in only two subjects in this condition, while the perception of cool and/or cold was absent in 14. In terms of the qualities of the TGI's painfulness, "sharp", "stinging", and "burning" were all frequently reported, while aching was seldom indicated. These four pain-related adjectives were used to describe the

grill a total of 42 times in the neutral adaptation condition, an average of 1.75 descriptors per run. See *Figure 3.4*.

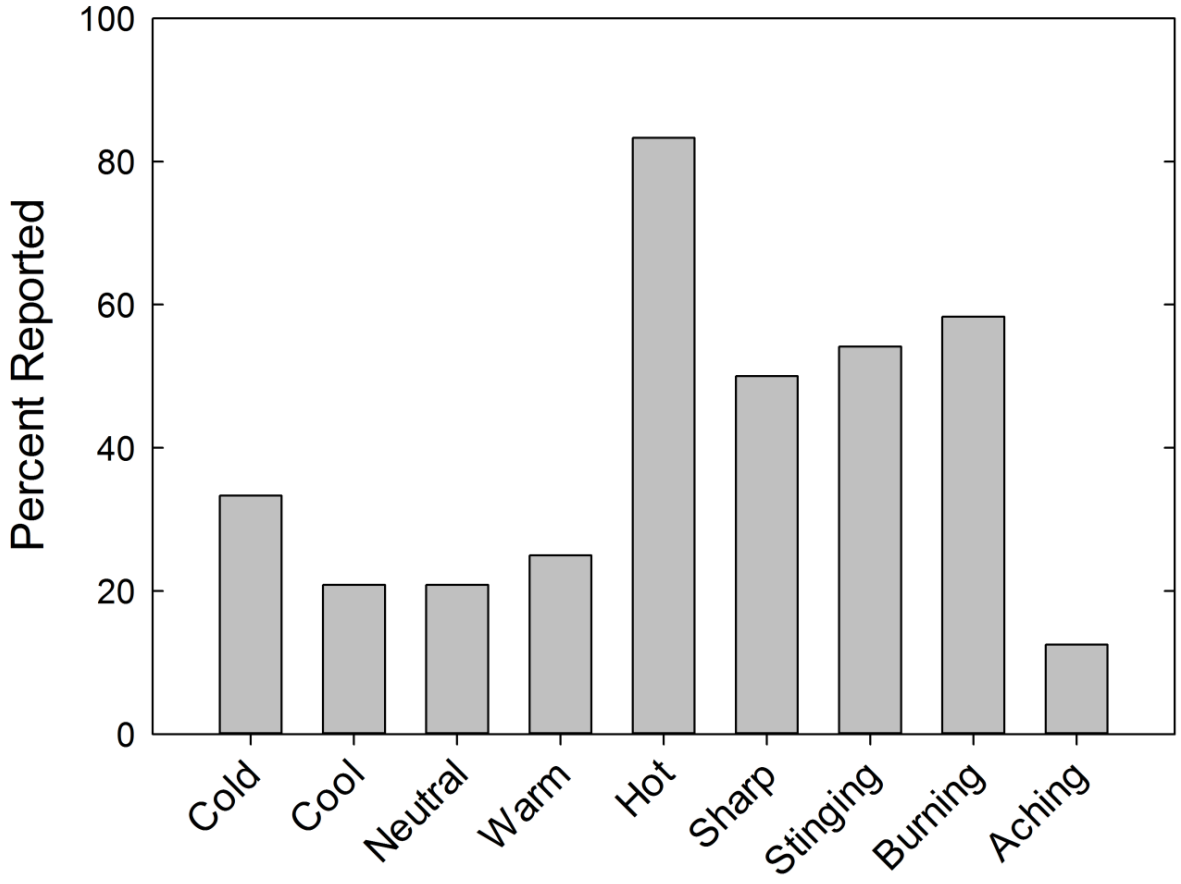

Figure 3.4 Sensations associated with the TGI following neutral adaptation.

The effects of cool and warm adaptation on the sensations associated with the TGI are shown in *Figure 3.5*. Non-parametric Wilcoxon signed ranks tests were used to determine whether adaptation changed the frequency of responses for each descriptor on the questionnaire. The neutral run was compared with the cool or warm adaptation runs in separate tests.

Cool adaptation increased the frequency of "warm" responses [Z=2.11, p=.035], while the decrease in "hot" responses approached statistical significance [Z=1.90, p=.058].

Cool adaptation significantly decreased the number of "sharp" responses [Z=2.33, p=.02] and trended towards decreasing "burning", "stinging", and "aching" [Z=1.73, p=.083, for all]. Pain-related descriptors were used a total of 20 times following cool adaptation, 0.83 times per run on average, which was significantly less frequently than they were used following neutral adaptation [Z=2.75, p=.006].

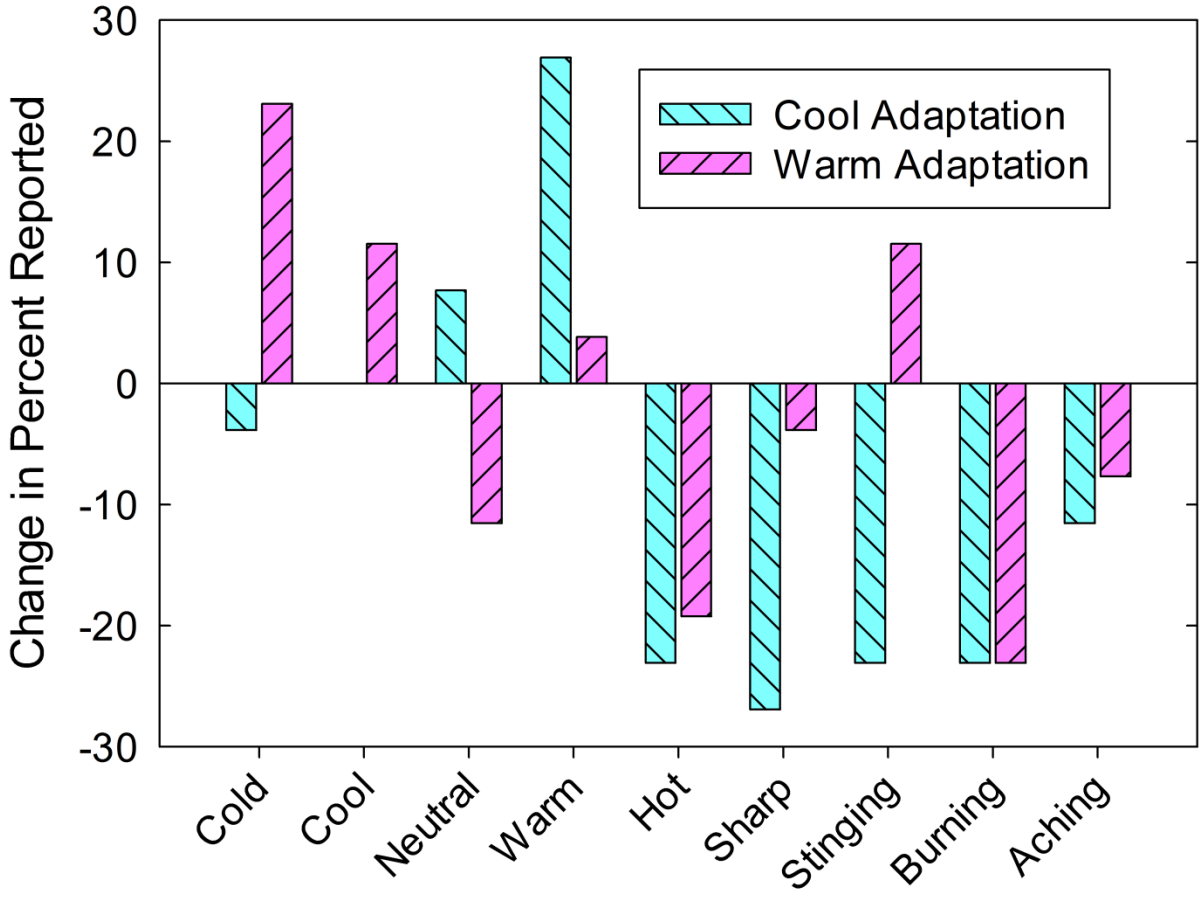

Figure 3.5 Change in reported sensations compared with the neutral run. Each bar represents the difference between the neutral run and one of the adaptation runs (i.e. Adaptation Run % - Neutral Run %).

Warm adaptation significantly increased the frequency of "cold" responses [Z=2.45, p=.014]. No other change in the frequency of reported thermal sensations occurred (p>.05 for all). Likewise, warm adaptation did not reduce the frequency of pain-related descriptors,

though the decrease in "burning" responses trended towards significance [Z=1.90, p=.058]. Pain-related descriptors were used a total of 36 times following warm adaptation, 1.5 per run, which was significantly greater than following cool adaptation [Z=2.30, p=.021] but not different from neutral adaptation [Z=0.87, p=.38]. Thus, while warm adaptation unmasked cold sensation, it was not effective in reducing the pain or the perceived heat of the TGI.

Effects of adaptation on thermal ratings of the TGI. The TGI often involves an experience of synthetic heat, meaning that the grill feels hotter than the temperature of the warm bars on their own. We therefore wished to determine whether adaptation to either warm or cool reduced the TGI's perceived thermal intensity.

Subjects rated thermal intensity of the TGI immediately following the 3-min adaptation period, using the same bi-directional VAS that was used to rate thermal sensation during adaptation. For the purposes of distinguishing between hot and cold ratings, responses on the left (cool/cold) side of the VAS were recorded as negative, and those on the right (warm/hot) side as positive. However, while a majority of subjects rated heat, some rated cold, and still others fluctuated between reporting heat and cold. Averaging raw data across subjects would therefore have clouded the interpretation of sensation intensity. To avoid this, absolute values were calculated in order to determine the overall thermal sensation intensity, without regard to whether it was heat or coldness. The absolute value of each data point (10/sec) was calculated and the values recorded during the last 10 sec of each thermal rating period were averaged. This provided a measure of sensation intensity of the TGI on a scale from no thermal sensation to the most intense thermal sensation (i.e. either hot or cold) imaginable.

The average perceived thermal intensity (without regard to warmth or coolness) during the neutral run was 48.2 (SD=25.9). Following warm and cool adaptation, average values were 41.3 (SD=21.7) and 31.9 (SD=18.0), respectively. See *Figure 3.6*. A repeated-measures ANOVA revealed a significant difference in perceived thermal intensity across conditions [F(2,46)=7.15, p=.002]. Post-hoc paired-samples t-tests showed that thermal

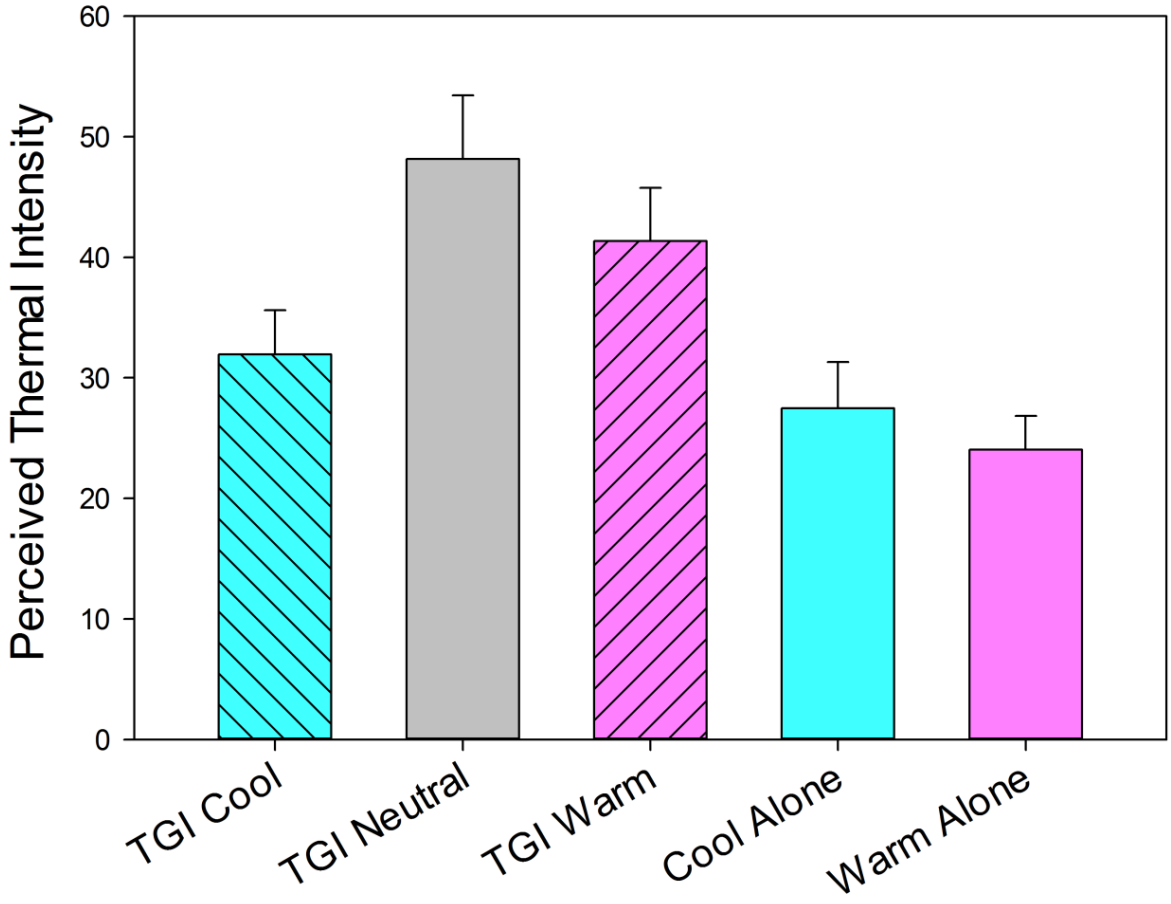

Figure 3.6 Perceived thermal intensity of the TGI and of cool and warm adaptation stimuli. Each bar represents the absolute value of thermal intensity, ranging from zero (no thermal sensation) to 100 (the most intense heat/cold imaginable). The left three bars represent average thermal ratings of the TGI across the three run types: For example, "TGI Cool" means the perceived thermal intensity of the TGI following cool adaptation. The right two bars show the average thermal rating during the beginning of thermal adaptation. Synthetic heat can be seen by comparing the Warm Alone and TGI Neutral bars. Error bars represent 1 SEM.

intensity following cool adaptation was significantly reduced compared to both the neutral [t(23)=3.4, p=.002] and warm [t(23)=2.5, p=.02] adaptation runs, while the warm and neutral adaptation runs were not significantly different from one another [t(23)=1.6, p=.13]. This pattern of results thus mirrored the alterations in painfulness of the TGI: Cool adaptation reduced not just pain but also perceived thermal intensity, while warm adaptation was without effect on either.

