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**COLD-EVOKED PAIN VARIES WITH SKIN TYPE AND COOLING RATE:
A PSYCHOPHYSICAL STUDY IN HUMANS**

by

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A thesis submitted in conformity with the requirements
for the degree of Master's of Science
Graduate Department of The Institute of Medical Sciences
University of Toronto

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Abstract

This thesis examined the effect of skin type and cooling rate on the intensity, quality and time-course of cold-evoked pain in normal human subjects.

Cold stimuli were delivered to the thenar eminence (glabrous skin) and the dorso-lateral hand (hairy skin) via a 9cm² Peltier-type stimulator. Cold and pain thresholds were determined by the method of limits. A computerized visual analogue scale was used to obtain continuous ratings of pain intensity and affect. The McGill Pain Questionnaire was used to assess the quality of cold-evoked pain. Supra-threshold stimuli (34°C base) were delivered at 0.5, 1 or 2°C/s to 2°C, held for 20s and returned to baseline at 9°C/s.

These studies revealed: (1) Cold and cold-induced pain thresholds were lower (e.g. occurred at a higher temperature) for hairy compared to glabrous skin. (2) Power function exponents for supra-threshold ratings were larger for glabrous skin than for hairy skin. (3) Pain indices were higher for slower cooling rates. (4) No differences were found for pain ratings of intensity and affect. (5) Cold-evoked painful paradoxical heat sensations occurred most often during innocuous cooling.

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List of Abbreviations

ACC - anterior cingulate cortex
ACh - acetylcholine
AMH - A-fibre mechanoheat receptor
ANOVA - analysis of variance
ATP - adenosine triphosphate
aVAS - affect visual analogue scale
CMH - C-fibre mechanoheat nociceptor
CMM - cross-modality matching
CNS - central nervous system
CV - conduction velocity
dFNE - dermal free nerve ending
DLH - dorso-lateral hand
DRG - dorsal root ganglion
eFNE - epidermal free nerve ending
FNE - free nerve endings
HCR - high-threshold cold receptor
HPC - heat, pinch, cold
iVAS - intensity visual analogue scale
JND - just noticeable difference
LCR - low-threshold cold receptor
MOL - method of limits
MPQ - McGill pain questionnaire
NE - norepinephrine
NS - nociceptive specific
NWC - number of words chosen
PGP9.5 - protein gene product 9.5
PH - paradoxical heat
PMN - polymodal nociceptor
PPI - present pain index
PRIT - pain rating index total
RF - receptive field
RSDS - reflex sympathetic dystrophy syndrome
SI - primary somatosensory cortex
SII - secondary somatosensory cortex
SA-M - slowly adapting mechanoreceptor
SEM - standard error of the mean
SNS - sympathetic nervous system
STT - spinothalamic tract
TENS - trans-cutaneous electrical nerve stimulation

VAS - visual analogue scale
VB - ventral basal nucleus
Vc - ventro-caudal nucleus
VDS - verbal descriptor scale
VMpo - ventro-medial nucleus, posterior aspect
WDR - wide dynamic range

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Section 1. Introduction and History

Controversy over whether noxious (pain) and thermal sensations are unique senses subserved by their own dedicated sensory systems has existed since the beginning of formal scientific study. Aristotle (384-322 B.C.) (see Weddell, 1947), who is credited with classifying the five senses, considered pain and pleasure to be related to the sense of touch, but not defined as senses but rather as "passions of the soul". Galen (c. 130-c. 200) (see Weddell, 1947) later hypothesized that cold and heat were indeed senses separate from touch, however he did not believe that pain was subserved by specialized terminals. Instead he suggested that pain sensations derived from a central interpretation of touch stimuli. Very few developments in these concepts occurred over the ensuing centuries.