Analysis of individual differences in the TGI. Several studies have noted a high degree of variability in the painfulness of the TGI across subjects (Bouhassira et al., 2005; Kern et al., 2008a,b). The present study also demonstrated this: TGI painfulness ranged from 0.9 to 91.1 on our 0-100 scale in the neutral adaptation run. Craig and Bushnell (1994) suggest that innocuous cool-signaling neurons are the key to inhibiting the nociceptive activation elicited by low temperatures. We reasoned that individuals who are better able to perceive coolness or coldness during the illusion might be less susceptible to the pain of the TGI, since this would presumably reflect the presence of stronger innocuous cool signals during the illusion.

To test this idea, I calculated the number of runs in which a subject reported feeling the TGI as cool and/or cold on the sensation questionnaire. Individual subjects felt these sensations in either zero (n=6), one (n=7), two (n=7), or all three (n=4) runs. *Figure 3.7* shows perceived pain of the TGI following the different adaptation periods, broken down into these four groups. A 3 X 4 mixed-model ANOVA revealed a significant main effect of *Run Type* [F(2,40)=7.26, p=.002], indicating the effect of adapting condition. The main effect of *Group* was also significant [F(3,20)=5.75, p=.005], revealing that the more times a subject was able to feel cool and/or cold during the illusion, the less pain he or she felt from the TGI.

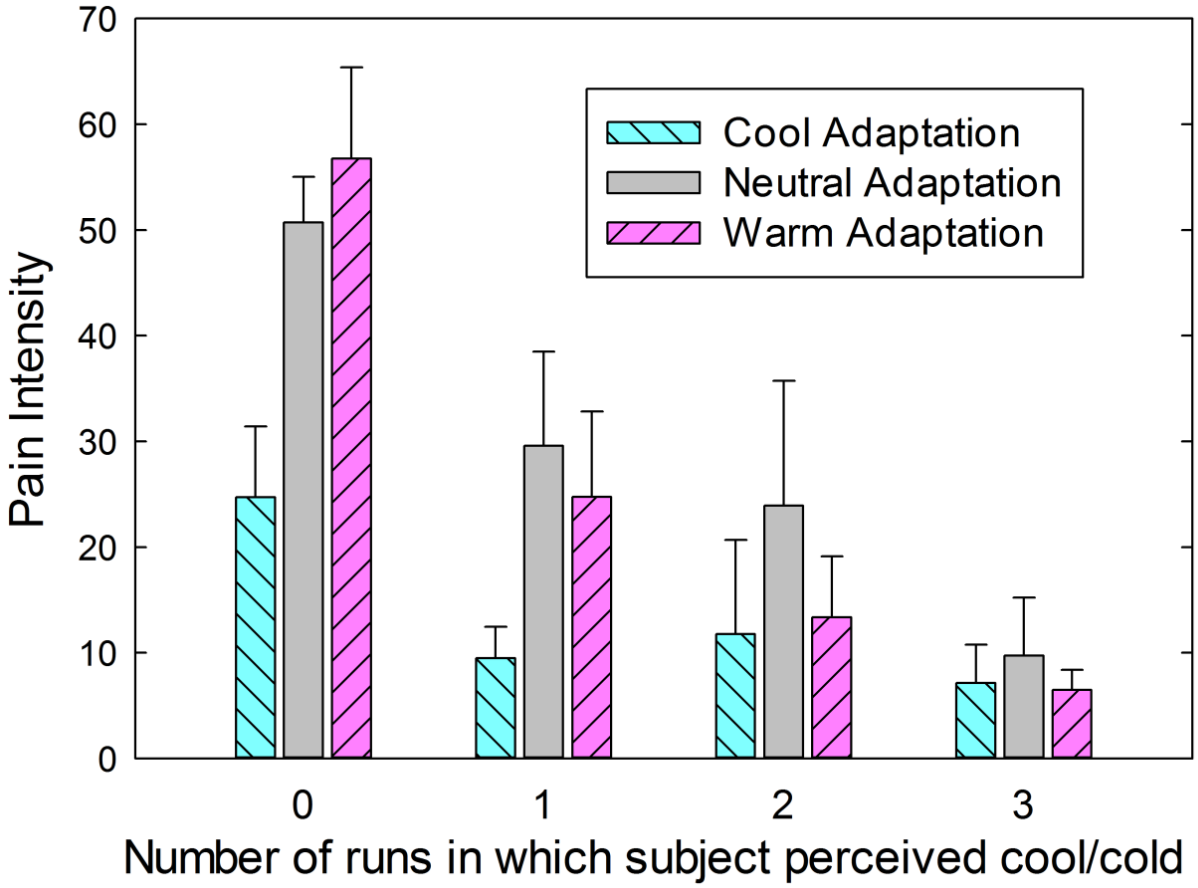

Figure 3.7 Feeling cool or cold during the TGI may protect one from its painfulness. Subjects were grouped based on the number of runs in which they reported feeling cool and/or cold on the sensation questionnaire. For example, six subjects never reported feeling the TGI to be cool or cold, while four others reported feeling either or both of these sensations during all three runs. The three bars for each group represent the average pain rating of the TGI, separated by run type. Error bars represent 1 SEM.

The interaction between these factors was not significant [$F(6,40)=1.74$, $p=.14$], suggesting no difference between groups in the ability of adaptation to alter the painfulness of the TGI.

Discussion

The warm and cool signals that contribute to the TGI were selectively reduced by adaptation to determine their relative influences on the illusion. The main finding was that cool adaptation attenuated the illusion while warm adaptation was without effect. This is at odds with theories of simple addition, which predict similar reductions in the illusion following both manipulations. The failure of warm adaptation to reduce the TGI also appears inconsistent with the disinhibition theory, although some other aspects of the results are compatible with this theory. Finally, analysis of individual differences in the data suggests that feeling coolness and/or coldness, instead of just heat, during the illusion may be an identifying characteristic of individuals who are relatively unsusceptible to its painfulness.

Addition theories of the TGI. One of the earliest theories of the TGI was based on a phenomenon called paradoxical cold, which involves the "paradoxical" activation of cold fibers by noxious heat. Alrutz (1898) reasoned that if noxious heat activates warm fibers through their normal means and cold fibers paradoxically, the perception of heat might result from dual activity in these channels. The grill's warm bars stimulate warm fibers and its cool bars activate cold fibers, producing a pattern of activity resembling that which occurs during exposure to noxious heat. According to Alrutz (1898), the simultaneous warm and cool sensations are perceptually added to one another to give the illusion of *synthetic heat*, a feeling that the object is hotter than its warm component stimulus. Synthetic heat, resulting from concurrent warm and cool stimulation, has been replicated in numerous studies (Alston, 1920; Bouhassira et al., 2005; Craig & Bushnell, 1994; Craig et al., 1996; Cutolo, 1918;

Fruhstorfer et al., 2003; Green, 2002; Gritman & Dallenbach, 1929; Kammers et al., 2010; Leung et al., 2005).

Some modern psychophysical evidence supports the idea that the TGI is an additive interaction between warm and cool. Using mild temperatures that generated non-painful synthetic heat, Green (2002) found that the perceived thermal intensity of the TGI is roughly equal to the sum of the perceived thermal intensities of the component warm and cool temperatures. Bouhassira and colleagues (2005), using a range of more extreme warm and cool temperatures, showed that additive processes might also underlie the painfulness of the TGI. They found that the painfulness of the illusion is increased if either the warm or the cool temperature is held constant while the other is made more extreme, suggesting similar contributions from warm and cool. If the TGI involves additive processes, it could be through a convergence of warm and cool signals onto wide dynamic range (WDR) neurons (Mendell, 1966), whose firing rates could summate to those that normally signal the presence of a noxious stimulus.

We found that the warm and cool stimuli used in the present study were perceived to be equally intense and that subjects adapted similarly to both. Therefore, if warm and cool signals add to one another to produce the illusion, adaptation to either of the grill's component temperatures should have resulted in a similar reduction in the TGI. Cool adaptation reduced the strength of the illusion, as predicted by the theory, but warm adaptation was without effect. The data, therefore, do not offer support for these additive theories of the illusion and suggest that the cool stimulus might have a privileged role in supplying the nociceptive qualities of the TGI.

The results also showed selective and significant reduction in perceived thermal intensity of the TGI following cool adaptation. Green's (2002) study, which showed evidence of additive processes at warm and cool temperatures that produced synthetic heat without pain, led him to conclude that the TGI might rely on different mechanisms at different temperatures. However, the present results suggest a single the mechanism for the synthetic heat and illusory pain of a painful TGI, since cool adaptation had similar effects on both. These findings do not, however, rule out the possibility that the synthetic heat associated with an innocuous TGI relies on additive mechanisms, as suggested by Green (2002), since we only tested a temperature combination intended to produce a painful TGI.

Disinhibition theory of the TGI. The theory rests on the premise that cool signals are inhibitory on pain (Craig & Bushnell, 1994). This idea is supported by other studies which have shown that selective block of A fibers, including those that transmit cool signals, leads to an absence of innocuous cool sensation and a transition in the perceived quality of cool stimuli to burning heat (Fruhstorfer et al., 1984; Wahren et al., 1989). These previous findings suggest that 1) cool temperatures are capable of stimulating some C-fiber nociceptors and 2) innocuous cool signals are normally able to inhibit these nociceptive signals, at least in the innocuous temperature range.

According to the disinhibition theory (Craig & Bushnell, 1994), the TGI is created by a selective inhibition of spinothalamic tract (STT) COOL neurons (i.e. those that transmit cool sensations) by the warm bars of the grill, which in turn unmasks the nociceptive STT signals carried in HPC neurons, which respond to noxious heat, noxious pinch, and both noxious (below 15°) and innocuous (15°-24°C) cold (Craig et al., 2001). The theory is supported by electrophysiological evidence in cats showing that, compared to a uniformly

cool grill, an alternating warm/cool grill reduces activity in COOL more than in HPC neurons (Craig & Bushnell, 1994). The warm/cool grill produced a difference in firing between the two classes (i.e. HPC minus COOL) that is comparable to that produced by a uniform 11°C stimulus, a temperature that is beyond the cold pain threshold. Furthermore, in line with these neurophysiological results, psychophysical matching results in humans showed that the TGI feels as painful as a uniform 10°C grill (Craig & Bushnell, 1994).

If the TGI is caused by higher signaling in HPC neurons than in (inhibited) COOL neurons, it would be expected that adaptation to cool would increase the intensity of the illusion. Normally, HPCs fire less than COOL neurons throughout the innocuous cool range (i.e. 29-15°C; Craig et al., 2001), which implies that a prolonged cool stimulus should adapt COOL neurons more strongly. This, in turn, should increase the TGI since it would add to the fairly specific inhibition of COOL cell firing caused by the interlaced warm stimuli.

Instead, the results of cool adaptation were the opposite of this prediction; perceived pain was robustly decreased by this manipulation. Although this result is difficult to reconcile with the disinhibition theory, the fact that cool adaptation reduced pain does suggest that the pain of the TGI is the result of activity generated by the grill's cool bars. This is compatible with Craig and Bushnell's (1994) finding that the cool bars of the grill activate HPC nociceptive neurons.

The ability of disinhibition theory (Craig & Bushnell, 1994) to capture our results is enhanced by the recent discovery of cutaneous low-threshold cool nociceptors – C fibers with lower thresholds than the traditional C-polymodal nociceptors that respond to cold. These newly discovered nociceptors have sensitivities similar to Aδ cool fibers, but project to nociceptive second order neurons (Green & Akirav, 2010; Zanotto et al., 2007). Given the

robust response of these afferents to cool temperatures (Campero et al., 2009; Campero & Bostock, 2010), Aδ cool and low-threshold C fibers might have adapted to the same degree in the present study. Equal reductions in the firing of these two populations would reduce the difference in activity between HPC and COOL neurons, and therefore reduce pain.

Since warmth has been shown to inhibit COOL neurons (Craig & Bushnell, 1994), adaptation to warmth was predicted to lift this inhibition, leading to an increased sensation of coolness and an increased ability of COOL neurons to inhibit HPC neurons. The first prediction was supported by the data: The majority of subjects rated the TGI as cool or cold following warm adaptation, suggesting that warm's influence on inhibiting COOL neurons and their resulting sensations waned. However, this adaptation failed to produce any measurable reduction in the pain of the TGI or in its thermal sensation magnitude, a result that is at odds with the disinhibition theory (Craig & Bushnell, 1994).

While speculative, it could be that a subpopulation of warm fibers contributes to the inhibition of COOL neurons but not to warm sensation, and that these fibers are not very susceptible to adaptation. Some C2 fibers in humans respond to innocuous warmth, and though nothing is known about their adaptation profiles, they are not thought to contribute to warm sensation (Campero et al., 2009). This or a similar situation might explain how adaptation to the warm bars could have reduced warm sensation without reducing pain, and would make the present findings compatible with the disinhibition theory.

Protection against the pain of the TGI. Bouhassira and colleagues (2005) found that roughly one third of the individuals they tested were insensitive to the TGI at all but the most extreme temperatures tested. In our study, we observed a similar proportion of weak responders – 8 of our 24 subjects rated the pain of the TGI below 10 out of 100. According to

the disinhibition theory (Craig & Bushnell, 1994), signals transmitted in COOL neurons (i.e. those that carry the underpinnings of cool sensation) are responsible for inhibiting the pain transmitted in HPCs. We reasoned that feeling coolness during the TGI might indicate immunity to its painfulness, since the former percept could be considered a gauge of COOL neuronal activity. We found that the more often a person reported feeling cool or cold during the TGI, the less pain he or she experienced. This result provides a further illustration of the fact that a lack of cold sensation, by selective A fiber block (Fruhstorfer et al., 1984; Wahren et al., 1989), some types of neuropathy (Craig, 2008; Ochoa & Yarnitsky, 1994), or (in the present case) the thermal grill, is associated with the induction of painful burning sensations from normally innocuous cool temperatures. In the case of the thermal grill, it could be that for some, the inhibition of cool signals by warmth is more effective than in others, leading to increased susceptibility to the TGI.

Conclusions and future directions. The results of this aim show that the TGI is more complex than a simple addition of warm and cool signals. The fact that cool adaptation reduced the painfulness of the TGI implies that the cool bars of the grill supply the nociceptive qualities of the illusion, as suggested by Craig and Bushnell (1994). On the other hand, feelings of coolness indicated immunity of some individuals to the painfulness of the TGI, in that feeling coolness during the illusion was associated with feeling less pain.

The lack of an effect of warm adaptation on the illusion is seemingly at odds with all existing theories of the TGI. While the lack of an effect could be due to dissociation between the fibers contributing to warm sensation and those inhibiting cool, I came to realize that the results might not be entirely due to adaptation. Thunberg (1896) noted that the illusion of burning heat is more robust when warmth precedes coolness. Although others have begun

the warm stimulus before the cool because of this (e.g. Craig & Bushnell, 1994), no one to date has systematically tested whether temporal effects can modulate the TGI. If they can, then 1) cool adaptation might have, in part, reduced pain because cool preceded warmth, and 2) the attenuating effects of warm adaptation might have been masked by the increase in the illusion caused when warmth precedes cool.

I discuss the results of a systematic alteration in temporal offset in the warm and cool stimuli in the following chapter. This examination is important not only to rule out the possibility of confounding in the present results, but also to test the accuracy of Thunberg's (1896) original claim.

CHAPTER 4: TEMPORAL OFFSET DOES NOT DISRUPT THE TGI

Abstract

The previous experiment showed that warm adaptation had no effect on the painfulness of the TGI, while cool adaptation significantly reduced it. However, the possibility that the results were due not to adaptation of warm and cool signals, but rather to the temporal order in which the TGI component temperatures were applied, could not be ruled out. Some researchers have noted that the TGI is enhanced when coolness is added to already-present warmth (Craig & Bushnell, 1994; Thunberg, 1896). If accurate, this could mean that the reduction in pain following cool "adaptation" was, to some degree, a product of cool preceding warmth. No study to date has systematically varied the onset times of the warm and cool component temperatures and observed its effects on the TGI. Therefore, the present study was carried out to see whether this variable has any bearing on the strength of the illusion, and in particular whether it was responsible for the alterations observed in the previous aim. The same experimental procedures were carried out as in the previous aim, with the exception of shortening the temporal offset between warm and cool to 15 sec. This period was chosen because it was long enough to allow a substantial thermal sensation to build but short enough to minimize thermal adaptation before the TGI was generated. The results show that temporal offset is not a significant factor in the TGI. The temporal-offset manipulation was without effect on all of the dependent variables examined, except for a greater tendency of subjects in the warm-first condition to report a cold sensation as part of

the TGI experience. These results show that the results of the previous aim were likely due to adaptation, and more generally show that the intensity of the TGI is not significantly affected by which stimulus comes first.

Introduction

Experiment 2 was concerned with distinguishing between the two spinal theories of the thermal grill illusion (TGI) by selectively adapting, and thus weakening, either the warm or cool signals before subjects experienced the TGI. The addition (Bouhassira et al., 2005; Green, 2002) theory predicted that adaptation to warm or cool should be equally effective in reducing the illusion. On the other hand, the disinhibition theory (Craig & Bushnell, 1994) predicted that adaptation to warm would reduce the illusion (by allowing cool neurons to once again inhibit pain), but that adaptation to cool would increase the illusion by adapting pain-inhibiting cool neurons.

However, the results of this experiment were not entirely compatible with the predictions of either theory. Cool adaptation significantly reduced the pain of the TGI, but warm adaptation did not. Since the hypotheses of both theories were found to be at odds with the results, I began to consider explanations for the effect that fall beyond the realm of these existing theories. One such explanation comes from Craig and Bushnell (1994), who began the warm stimulus first in their experiment based on the idea that the TGI is enhanced when warmth stimulates the skin before the cool stimulus is added. Their idea was based on one of Thunberg's initial impressions of the illusion:

> If you suitably time the cold thermal stimulus so that it occurs when the heat sensation is most intense and sensitive, it is as if the temperature suddenly rises and a strong scorching sensation begins – you almost expect that you will be burned at any moment. (Thunberg, 1896, pp. 492-493)

If Thunberg's personal observations of the illusion are correct, the results of the previous experiment could be due, at least in part, to the temporal offset between the warm and cool stimulation. Specifically, it might not have been that cool *adaptation* reduced the

illusion, but instead that cool stimulation (in the form of the cool adapting stimulus) preceding warm stimulation did. While temporal factors may play a role in the strength of the TGI, no one to date has formally tested it.

Several early investigations of the TGI found that when warm and cool are applied simultaneously, cool sensation predominates initially before the illusion of heat that is characteristic of the TGI is apparent (Alston, 1920; Burnett & Dallenbach, 1927; Cutolo, 1918). Thunberg (1896) found that when he applied the warm and cool simultaneously, his perception would shift back and forth between the two stimuli rather than combine them into a synthetic experience. A more recent investigation of the TGI, using a replica of Thunberg's original apparatus, also observed both initial percepts of coolness and an incomplete blending of sensations (Bach et al., 2011). Green (2002), who was able to generate a synthetic experience of heat in his experiment, chose a higher rate of temperature change for the warm (e.g. 2.0°C/s) than the cool elements (e.g. 1.5°C/s), because pilot experiments revealed an initial predominance of cool sensation when using equal rates. The initial perception of coolness with simultaneous stimulation is now thought to be due to the faster conduction velocity of cool fibers (Green, 2002). Although the initial perception of coolness during the TGI can be eliminated, it is not known whether the illusion is enhanced by doing so.

To test whether the illusion is affected by temporal offset, procedures identical to those of the second Aim will be employed, except warmth and coolness will precede one another by a period of 15 sec instead of 3 min. This amount of offset is expected to allow the apparatus to produce a robust warm or cool sensation in the initial period (i.e. before the two temperatures are simultaneously present) without incurring significant thermal adaptation.

Methods

Participants. Twenty-four undergraduate students participated in the study. Recruiting was carried out through the UNC Psychology Department's participant pool website. Age of participants ranged from 18 to 21 years (M=18.6; SD=1.0). All but two subjects were right handed. The study was approved by the University's IRB and written informed consent was obtained. Participants were compensated with research participation credit for an Introductory Psychology course.

Experimental design. The design of this experiment was identical to that of Aim 2, except what I previously called the "adaptation" phase was shortened to 15 sec. Since this was period was short enough to avoid any substantial adaptation (refer back to Figure 3.2), it will be referred to as the initial phase. Each subject participated in three separate runs. Each run consisted of a 15 sec initial phase, followed by a test phase. The test phase was identical in all three runs. During the cool initial phase half of the bars were at the cool temperature (18°C) that was later used to produce the TGI, while the interlaced bars were held at a neutral temperature (32°C). The warm initial phase used warm and neutral bars (42°C + 32°C). Following the initial period in each of these runs, the neutral bars were heated or cooled to produce the test stimulus (i.e. 18°C bars interlaced with 42°C bars). A neutral initial phase was also included as a control (i.e. 32°C during the initial period). Again, each subject's forearm remained in place throughout the run, so that the same areas of skin were stimulated during the initial and test phases of a run. The order of runs was counterbalanced across subjects. Refer back to *Figure 3.1*, in the previous chapter, for a visual depiction of bar temperatures during the two phases.

Apparatus.

***Thermal grill*.** The thermal grill apparatus was the same as that used in Aim 2. The warm (42°C) and cool (18°C) bar temperatures used in this experiment produced skin temperatures of ~41°C and ~20°C, respectively.

Pain and thermal ratings. Thermal and pain ratings were again obtained continuously using the same custom-designed visual analogue scales (VAS) that were used in the previous aim. The screen looked identical to the last experiment, except the initial thermal rating period was shortened.

Questionnaires. Subjects again completed a demographics questionnaire at the beginning of the session and a sensation questionnaire immediately following each run. The same descriptors were used in this experiment: cold, cool, neutral, warm, hot, stinging, sharp, burning, aching.

Procedure. The procedures were very similar to those of the previous experiment. Subjects underwent the same training procedure for rating sensations using the VAS and received identical instructions on how to interact with the apparatus.

After the temperatures had stabilized (and at the end of a 5-sec countdown), the subject placed his or her left volar forearm onto the grill. Pretest Initial temperatures (See *Table 4.1*) were recorded. The initial temperatures were only held constant for a period of 15 sec, to ensure a substantial thermal sensation but minimize adaptation. Pretest Final temperatures were recorded at the end of this 15-sec period. Bar temperatures gradually changed to the new set-points over the next 30 sec. The subject continued to rate thermal intensity, now of the TGI, for the next 30 sec while the temperatures of the grill stabilized at the new setting.

Following the 30-sec temperature stabilization period (i.e. 45 sec into the run), the Test Initial temperatures were recorded and the thermal VAS was replaced on the screen with the pain VAS. This VAS was activated at the end of a 5-sec countdown, and the subject rated TGI pain intensity during the final 15 sec of the run. After these ratings were obtained, the Test Final temperatures were recorded and the subject removed his or her forearm from the grill. The sensation questionnaire was then administered.

The subject took a 15 min break between runs. The second and third runs were carried out using the same procedures as the first, except using different temperatures during the initial phase. After the participant filled out the third sensation questionnaire, he or she was debriefed and awarded credit for his or her participation. The order in which subjects underwent the three conditions was counterbalanced.

		Pretest Initial	**Pretest Final**	**Test Initial**	**Test Final**	**Test Final Difference**
Cool First	Cool Set	17.9 (0.2)	17.9 (0.1)	17.9 (0.2)	18.0 (0.2)	23.8 (0.2)
	Warm Set	31.9 (0.1)	31.9 (0.1)	41.3 (0.2)	41.8 (0.1)	
Neutral First	Cool Set	32.0 (0.1)	32.0 (0.1)	18.8 (0.2)	18.2 (0.1)	23.7 (0.2)
	Warm Set	32.2 (0.2)	32.2 (0.2)	41.6 (0.3)	41.9 (0.2)	
Warm First	Cool Set	32.2 (0.2)	32.2 (0.2)	18.9 (0.3)	18.2 (0.2)	23.8 (0.3)
	Warm Set	42.1 (0.2)	42.1 (0.2)	42.1 (0.2)	42.0 (0.2)	

Table 4.1 Bar temperatures at various time points. Depicted above are the temperature of the bars recorded at the beginning and end of the pretest and test periods and the difference between the temperatures of the warm and cool bars.

Results

Effects of initial temperature on the pain of the TGI. Subjects rated the painfulness of the TGI for a period of 15 sec before the end of each run. See *Figure 4.1*.