In 1794 Erasmus Darwin observed a patient who could feel neither pin-prick nor pinch presented to the sole of the foot, but who could feel a red-hot poker when it was held near the skin. He concluded that although the nerves subserving touch sensations had become paralyzed, the nerves subserving heat sensation had retained their function; implying that touch and heat sensations were subserved by different systems of nerves. Pain, along with pleasure, were still thought to be due to central interpretation of touch input (Darwin, 1794).

In 1840 Johannes Müller presented the "Theory of Specific Sensory Energies", in which he proposed that the quality of sensation depended on the sense organ that was excited by a stimulus rather than on the stimulus itself. Although Müller subdivided touch into tickle, itch, shudder, pleasure, pain, fatigue, suffocation, warmth, cold, and movement, he did not suggest that these qualities were subserved by different systems of nerve-fibres. Instead he proposed that these qualities were dependant upon the mode of arousal and the state of the organism when the sensations were elicited (Müller, 1837). Although Müller's concept of pain and thermal sensations had not radically changed from Aristotle's, his theory suggested the important hypothesis that if fibres were capable of eliciting particular sensations regardless of the mode of stimulation, perhaps different systems of nerves were devoted to each of these subtypes of touch.

Soon after, Goldscheider (1881) and Blix (1884) independently reported separate spots of skin which, however stimulated, evoked sensations of warmth, cold, touch or pain. This evidence seemed to confirm that the sense of touch was actually made up of several unique modalities.

Despite this evidence, Goldscheider continued to maintain that pain sensations were the result of intense stimulation of touch fibres and central summation of impulses and was not a separate modality (Goldscheider, 1898).

One of the most important findings that established the concept of specificity came from Max von Frey. Von Frey (1896) used fine hairs applied to the skin to systematically study pain sensations elicited in human subjects. As opposed to Goldscheider, von Frey considered even the weakest sting or itch to be a painful sensation. From his experiments, he concluded that peripheral nerves devoted to conducting noxious and thermal messages were primarily responsible for pain and thermal sensations respectively. Although he had no histological evidence, von Frey ascribed sensations of pain and cold to free nerve endings and Krause's end bulbs respectively. Specific nerve fibres devoted to sensations of pain (Gasser & Erlanger, 1929; Adrian, 1932) and cold (Zotterman, 1936) were subsequently identified physiologically. These findings firmly established that pain and thermal sensations were senses in their own right.

Nevertheless, the issue of specificity for thermal and pain sensations still remains controversial today. For instance, modern experiments have demonstrated polymodal nociceptors which respond to intense touch and thermal stimulation. In addition, wide dynamic range neurons of the spinal cord dorsal horn, which respond both to innocuous and noxious stimulation, have also recently been characterized. These findings once again raise the issue of whether noxious and thermal sensations are mediated by their own neural circuitry.

Section 2. Literature Review

(I) Anatomy and Physiology of Structures Involved in Cold and Cold Pain Sensations

1. The Peripheral Nervous System

1.1. Anatomy of PNS Structures

1.1.1. The Epidermis of Hairy and Glabrous Skin

The epidermis is the avascular outer layer of the body that acts as a barrier to prevent certain foreign substances from entering the body while allowing others, such as sweat, to be expelled. The epidermis of most mammals can be sub-divided into two main types: glabrous and hairy. Glabrous skin is exclusively found on the palms of the hands, the soles of the feet, and the lips, the rest of the body consists of hairy skin. Although the epidermis does not generally exceed 0.12 mm over the entire body surface, it may be considerably thicker in areas subjected to constant pressure or friction such as the glabrous skin of the palms and soles of the feet (Cormack, 1987; Lever & Schaumburg-Lever, 1990). Furthermore, the epidermis may be thicker in males than in females (Southwood, 1955). The most common cell type in the epidermis are keratinocytes which change both shape and function as they travel from the basal layer, where they are formed, to the superficial layer where they are then lost to the external world. These morphological and histological changes allow several layers, or strata, to be defined which often differ between hairy and glabrous skin sites (Cormack, 1987; Lever & Schaumburg-Lever, 1990; Spence & Mason, 1992c).