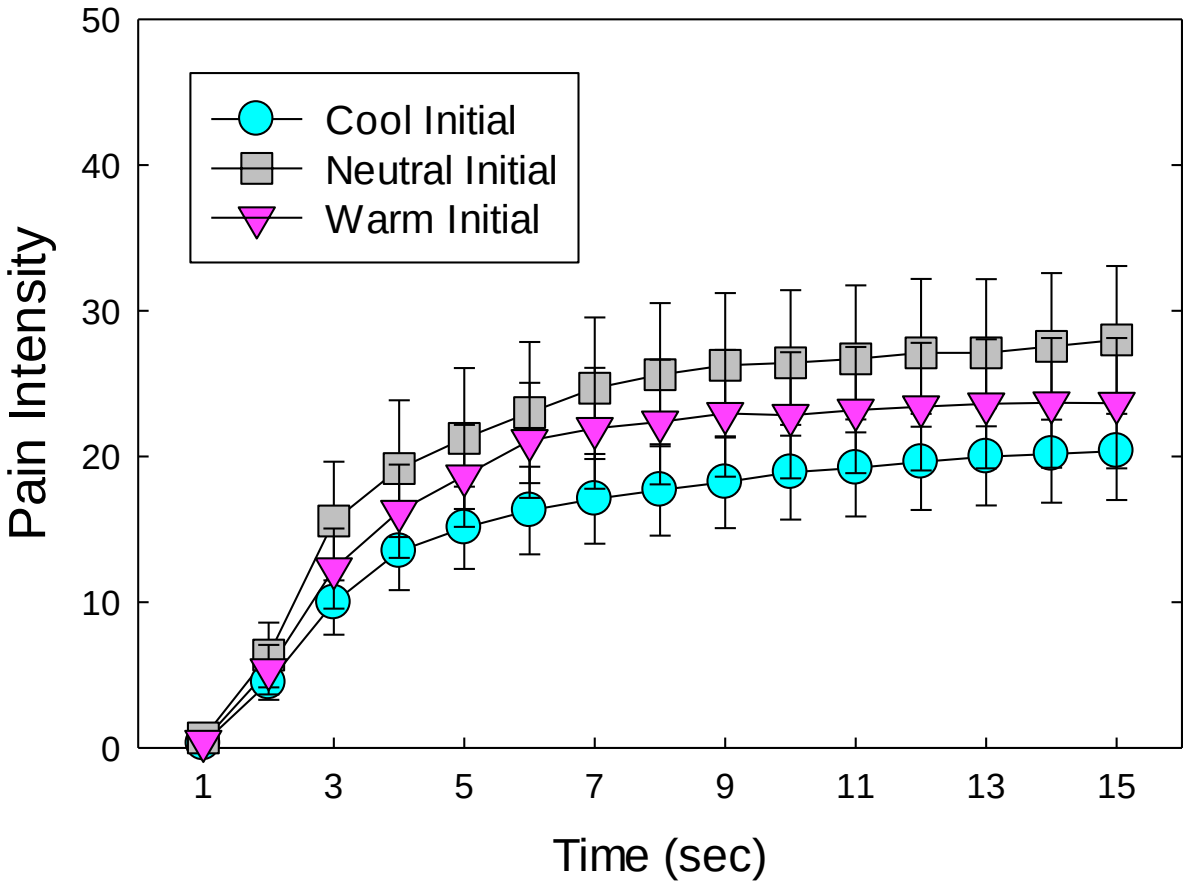

Figure 4.1 Perceived pain of the TGI. Subjects indicated pain using a VAS scale ranging from "No Pain" to "The Most Intense Pain Imaginable", which correspond to values of zero and 100, respectively. Error bars represent +/-1 SEM.

Ratings recorded during the final 10 sec of the 15-sec pain rating period were averaged for statistical analysis. A repeated-measures ANOVA showed that the difference in pain across the three run types was not significant [$F(2,46)=1.51$, $p=.23$]. Post-hoc paired samples t-tests showed that pain in the cool initial condition was no different from either the

neutral initial condition [t(23)=-1.61, p=.12] or the warm initial condition [t(23)=-.94, p=.36]. Similarly, there was no significant difference in pain between the warm and neutral adaptation conditions [t(23)=.88, p=.39]. These results show that the temporal order of warm and cool does not significantly affect the pain of the TGI.

Sensations attributed to the TGI. In the neutral initial condition, 17 of the 24 subjects (70.8%) reported the TGI as feeling "hot" on the sensation questionnaire. See *Figure 4.2*. Nine subjects reported feeling cool and/or cold during this run, and 7 of them also reported feeling warmth and/or heat. The perception of warmth and/or heat from the thermal

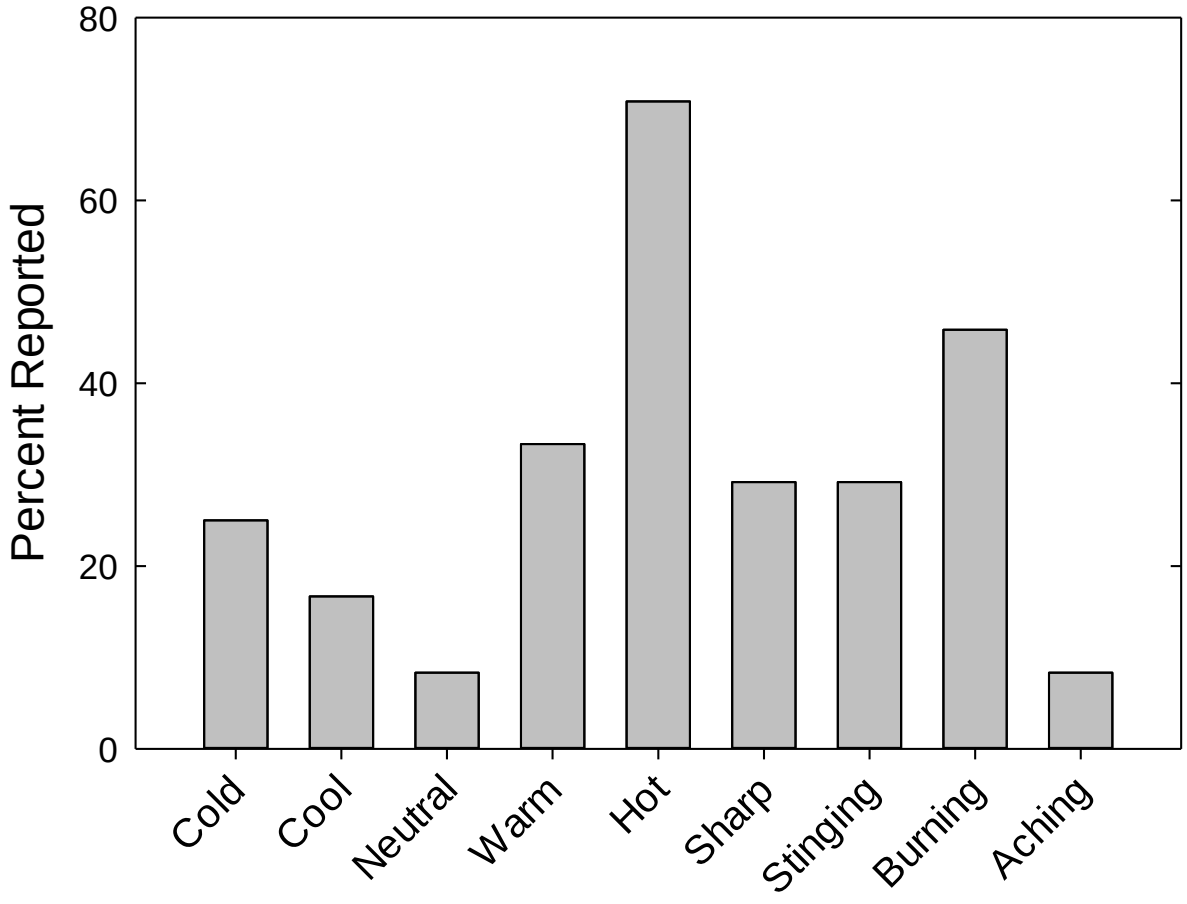

Figure 4.2 Sensations associated with the TGI in the neutral-first condition.

grill was absent in only three subjects, while the perception of cool and/or cold was absent in 15.

The perception of warmth and/or heat from the thermal grill was absent in only three subjects, while the perception of cool and/or cold was absent in 15. The TGI was often attributed pain descriptors including “sharp”, “stinging”, and “burning”, but aching was not often indicated. These pain-related adjectives were used to describe the grill a total of 27 times in the neutral adaptation condition, an average of 1.13 descriptors per run.

The effects of beginning with cool or warm on the sensations associated with the TGI were statistically tested with Non-parametric Wilcoxon signed ranks tests. See *Figure 4.3*. Separate comparisons, including Neutral vs. Warm, Neutral vs. Cool, and Warm vs. Cool, were made to determine whether any of the three conditions differed from another.

Cool being present initially failed to significantly alter the frequency of any of the descriptors compared to the neutral-first run ($p > .10$ for all), with the exception of a decrease in “Cold” approaching significance [$Z=1.89$, $p=.059$]. Pain-related descriptors were used a total of 22 times in this condition, 0.92 times per run on average, which was not significantly different compared to the number used in the neutral condition [$Z=-.99$, $p=.32$].

The initial presence of warmth failed to produce any significant changes in the frequency of descriptors used to describe the illusion compared with the neutral-first run ($p > .10$ for all). Pain-related descriptors were used a total of 30 times following warm adaptation, 1.25 per run, which was not significantly different from the cool or neutral initial conditions [$p > .10$ for both]. Comparisons between the warm first and cool first runs revealed a significant difference in the frequency of “Cold” responses [$Z=-2.4$, $p=.01$], indicating

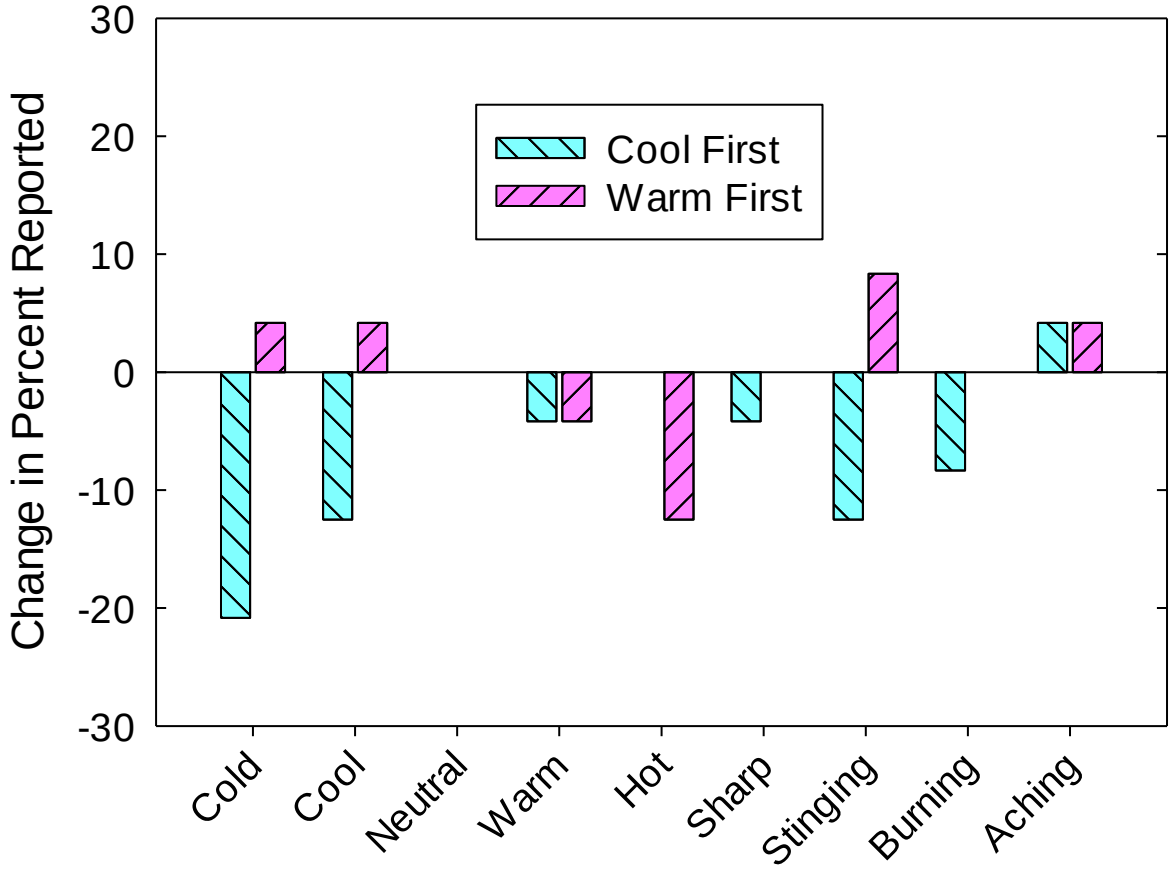

Figure 4.3 Change in reported sensations compared with the neutral-first condition. Each bar represents the difference between the neutral run and one of the adaptation runs (i.e. Adaptation Run % - Neutral Run %).

significantly fewer responses in the cool first condition. No other difference was significant.

In line with the lack of effects of temporal order on the painfulness of the TGI, the results of the sensation questionnaire also indicate that the manipulation was not an effective means of altering the illusion.

Effects of temporal order on thermal ratings of the TGI. Subjects rated thermal sensations during the 15-sec initial period and kept rating these sensations, now of the TGI,

for 30sec while the TGI temperatures stabilized. For the purposes of distinguishing between hot and cold ratings, responses on the left (cool/cold) side of the VAS were recorded as negative, and those on the right (warm/hot) side as positive. To directly compare the values from each side of the scale, absolute values were utilized. The instantaneous ratings recorded at 3-sec intervals are plotted in *Figure 4.4*.

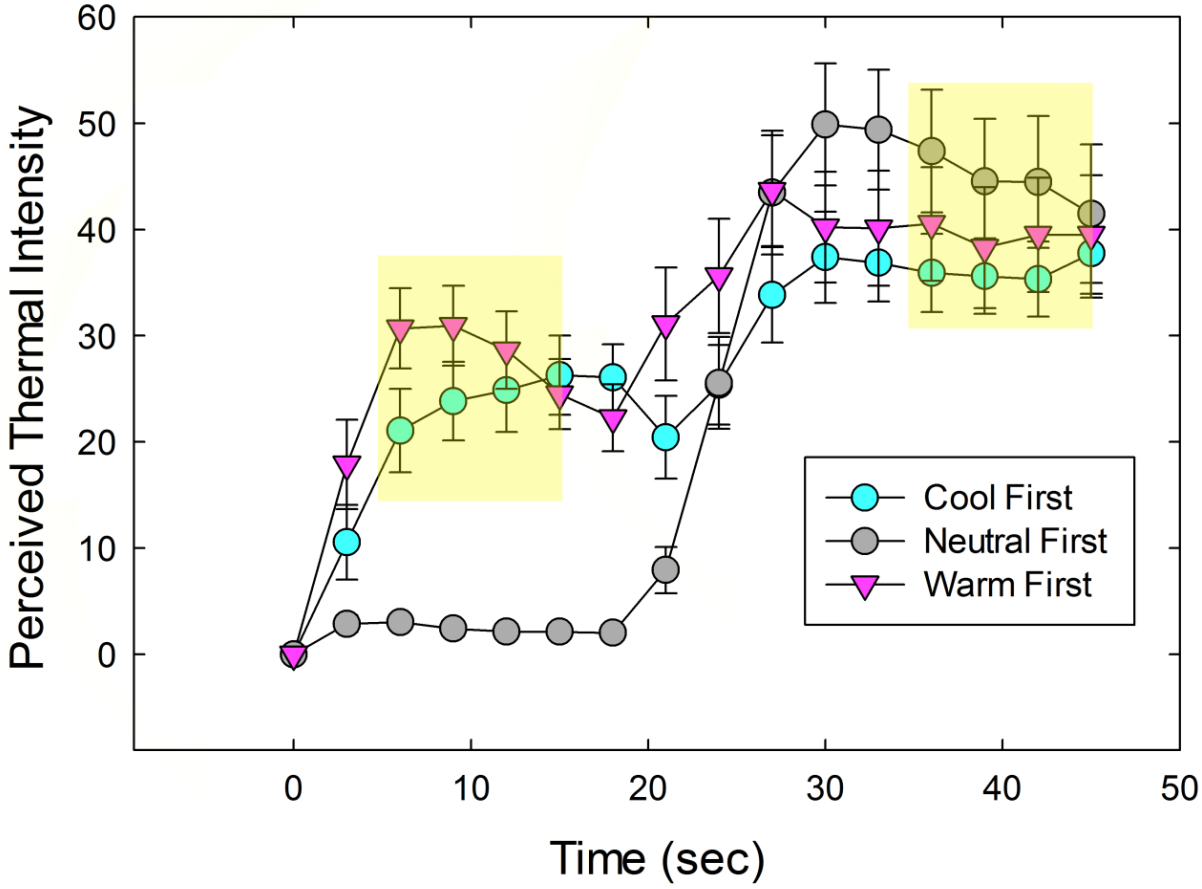

Figure 4.4 Absolute value of thermal ratings. Each point represents an instantaneous rating, recorded at 3-sec intervals. The yellow box on the left indicates the time period (5-15sec) that was averaged to determine whether perceived thermal intensity differed for the warm and cool stimulus. The yellow box on the right indicates the time period (35-45sec) that was averaged and used to test whether perceived thermal intensity of the TGI differed across conditions. Note that every rating recorded during these periods (i.e. 10/sec) was used in these analyses, not just the instantaneous points shown in the figure. Error bars represent +/- 1 SEM.

The absolute values of the responses (10/sec) recorded during the last 10 sec of the warm and cool first initial periods were averaged to determine whether subjects perceived a similar thermal sensation magnitude for the warm and cool stimuli. The period that was statistically compared is denoted in the left yellow box of *Figure 4.4*. A paired-samples t-test showed that there was no difference in perceived intensity between the warm and cool stimuli [$t(23) = -1.12$, $p=.27$].

Next, the absolute value data were analyzed to determine whether temporal offset affected the perceived thermal intensity of the TGI. The average perceived thermal intensities during the TGI phase of the neutral-first, warm-first, and cool-first runs were 45.3 (SD=29.1), 39.5 (26.0) and 35.8 (17.5), respectively. For a depiction of what values were used, refer to the right yellow box in *Figure 4.4*. A repeated-measures ANOVA showed that these values were not statistically different from one another [$F(2,46)=1.7$, $p=.19$]. Thus, this pattern of results combined with the lack of an effect of temporal offset on the painfulness and the qualitative components of the TGI suggest that the temporal order of warm and cool is not a significant contributor to the strength of the illusion.

Analysis of individual differences in the TGI. Like those of the previous experiments, the results again reflected a high degree of variability in susceptibility to the painfulness of the TGI. In the neutral initial run, TGI painfulness ranged from 0.0 to 65.2 (M=26.2, SD=24.3). Nine of the 24 subjects reported pain of less than 10 out of 100 in the neutral first run (37.5%), again suggesting that roughly 1/3 of the population may be insensitive to the painfulness of the TGI.

I conducted a variety of post-hoc tests on the present data in an attempt to determine potential characteristics that relate to one's susceptibility to the TGI. The previous aim

showed that the number of runs in which a subject reported feeling cool and/or cold was significantly related to the amount of pain he or she felt from the TGI, with a higher incidence of coolness and coldness being associated with less pain from the illusion. In the present aim, this same comparison did not reveal any significant difference across subjects. The reason for this is unclear, but perhaps the prolonged adaptation to stimuli in the previous experiment teased this factor out. In this experiment, only seven subjects reported feeling cool and or cold during two or more runs, compared to 11 in the previous aim.

Discussion

This experiment shows that the temporal order in which the warm and cool stimuli are initiated to produce the TGI does not significantly affect it. Based on this result, it rules out the possibility that cool adaptation's attenuating effect on the TGI revealed in the previous aim was due to the fact that it preceded the warm stimulus. Thus, confidence in the claim that cool adaptation reduces the painfulness of the TGI is greatly strengthened.

Temporal offset and the perceived intensity of the TGI. According to Craig and Bushnell (1994), Thunberg stated that the TGI is stronger if the warm stimulus precedes the cool stimulus, which led them to warm the warm bars of their apparatus 5 sec before cooling the cool bars. Following a 50 sec presentation of the thermal grill (40°C + 20°C), subjects indicated the amount of heat, cold, and pain on a scale from zero (i.e. not at all) to 50 (i.e. extremely). The average pain rating under these conditions, when warmth preceded cool, was 12.5. Although no other studies have systematically varied the offset of the warm and cool stimuli, others have shown comparable pain ratings with similar intensity and duration of stimulation. For example, Kern et al. (2008a) found that subjects rated the painfulness of their lab's grill approximately 24 out of 100 following a 30-sec application of preset

temperatures (~39°C + ~ 22°C) close to those Craig and Bushnell (1994) used. Also, in the first aim of this dissertation, the average pain from a 5-sec thermal grill application was 27.1 out of 100. Nevertheless, it is difficult to glean anything concrete from direct comparisons across these studies, since variations in stimulus duration, grill construction, individual differences in susceptibility to the TGI (Bouhassira et al., 2005), and numerous others factors could have produced differential results.

The present study eliminated these potential confounding factors in a within-subjects test of the effects of temporal offset. The 15-sec offset enabled substantial sensations of warmth and coolness to build before the second temperature was added, but was short enough to minimize adaptation. Multiple measures of the TGI, including pain, thermal intensity, and pain-related sensations failed to be significantly altered by the offset of the warm and cool stimuli, suggesting that their order is relatively unimportant in determining the strength of the illusion.

Temporal offset and the thermal qualities of the TGI. The idea that temporal order of warm and cool can affect the TGI has been around since the discovery of the illusion. Thunberg (1896) said that when the skin is warmed and cooled simultaneously, one first experiences coldness followed by heat. The initial appearance of cold sensation with simultaneous warm + cool stimulation has been reported in numerous experiments (Bach et al., 2011; Burnett & Dallenbach, 1927; Cutolo et al., 1918; Ferrall & Dallenbach, 1930; Green, 2002), and is thought to be due to the faster conduction velocity of the peripheral fibers that convey cool, compared to those that transmit warm, sensation (Fruhstorfer et al., 2003; Green, 2002). Whereas previous studies of the TGI relied on post-stimulus descriptions of the cold-first phenomenon, the use of a continuous VAS in the present study

confirms, in real-time, that the majority of individuals do perceive an initial period of coolness before the warmth and/or heat dominates perception, as originally suggested by Thunberg (1896). In addition, these data show that, like Thunberg (1896) and many of Jenkins' (1938a,b,c) subjects, some individuals on occasion experience an alternation of warm and cool sensation, rather than their fusion into heat.

Is the TGI hot or cold? Subject-by-subject analysis of the raw VAS data shows that temporal offset can affect whether one reports coldness or heat from the TGI. No subject ever rated the TGI on the cold side of the scale in the cool first condition, whereas 62.5% of subjects did for at least some time in the warm first condition, a significant difference. Despite this difference, thermal sensation intensity, regardless of cold or heat, was not significantly different across conditions. The lack of a difference in pain and thermal sensation intensity in conditions where subjects perceived different thermal qualities suggests that the TGI is perceived to be similarly intense whether the innocuous cool or warm thermal signals are more salient to the observer. The TGI normally includes an illusion of synthetic heat; however, at least in some conditions, the perception could be described as *synthetic cold*, a perception more extreme than the thermal sensation associated with the cool stimulus alone.

Was Thunberg's enhancement of the TGI due to stimulus offset? Thunberg's (1896) observance of a scorching sensation when cool was added to a warm sensation might not have been due to warmth preceding cool in the sense that Craig and Bushnell (1994) interpreted. The means through which Thunberg produced this effect were slightly more complicated than simply offsetting the stimuli. Thunberg's (1896) actual manipulation was to run warm water through both sets of pipes, apply the apparatus to the skin and allow the

sensation to build, and finally allow cold water to run through one set of bars. Therefore, the skin to which cold was applied was preheated with a warm temperature. The enhancement of the TGI under these conditions could have been due to a separate, but possibly related (Campero et al., 2009), thermal phenomenon called paradoxical warmth (Jenkins & Karr, 1957) or paradoxical heat (Hamalainen et al., 1982; Hansen et al., 1996; Susser et al., 1999; Yosipovitch et al., 1995)). In 1884, Goldscheider wrote that a sensation of warmth can sometimes be evoked, paradoxically, by cooling a warm spot on the skin (Green, 2004). Jenkins and Karr (1957) showed that paradoxical warmth is more likely to occur if a warm spot is warmed before it is cooled. Using a larger stimulus that covered numerous warm spots, Hamalainen et al. (1982) showed that a paradoxical thermal sensation can occur when the skin is cooled, but their subjects were more likely to report the resulting sensation as heat than warmth. Since Thunberg (1896) preheated the cool bars of his apparatus, he was likely initiating the TGI and paradoxical heat simultaneously, in effect increasing the effectiveness of the cool stimulus to elicit the perception of heat.

Limitations of the present study. The present study was designed to accomplish two main goals: 1) Determine whether temporal offset affects the TGI and 2) determine whether temporal offset might have affected the results of the previous aim of this dissertation. Because the latter goal required a protocol that was as similar as possible to that of Aim 2, the conclusions concerning the first goal are somewhat limited in scope. While I have shown that temporal offset does not affect the illusion under the conditions I employed, this experiment does not completely rule out the possibility that shorter offset times may enhance the illusion when warmth precedes cool. However, I have shown that offset could not have been responsible for the previously reported results of thermal adaptation.

Furthermore, Thunberg's original claim of an enhancement could very well have been more the result of paradoxical heat than a simple effect of offsetting warm and cool. Future studies should therefore systematically test the extent to which paradoxical heat can add to the TGI.

Conclusions. This experiment shows that temporal offset of the stimuli used to generate the TGI has little effect on the illusion. Temporal offset, while potentially important for the perceived thermal quality of the illusion, does not contribute to its perceived intensity. This experiment utilized the same procedures as those of the previous aim, in order to directly compare the results. Both experiments also had the same sample size. The lack of effect of a slight temporal offset on the painfulness or perceived thermal intensity is a clear indication that the differences observed in the previous aim are due to selective adaptation.

CHAPTER 5: GENERAL DISCUSSION

The experiments reported herein were designed to increase understanding of the TGI by putting the theories of the illusion at odds with one another. Furthermore, these experiments were intended to generate new knowledge concerning the various factors associated with the non-painful aspects of the TGI (e.g. perceived thermal intensity) and how they relate to the TGI's painful component. Taken together, these experiments confirm many previous demonstrations that the TGI is most often described as painful, burning heat, although a subset of individuals are relatively insensitive to the illusion. The results of this dissertation are at odds with the idea that the TGI is due to an additive process. Instead, they show that the cool bars of the grill generate a pain signal that is processed in spinal nociceptive neurons. Of the current theories, the results most closely support the disinhibition theory proposed by Craig and Bushnell (1994). Before discussing the more general theoretical implications of my experiments, I will first review the three experimental aims of this dissertation.

Summary of Experiments

The TGI is disrupted by descending pain inhibition. Alrutz (1898) proposed that the TGI results from a perceptual fusion of warm and cold sensations. In modern terms, this cognitive addition theory therefore implies that synthetic heat of the TGI results from a high-level summation, presumably in the cortex, of the output of innocuous WARM and COOL channels in the spinal cord. Conditioned pain modulation (CPM) was used as a tool to test the accuracy of this idea, since CPM's descending inhibition selectively attenuates

nociceptive signals at the level of the spinal cord (Heinricher et al., 2009; Le Bars et al., 1979a,b; Leith et al., 2010; Waters & Lumb, 1997).