The basal cell layer, or stratum germinativum, is the deepest epidermal layer, directly attached to the underlying dermis by a basement membrane. This layer, which is similar in hairy and glabrous skin, is the site of epidermal cell genesis. Immediately superficial is found the squamous cell layer, or the stratum spinosum, a layer of polygonal-shaped keratinocytes. This layer is usually 5-10 cells thick, however it is generally thinner in hairy skin sites. Above is found the granular layer, or stratum granulosum, consisting of flat diamond-shaped and granulated cells acting to increase the strength and stability of the epidermis. This layer is also thicker in glabrous skin sites, reaching 10 cells thick in the palms and soles of the feet. The horny layer is the most superficial layer in the epidermis, containing anuclear, closely packed, dead

skin cells which act as the primary barrier of the skin. The deepest portion of the horny layer is known as the stratum lucidum, which can often be identified only in glabrous skin sites and contains many organelles known as Odland bodies. The lipids found in these small organelles prevent water loss from the body and mediate attachment to the above lying stratum corneum. The stratum corneum, which constitutes the upper portion of the horny layer, varies from 15-20 cells thick, although it is generally thicker in glabrous skin. These epidermal layers are shown schematically in Figure 1. A characteristic finger-print pattern of grooves and ridges can be seen on certain portions of glabrous skin which increases the friction of the epidermal surface, enhancing the gripping ability of the areas (Southwood, 1955; Cormack, 1987; Lever & Schaumburg-Lever, 1990; Spence & Mason, 1992c).

1.1.2. The Dermis of Hairy and Glabrous Skin

In contrast to the epidermis, the dermis is well vascularized and comprises much of the thickness of human skin. The dermis consists mainly of fibrocytes although several other cell types may be present including macrophages, adipocytes, and mast cells. The fibrocytes create two major layers of loose connective tissue. The papillary layer, which lies just inferior to the basal layer of the epidermis, is named for its papillae which pierce the overlying epidermis. The numerous papillae of glabrous skin approximate 0.2mm high cones, while those in hairy skin are flatter, rounder, less numerous, and do not project as high into the epidermis. Below this layer is the reticular layer which is much thicker and consists of a weaving or netlike pattern of connective tissue. Capillaries are found in abundance in this layer, mostly in association with epidermal appendages such as hair follicles and various glands (Southwood, 1955; Cormack, 1987; Lever & Schaumburg-Lever, 1990; Spence & Mason, 1992c).

1.1.3. The Cutaneous Vasculature

The avascular epidermis receives its blood supply from an extensive plexus of vessels that originate from the subcutaneous tissue. This plexus ascends into the dermis to form three important dermal plexuses. The subpapillary plexus consists of a flat network of vessels along the epidermal-dermal border from which capillary loops ascend into the dermal papillae

Figure 1: The Human Epidermis. A schematic representation of a photomicrograph of a section of glabrous skin. From outer to inner, the layers are: (A) Stratum corneum. (B) Stratum granulosum. (C) Stratum spinosum. (D) Stratum basale.

Adapted from Spence and Mason, 1992. p.134.

nourishing the skin and sebaceous glands. Typically each dermal papilla is innervated by a single capillary loop. The other two plexuses form similar networks around hair follicles and sweat glands (Cormack, 1987; Lever & Schaumburg-Lever, 1990).

Histologically, blood vessel walls can be divided into three main tissue layers. The inner layer, known as the tunica intima, is composed of endothelial cells and an internal elastic lamina. The tunica media consist mainly of layers of smooth muscle cells that act to constrict and dilate the vessel. Finally, the tunica adventitia surrounds the entire vessel with connective tissue. Veins and arteries are quite similar anatomically, however the walls of veins are generally thinner than those of arteries (Lever & Schaumburg-Lever, 1990; Spence & Mason, 1992a).