CPM significantly and similarly attenuated the perception of noxious heat and the TGI, suggesting that the painfulness, unpleasantness, and synthetic heat of the illusion are carried in spinal nociceptive neurons. CPM engages DNIC, a descending inhibitory mechanism (Le Bars et al., 1979a), but it can also involve supraspinal inhibition of pain (Sprenger et al., 2011). Since a fraction of CPM's pain-inhibitory effects are likely mediated by structures in the brain (e.g. ACC), CPM could be expected to attenuate the TGI to some degree even if the integration into the nociceptive system occurs at a high level. However, the equivalent effects of CPM on both types of pain provide strong evidence that the TGI's ascending signals are attenuated by CPM at *all* levels of inhibition, including the spinal cord. The cognitive addition theory is therefore at odds with this result.

To be fair to Alrutz (1898), his cognitive addition theory was proposed to explain the perception of heat, not pain. However, the effect of CPM on the thermal reports of the TGI also presents problems for the idea that its synthetic heat results from a perceptual addition of warm and cool sensations. In the baseline condition (i.e. no CPM), the majority of subjects reported that the TGI and noxious heat felt "hot" on the sensation questionnaire. Under the influence of CPM, the frequency of "hot" responses significantly decreased for both grill stimuli, while the frequency of "warm" responses significantly increased and became the majority response. CPM's attenuation of the perceived heat implies that the synthetic heat of the TGI is also (i.e. in addition to its pain) carried in nociceptive neurons at the level of the spinal cord. Thus, the results of Aim 1 are at odds with the notion that any aspect of the TGI is a product of cognitive addition of innocuous signals.

While this study ruled out the possibility of a cognitive addition, the TGI's constituent signals still might add to one another at the spinal level, as suggested by Green (2002) and Bouhassira et al. (2005). Alternatively, the illusion could result from a stimulation of peripheral nociceptors whose activity is unmasked centrally during simultaneous warm and cool stimulation (Craig & Bushnell, 1994). These two ideas are compatible with the results of Experiment 1 since they both suggest a low-level integration into the nociceptive system. Experiment 2 was therefore carried out to distinguish between them.

The TGI is disrupted by cool adaptation. In Experiment 2, the contributions of warm and cool signals to the TGI were determined by selectively adapting them before application of the canonical thermal grill. In addition to ratings of the TGI's painfulness and its elicited sensations, this experiment employed a computerized VAS that enabled continuous recording of pain and thermal sensation ratings. The main result was that cool adaptation attenuated the pain of the TGI, while warm adaptation was without effect. The selective effects of cool adaptation also extended to the TGI's perceived thermal intensity. Much like the effects of CPM in Experiment 1, cool adaptation shifted responses on the sensation questionnaire from "hot" to "warm". In addition, the VAS magnitude estimates of thermal sensation collected in this experiment showed a significant reduction by cool adaptation. The parallel reductions in pain and perceived thermal intensity of the TGI suggest that these two aspects may rely on the same underlying mechanism, an idea that will be discussed further in the general discussion.

The differential effect of cool adaptation observed in Experiment 2 is at odds with both the cognitive and spinal addition theories, and instead shows that the illusion is more

complex than a simple addition of warm and cool signals. Specifically, the present results imply that the pain and synthetic heat of the TGI are elicited by signals transduced from the grill's cool bars, a result that is entirely in line with Craig and Bushnell's (1994) disinhibition theory. Further support for the disinhibition theory came from finding that, in general, the more often a subject felt the cool stimulus during the illusion, the less pain he or she felt from it. COOL neurons are inhibited during the TGI (Craig & Bushnell, 1994), which probably explains why most individuals are unaware of the presence of the cool stimulus when feeling the illusion. The greater ability of some subjects to perceive coolness or coldness during the TGI likely reflects a higher integrity of their COOL signals under warm + cool conditions. Since the disinhibition theory suggests that COOL signals also inhibit pain (Craig & Bushnell, 1994) intact cool sensation would be predicted to coincide with reduced pain during the TGI.

Although the results of cool adaptation can be reconciled with the disinhibition theory (Craig & Bushnell, 1994), the results of warm adaptation are at odds with it. Since the disinhibition theory states that warmth inhibits the firing of pain-inhibitory COOL neurons, adaptation to warmth was expected to reduce this inhibition on COOL neurons and decrease pain. The perception of warmth significantly adapted, as indicated by the continuous thermal VAS ratings, but the TGI remained robust. To reconcile this unexpected result with the disinhibition theory, one could postulate that there are separate populations of peripheral warmth-activated afferents that project to different spinal cord populations, and that these populations are more or less susceptible to thermal adaptation. Specifically, it might be that the afferents conveying warm sensation adapt readily (explaining the observed adaptation) but those that inhibit COOL neurons are relatively unadaptable (explaining the lack of an

effect of warm adaptation on the TGI). There are multiple populations of warmth-responsive peripheral afferents in humans (Campero et al., 2009). However, this explanation seems unlikely considering that warm adaptation did significantly increase the frequency of "cold" descriptions of the TGI, an effect that is suggestive of a release of inhibition on COOL neurons.

Another possible explanation is that the effects were due to a combination of adaptation and another phenomenon. Craig and Bushnell (1994) stated that the illusion is stronger when warmth precedes coolness; if accurate, this could have counteracted the pain-relieving effects of warm adaptation and led to an insignificant net effect on TGI pain. Furthermore, if the TGI is less amenable to situations in which coolness precedes warmth, the large effect of cool adaptation on pain could have been due, at least in part, to this temporal factor. Experiment 3 was therefore carried out to rule out this possible confounding factor in the present aim and to more generally test whether temporal order of the warm and cool matters for the perception of the TGI.

The TGI is not affected by temporal order of warm and cool. Experiment 3 tested the effects of a slight temporal offset (15 sec) between the warm and cool stimuli on the resulting perception of the TGI. With the exception of the shorter pre-TGI duration, the procedures were similar to those of Experiment 2. The results showed that the pain and perceived thermal intensity of the TGI were not affected by the temporal precedence of either constituent temperature. Thus, the difference in the temporal order of the stimuli in the previous experiment cannot explain those results. More generally, this experiment shows that while the TGI might be enhanced by preheating the grill's cool bars, as suggested by

Thunberg (1896), the illusion is not enhanced when the temperature of the warm bars is changed first, as suggested by Craig and Bushnell (1994).

Taken together, the results of these three experiments suggest that Craig and Bushnell's (1994) disinhibition theory is the most likely candidate for explaining the TGI. The present experiments have uncovered interesting effects that have numerous theoretical implications, not just for the TGI, but also for the perception of thermal stimuli under normal conditions.

General Theoretical Discussion

The TGI as an illusion of heat vs. pain. Pain was seldom mentioned in the early TGI literature. While the TGI was discovered by Thunberg (1896), Alrutz provided its first theory, and more generally a theory of heat. The paradoxical cold phenomenon meant that hot temperatures generate activity at both warm and cold spots, and thus Alrutz (1898) reasoned that the synthetic heat of the TGI could be due to simultaneous activity in these channels. The Alrutz theory found support from other labs (Alston, 1920; Burnett & Dallenbach, 1927, 1928; Cutolo, 1918; Ferrall & Dallenbach, 1930; Gritman & Dallenbach, 1929; Lowenstein & Dallenbach, 1930) and was generally accepted (Boring, 1942) until the work of Jenkins (1938d), who showed that the perception of heat can arise from places on the skin that are devoid of cold sensation. Because of this, Jenkins (1938d) argued, cold sensation is not a necessary precursor of the sensation of heat. It was also evident that some subjects report heat at temperatures lower than the threshold for eliciting paradoxical cold (Lowenstein & Dallenbach, 1930).

Following the dismantling of the Alrutz (1898) theory of heat, the TGI lost its status as an important experimental device for decades. Craig and Bushnell (1994) revived

experimental interest in the illusion by completely reframing it. The TGI was no longer an illusion of heat; it became an illusion of pain. The majority of the current literature has treated it as such, but there has been some resistance (Bach et al., 2011; Fruhstorfer et al., 2003). The present research does show, as some previous studies have (Averbeck et al., 2012; Bouhassira et al., 2005; Kern et al., 2008a,b), that pain is not a part of the TGI experience for all individuals, even when extreme innocuous temperatures are used. Green (2002), who observed results that were not in line with Craig and Bushnell's (1994) theory, suggested that there might be separate mechanisms for the TGI's synthetic heat (additive) and its pain (disinhibitory).

The results of this dissertation shed some light on the debate over whether the TGI is more appropriately regarded as an illusion of heat or pain and also suggest that the mechanism behind the two effects is likely the same.

First, the present studies show that the majority of individuals (~2/3) indicate non-negligible pain (> 10/100) from simultaneous warm and cool stimulation, at temperatures close to those utilized by Craig and Bushnell (1994). Fruhstorfer et al. (2003) took issue with Craig and Bushnell's (1994) instructions to subjects, which defined pain as "any uncomfortable sensation… even if the stimulus is tolerable" (p 254) and their administration of the McGill Pain Questionnaire (Melzack, 1975), as these factors might have biased subjects toward reporting pain. In the present studies, while subjects were often asked to report pain, they 1) were instructed that the stimuli may or may not be painful and 2) were also asked, in many cases, to rate non-painful aspects of the stimulation (e.g. heat or coldness). A bias toward reporting pain could have potentially caused some individuals to give low pain ratings to stimuli that were actually just unpleasant, but this could not explain

the response of individuals who reported substantial pain (e.g. 91/100) from the TGI. Furthermore, the cold pressor and cool adaptation caused some subjects to report negligible pain from the TGI, while they reported the TGI as substantially painful in the control run. It is unclear why subjects would have reported pain from the TGI in one condition but not in another due to a general bias created by the procedures. For these reasons, it is evident that the TGI involves sensations of pain in most subjects, at least when the warm and cool stimuli are moderately close to, but less extreme than, thermal pain thresholds.

Multiple lines of evidence from the present studies suggest that the TGI's synthetic heat and illusory pain might rely on the same mechanism. First, there were significant correlations between the TGI's painfulness and its perceived thermal intensity in the control runs of the second [$r = .55$, $p = .005$] and third [$r = .62$, $p < .001$] experiments. Thus, subjects who perceived a great deal of pain from the TGI also perceived it to be more intense thermally. Second, in Experiment 2, the amount that cool adaptation reduced pain was highly correlated with the amount of reduction in perceived thermal intensity [$r = .71$, $p < .001$], illustrating that the manipulation had a similar effect on the two variables for a given subject. See *Figure 5.1*. This dissertation's novel use of both rating scales in the same subjects has thus revealed that the synthetic thermal intensity and the pain of the TGI are highly related.

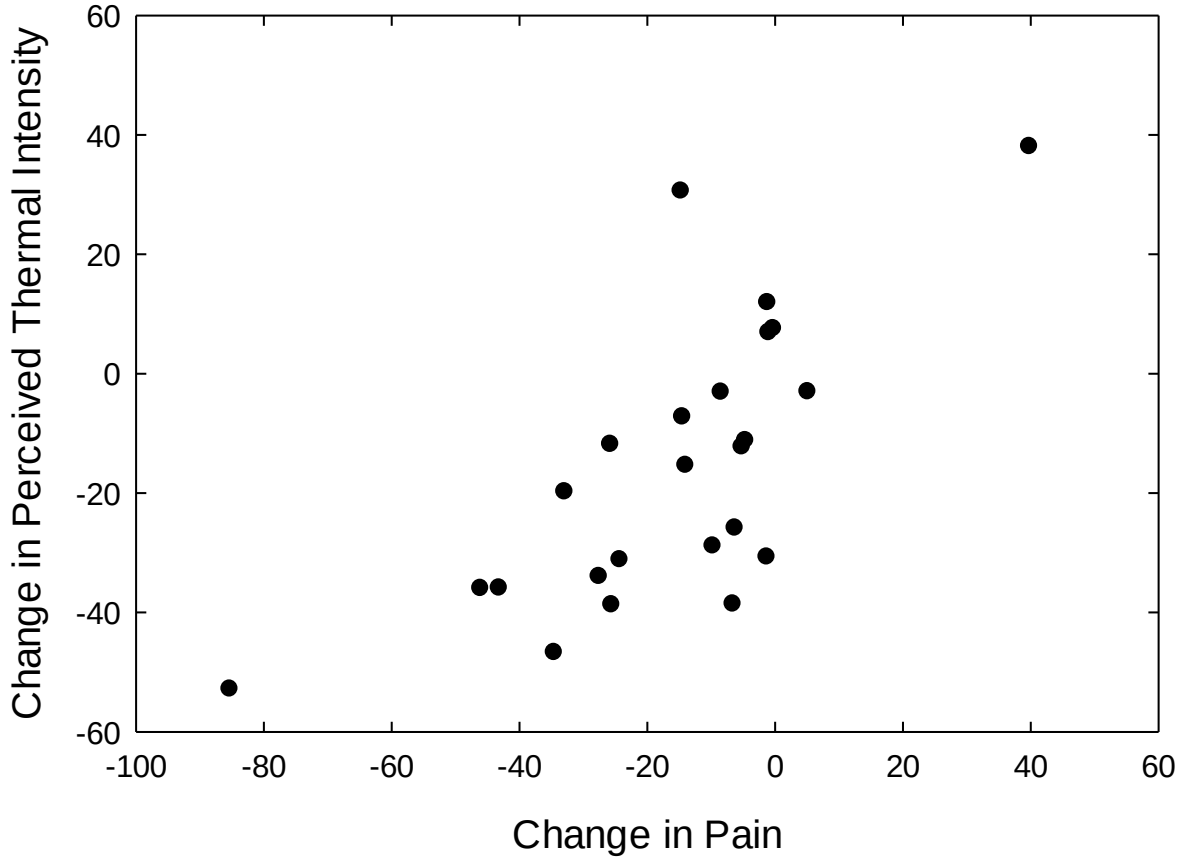

Figure 5.1 Change in pain and thermal intensity following cool adaptation. Data were calculated by subtracting ratings in the neutral adaptation run from corresponding ratings in the cool adaptation run; therefore, negative values indicate a decrease due to cool adaptation.

Although VAS data on the TGI's perceived thermal intensity were not collected in Experiment 1, a final piece of evidence from that experiment adds to the argument that the mechanism of pain and synthetic heat is shared. Subjects did report thermal descriptions of the TGI following each run on the sensation questionnaire; as reported in Chapter 2, CPM significantly decreased the frequency of "hot" responses and increased "warm" responses. In Experiment 2, cool adaptation had a similar effect on the sensation questionnaire. See *Figure*

5.2. This indicates that the manipulations that reduced the pain of the TGI, CPM and cool adaptation, also reduced its perceived thermal intensity, while those that were without effect on pain (e.g. warm adaptation) were also without effect on thermal intensity.

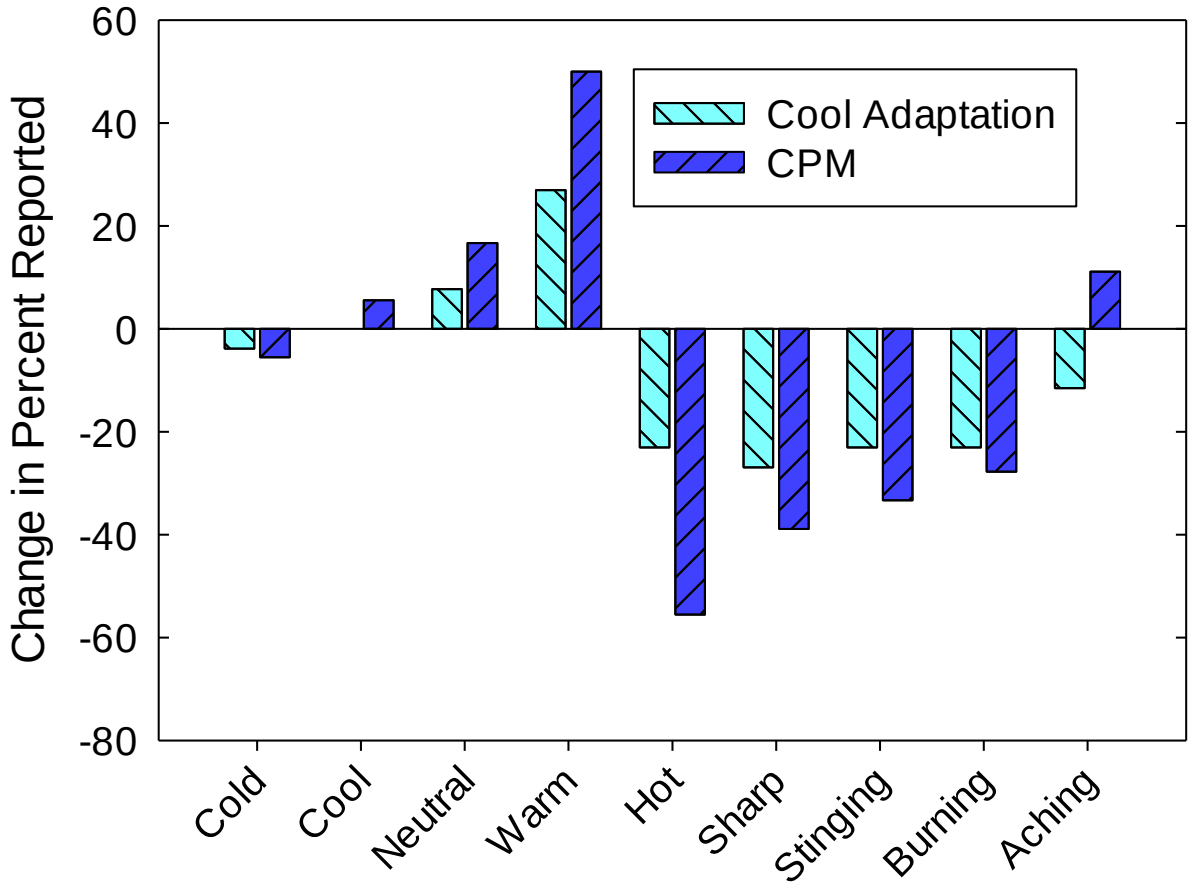

Figure 5.2 Effects of cool adaptation and CPM on sensations associated with the TGI. The data in the light blue bar compare the neutral and Cool Adaptation runs in Aim 2, while the dark blue bars compare the Control and CPM runs in Aim 1. Each bar represents the difference between the experimental run denoted and the neutral/ baseline run (i.e. Adaptation Run % - Neutral Run %).

In summary, each experiment presented in this dissertation provides some evidence that the synthetic heat of the TGI and its pain are related.

The underlying physiology of the TGI. Craig and Bushnell (1994) suggested that C-polymodal nociceptors, whose mean threshold is ~24°C, supply the pain of the TGI. However, Green (2002) obtained synthetic heat by pairing mild cool temperatures (e.g. 29°C) with warmth, indicating that this aspect of the TGI does not require activation of C-polymodal nociceptors. Based on this incompatibility with the disinhibition theory, Green (2002) originally proposed that there may be different mechanisms for the synthetic heat and illusory pain of the TGI.

HPC dorsal horn neurons, which Craig and Bushnell (1994) theorized to be the central targets of C polymodal afferents, have a mean threshold of ~25°C (Craig et al., 2001). However, 40% of the HPC neurons Craig and colleagues (2001) studied fired, to some degree, at one of their least extreme temperatures tested 28.7°C. Therefore, their activity could explain the illusion of synthetic heat at mild temperatures (Green, 2002). Furthermore, peripheral C fibers (called C2) have been discovered in the skin of human subjects that respond to mild cooling (Campero et al., 2009). Although the central connections of these C2 fibers are unknown, they may very well project to HPC or WDR neurons.

Since HPCs respond at least minimally at mild cool temperatures and increase their responses throughout the range of innocuous and noxious cold (Craig et al., 2001), they could lead to an experience of synthetic heat at mild temperatures and synthetic heat with pain at more extreme temperatures. The present data support this possibility, in that the manipulations that reduced the painfulness of the TGI also reduced its synthetic heat. In the case of CPM, it might have reduced the firing rates of HPC neurons, making the both the pain and the synthetic heat of the TGI less extreme. CPM is known to selectively attenuate nociceptive, but not innocuous signals, which provides strong evidence that the nociceptive

signals contribute to synthetic heat. If nociceptive signals do not contribute to the perception of heat, CPM would not have changed the predominant response from "hot" to "warm".

The fact that cool, but not warm, adaptation reduced the painfulness and synthetic heat of the TGI is in line with a role for HPC neurons in coding both aspects of the illusion. HPCs respond to the grill's cool temperature (20°C), but their mean threshold for heat (45°C) is well above the warm temperature (41°C) I used (Craig et al., 2001). Assuming that the painfulness and synthetic heat of the TGI are carried by HPCs, prolonged exposure to the cool stimulus should have reduced pain (by reducing the responsiveness of HPCs) but prolonged exposure to warmth should not have, which was exactly the effect I obtained.

Thus, the parallel alterations in the synthetic heat and the pain of the TGI during CPM and following cool adaptation were probably due to a decrease in ascending nociceptive drive following each manipulation. With a weaker pain signal reaching consciousness, the temperature felt less extreme. That nociceptive afferents contribute to perceived thermal intensity is apparent when one considers the responsiveness of innocuous thermosensory STT neurons: the responses of COOL and WARM neurons plateau at temperatures that are normally considered to be innocuous (15°C and 38°C, respectively). When one experiences a thermal stimulus that cools or heats the skin beyond pain threshold, the perceived thermal intensity does not plateau. Instead, the object seems to get colder or hotter as skin temperature progressively gets more extreme and elicits larger responses in nociceptors. Since innocuous thermal signals do not discriminate temperatures that are above pain threshold, nociceptive signals are the only ones that can.

Based on the idea that nociceptive signals contribute to perceived thermal intensity, it follows that a small amount of nociceptive activity (from the grill's cool bars) paired with

activity in WARM neurons (from the grill's warm bars) would heighten the perceived thermal intensity of the warm stimulus. Knight (1918) showed that pairing mechanical pain (pin prick) with a warm stimulus elicited responses of burning, painful heat. Integration of WARM and nociceptive signals is the most likely explanation for synthetic heat. In the case of the TGI, the nociceptive signals are provided by the grill's cool bars rather than a mechanical pain stimulus. As the nociceptive signals are increased, the perceived thermal intensity increases, and with enough nociceptive activity the person perceives pain.

Alrutz (1896) believed that the cool sensation elicited by the cool bars of the grill was responsible with fusing with warm sensation to form "heat". Based on current knowledge, it appears that he was not far off in his assessment. However, instead of cool sensations, which we now know to be inhibited by warm signals (Craig & Bushnell, 1994), the fusion is more appropriately regarded as one between nociceptive and warm signals. This presumably explains why Jenkins (1938d) was able to obtain perceptions of heat in spots that were devoid of cold receptors – they were likely spots containing both warm and pain receptors.

Do thermal signals contribute to pain? As discussed in the previous section, nociceptive signals likely contribute to the perceived magnitude of thermal stimuli. It is also reasonable to ask what effect, if any, innocuous thermal signals have on pain. The spinal disinhibition theory holds that "burning" pain, whether elicited by the TGI, noxious cold, or noxious heat, is solely due to HPC activity (Craig, 2004; Craig & Bushnell, 1994). With the exception of NS cells, which are only active during noxious heat, the majority of pain receptors (e.g. C-polymodals) and dorsal horn projection neurons (i.e. HPCs and WDRs) respond to a variety of peripheral inputs (Campero et al., 2009; Craig et al., 2001; Khasabov et al., 2001). However, there is something profoundly different in the *qualitative* percept of a

heat pain stimulus and a pressure pain stimulus, for example. Since the response in HPCs and WDRs is inherently ambiguous (i.e. they both respond to pressure, heat, and cold), there must be other signals the brain uses to determine the quality of the pain. It seems likely that pressure pain may be colored by the innocuous tactile signals associated with noxious pressure stimulation and, as this dissertation suggests, that innocuous thermal signals contribute to the quality of thermal pain.

The existing evidence is as follows. Ochoa and Torebjörk (1989) found that intraneural microstimulation of C polymodal nociceptors in humans sometimes results in sensations of burning and other times stinging, showing that this population of pain fibers can convey multiple qualities of pain. There was no discernable rule for whether a fiber conveyed one or the other sensation, and the variability in pain quality might have been due to the lack of concurrent innocuous signals. In the case of thermal pain, evidence from areas devoid of thermal sensation suggests that innocuous thermal signals do play a role in coding pain quality. Defrin et al. (2002) studied patients with neuropathy; some of them had intact warm sensation without cool sensation, while others had the opposite. The patients with no warm sensation did not perceive heating of the skin until pain threshold was reached, and they perceived painful heat to be painfully cold. Those without cold sensation, instead, perceived painfully cold stimuli as painfully hot. In patients with no intact innocuous thermal sensibility, noxious heat and cold elicited pricking sensations without any perceived thermal component. These results of Defrin and colleagues (2002) strongly suggest that the pain quality of noxious temperatures is coded by innocuous thermosensory populations.

Experiment 2 adds to the idea that innocuous thermal signals influence the quality of painful sensations in healthy subjects. Warm adaptation had no effect on the intensity of pain

of the TGI and was also without significant effect on the total number of pain-related descriptors given on the sensation questionnaire. However, warm adaptation significantly increased the frequency of "cold" responses, suggesting an unmasking of cold sensation. Although warm adaptation did not significantly affect any of the pain-related descriptors when viewed individually, it tended to reduce the number of "burning" responses and increase the number of "stinging" responses. To evaluate this shift, Wilcoxon signed-ranks tests were used. These showed that following neutral adaptation, there was no difference between the number of "burning" and "stinging" responses [$Z = .30$, $p = .76$], nor was there following cool adaptation [$Z = .33$, $p = .74$]. However, after adaptation to the warm stimulus, there were significantly more stinging than burning responses [$Z = 2.31$, $p = .02$]. See *Figure 5.3*. This implies, like the results of Defrin et al. (2002), that the burning quality of a painfully hot stimulus is carried in warm fibers. If they are damaged or adapted, the pain is more likely to have a stinging or pricking quality.

The theoretical implications of this dissertation can be summarized as follows. Craig and Bushnell were generally correct. The pain of the TGI is generated by an inhibition of COOL neurons by the grill's warm bars, which disinhibits nociceptive (e.g. HPC) neurons. This dissertation adds to this idea in suggesting that a disinhibition of nociceptive signals and their pairing with WARM signals can also explain the synthetic heat of the illusion. See *Figure 5.4*. The first aim confirmed that the nociceptive signals are present at the level of the spinal cord, while the second and third aims yielded evidence that they are generated by the grill's cool bars. These nociceptive signals in the spinal cord probably result from signals in peripheral C afferents that are sensitive to low temperature (Campero & Bostock, 2010;

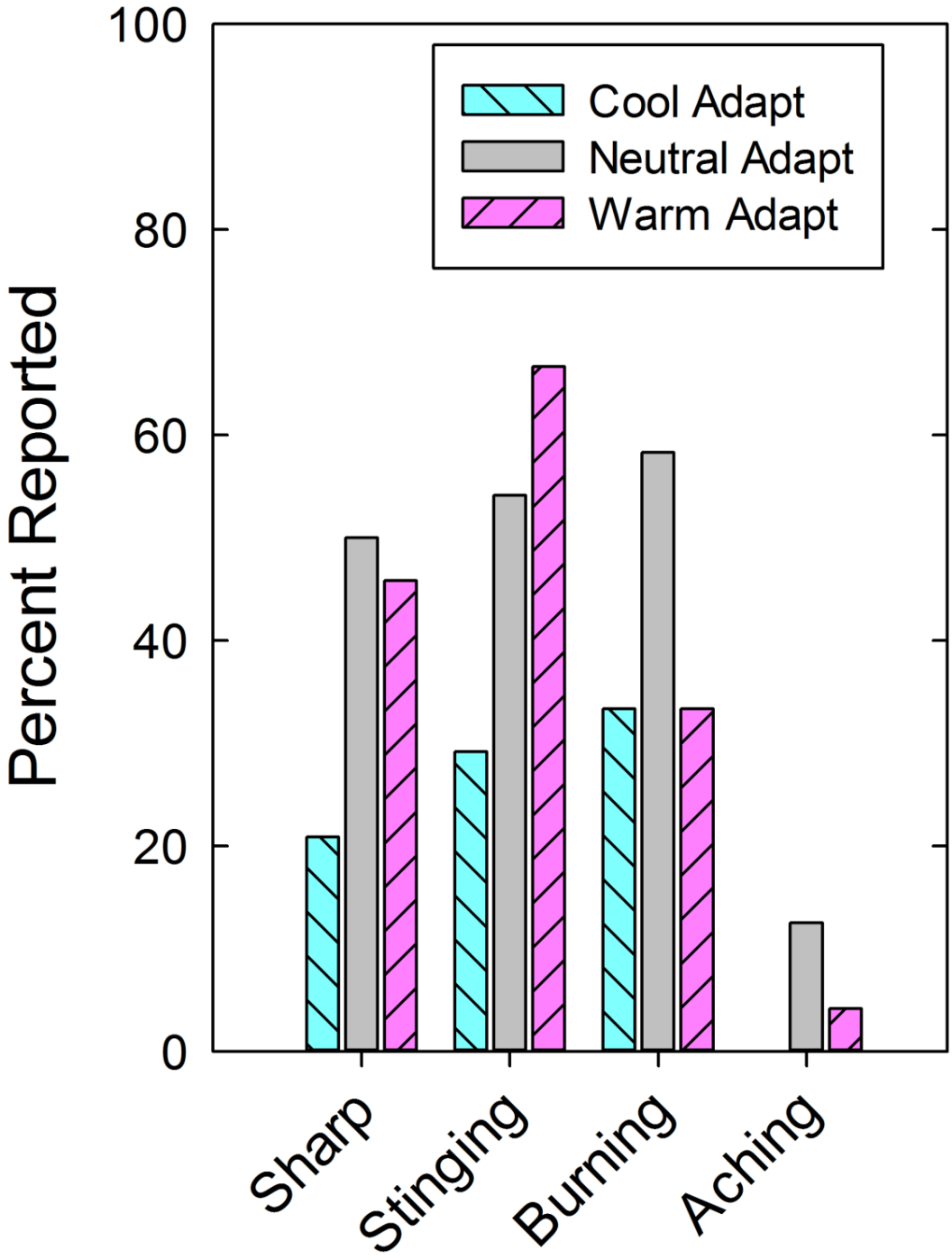

Figure 5.3 Frequency of pain descriptors attributed to the TGI following selective thermal adaptation. "Burning" was significantly less frequently reported than "Stinging" following warm adaptation.

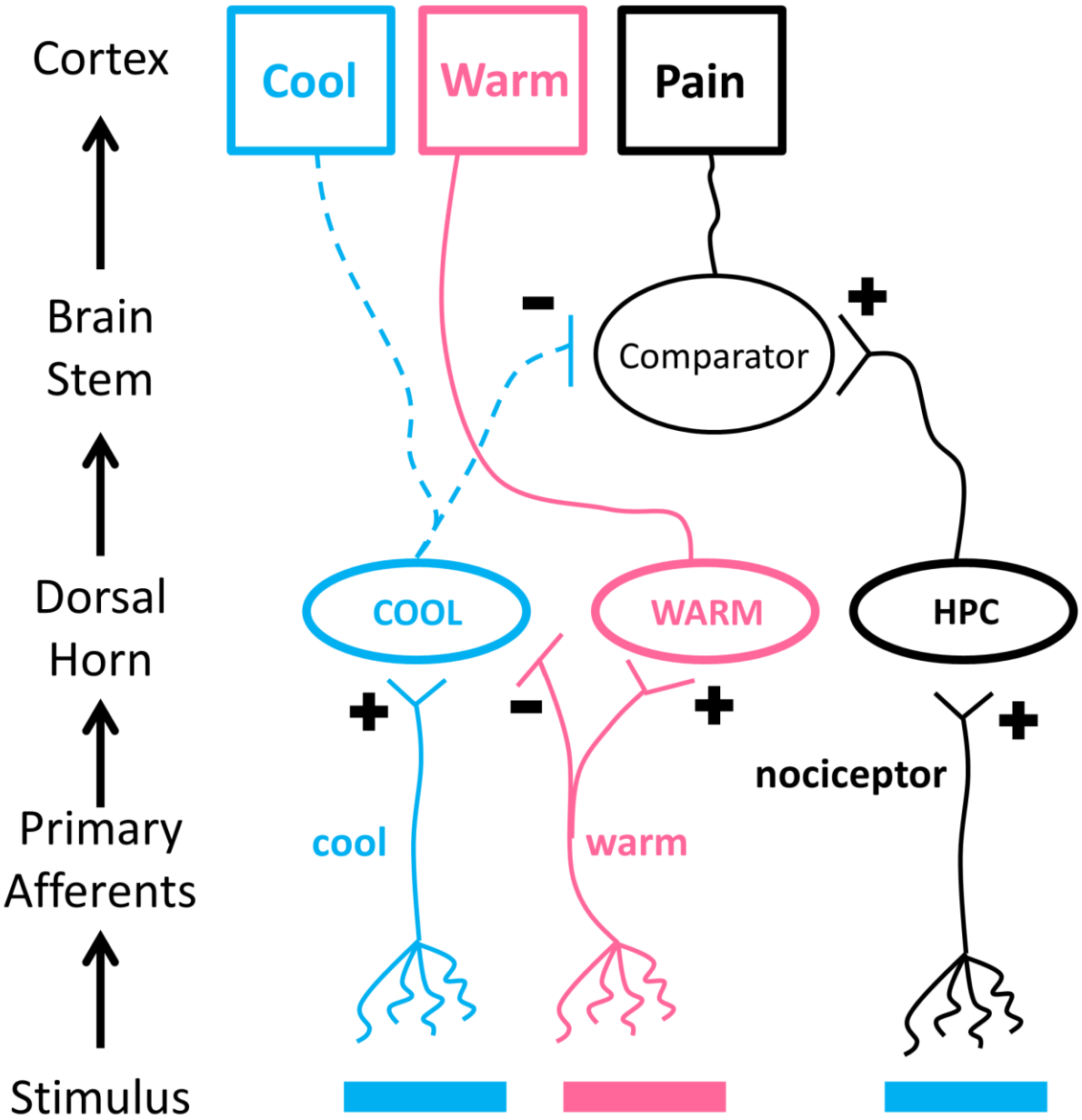

Figure 5.4 The disinhibition theory updated. This dissertation has added to evidence suggesting that innocuous thermal signals contribute to pain quality. The dashed lines projecting from the COOL dorsal horn neuron are used to indicate signals that are proposed to be inhibited during the TGI. The simultaneous sensations of warmth and pain from the grill's warm and cool bars, respectively, are theorized to blend into sensations of synthetic heat and, with the use of more extreme innocuous temperatures, burning pain. This would explain why the TGI feels hot, rather than cold, to most individuals.

Campero et al., 1996), since synthetic heat and paradoxical heat persist following A fiber block (Fruhstorfer et al., 2004; Susser et al., 1999). Different populations of C fibers might contribute to the TGI depending on the cool temperature used, including C2 afferents at mild cool temperatures (Campero et al., 2009) and C-polymodal nociceptors at temperatures cooler than ~24°C (Campero et al., 1996; Craig & Bushnell, 1994). Finally, the reduction in burning and increase in stinging sensations following warm adaptation provides further evidence that innocuous thermal signals participate in coding the perceived quality of painful stimuli.

Future Directions

Although this dissertation has made substantial headway in pinpointing the underpinnings of the TGI, many burning questions remain.

First, the idea that some individuals possess stronger pain-inhibitory COOL input, protecting them against the painfulness of the TGI, certainly warrants further investigation, as it may indicate a subpopulation of individuals who are less likely to develop chronic pain following nervous system injury.

Second, the theories of the codes for heat and burning heat I have presented could be corroborated by single-unit recordings in the dorsal horn of animals. First, it would be of interest to determine whether HPCs and WDRs respond equally well to thermal grill stimulation, and whether the effects of DNIC on these neuronal populations' coding of the thermal grill mirror the reductions in pain seen in human subjects. Second, C polymodal nociceptors are likely are the peripheral source of the painful component of the TGI at more extreme innocuous warm and cool temperatures, since they are known to synapse on HPC neurons (Craig et al., 2001). The spinal cord targets of C2 afferents, whose milder thresholds

make them a prime candidate for carrying the signals generating synthetic heat in the non-painful TGI (Campero et al., 2009), have not been identified. They too might synapse on HPCs, which would corroborate my idea that synthetic heat at mild grill temperatures shares the same pathway as painful heat at more extreme grill temperatures. It would also be interesting to examine the effects of thermal adaptation on the spinal-cord neuronal populations involved in the TGI, which might shed light on the question of why warm adaptation failed to reduce the illusion.

Finally, one of the main challenges for TGI research is the lack of a commercially available apparatus to produce the illusion. To ensure that the TGI realizes its full potential in the field of pain research, experts on the illusion should work closely with engineers to create a device that could be disseminated to labs and pain clinics across the world. A high degree of portability for a device such as this would be ideal, as it would open up the illusion for study in a variety of clinical populations, a realm of research for the TGI that is severely lacking to date.

Concluding Remarks

The experiments in this dissertation show that the TGI is an *illusion* of pain only in the sense that pain occurs at temperatures that are normally not perceived to be painful. Evidence presented here indicates that signals coding the painfulness of the illusion ascend in nociceptive neurons in the dorsal horn of the spinal cord, where they are subject to descending inhibition from the brainstem. The pain signals are triggered by the cool bars of the thermal grill, as evidenced by a reduction in the TGI's painfulness following adaptation to them. Warm signals are likely the inhibitors of COOL neurons and cool sensation, as indicated by the results of these experiments.

Like many previous studies of the TGI, these experiments have shown that the illusion of pain is a robust phenomenon that is repeatable in naïve subjects. Previous studies have pointed towards the robustness of the illusion in that stimulation site, number of bars, and a variety of other factors fail to significantly affect the illusion. The temporal order of the onsets of warm and cool stimulation is another factor that does not have a significant effect on the intensity or the quality of the illusion.

Although the TGI is robust and reproducible, the current results confirm previous investigations suggesting that roughly 1/3 of individuals are insensitive to the painfulness of the illusion (Bouhassira et al., 2005; Kern et al., 2008a,b). A related individual difference is the presence of COOL sensations during the TGI, inferred from subject's descriptions. This may be a protective factor against feeling the illusion as painful. This result may have clinical relevance for those who experience cold allodynia: A restoration of COOL activity could potentially reverse this undesirable consequence of several forms of neuropathic pain. It may also be that individuals who are more sensitive to the TGI have a higher propensity to develop cold allodynia following nerve injury.

While there are several experimental models of neuropathic pain in animals, models of neuropathic pain in humans are lacking due to the necessity of invasive procedures to induce the symptoms. For example, the most common methods of inducing cold allodynia in healthy subjects are subcutaneous injections of menthol and nerve block via compression or ischemia (Klein et al., 2005). Due to its similarities with cold allodynia, the TGI is arguably the safest, most efficient way to induce a neuropathic-like pain in pain free individuals while minimizing unwanted side effects of the treatment.

Finally, since the TGI is similar to the burning pain experienced in some neuropathies (Ochoa & Yarnitsky, 1994), it should prove to be a useful model for generating a reversible neuropathic-like pain in healthy subjects. It could, therefore, have promise in testing the effectiveness of different analgesics in reducing neuropathic pain. Morphine has already been found to significantly reduce the pain of the TGI (Kern et al., 2008b) and ketamine was shown to virtually eliminate the TGI while having no effect on the perception of normal, suprathreshold heat and cold pain (Kern et al., 2008a). As scientific interest in the TGI and our knowledge of its mechanisms continue to grow, in the coming years the TGI could very well find a place in diagnosing and treating neuropathic pain conditions.

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