Regulation of body temperature is mediated by the smooth muscle tone in the vessels within the papillary and subpapillary plexuses. In addition, specialized arteriovenous shunts, known as glomus formations, are found in abundance in the dermal reticulum of the glabrous skin of the fingers, palms and soles of the feet. These shunts consist of large capillary networks that, when open, dramatically increase the dermal blood flow thereby allowing heat to be released (Lever & Schaumburg-Lever, 1990).

1.1.4. The Cutaneous Branch of the Sympathetic Nervous System

The sympathetic nervous system (SNS) is one part of the autonomic nervous system which innervates all the organs of the body and has the general function of "fight or flight". Preganglionic sympathetic axons exit the CNS via the ventral roots and terminate in either the paravertebral or prevertebral ganglia (Roberts & Kramis, 1990). The postganglionic efferents then travel distally and, near their terminae, form a network of thin unmyelinated fibres with periodic varicosities $\sim 1\mu\text{m}$ in diameter (Vanhoutte & Shepherd, 1993). These efferents are either vasomotor or sudomotor and release adrenergic (NE) and cholinergic (ACh) transmitters respectively (Verrill, 1989). SNS fibres that innervate the cutaneous blood vessels are most commonly found at the border between the tunica adventitia and media, however in veins they may penetrate into the media itself (Vanhoutte & Shepherd, 1993), thereby situating them in an ideal position to control vasomotor activity. Besides the blood vessels, SNS fibres innervate several cutaneous structures such as the arrector pilorum of the hair follicles, and the eccrine and

apocrine but not sebaceous glands (Lever & Schaumburg-Lever, 1990). In addition to NE and ACh, the chemical transmitters traditionally associated with the SNS, several co-transmitters may also be released either directly or indirectly from SNS activity including: neuropeptide Y, ATP, prostaglandins, histamine, and nitric oxide (Roberts & Kramis, 1990; Vanhoutte & Shepherd, 1993; Raja, 1995).

1.2. Innervation of the Tissues Involved in Sensations of Cold Pain

It is generally accepted that while many modalities of cutaneous touch are subserved by encapsulated receptors located below their receptive fields, thermal and pain sensations arise from activation of non-encapsulated or "free" nerve endings. Where exactly these endings or terminations are located has been the subject of much research and debate. Research suggests that the receptors encoding cold pain most likely reside in the epidermal/dermal tissue layers or in the vein walls.

Many histological methods have been used to visualize the innervation pattern of cutaneous tissues. Methylene blue stains the axon contents of both autonomic and PNS components without also staining melanocytes and Langerhans cells which are found in abundance in the cutaneous tissue (Arthur & Shelley, 1959). Cholinesterase improves upon older metal staining techniques, for instance silver nitrate, as nerve tissues but not the surrounding connective tissue is impregnated by the chemical (Winkelman, 1988). Finally, many immunological stains use antibodies for substances such as protein gene product 9.5 (PGP 9.5), which labels even minute twigs emitted from axons (Stankovic *et al.* 1996), and substance-P which is absent in patients with diminished pain sensitivity (Nguyen-Legros & Cesaro, 1984).

1.2.1. Epidermal Innervation

The existence of epidermal free nerve endings (eFNEs) has been debated since the beginning of the century. The generally held belief at the turn of the century that the epidermis was the seat of pain was questioned by Waterson (1933), who reported that shaving off the stratum mucosum of the epidermis elicited sensations of touch but not pain. Since cutting through the capillary loops of the dermal papillae elicited extremely painful sensations, he

concluded that nociceptors reside in the dermis while touch receptors are found in the epidermis.

Subsequent studies using light and electron microscopy demonstrated eFNEs in the rat snout (MacIntosh, 1975) and in rat cutaneous skin stained with antibodies for PGP 9.5 (Stankovic *et al.* 1996), toluidine blue (Kruger *et al.* 1981) and methylene blue (Hensel *et al.* 1974). Glabrous but not hairy eFNEs were identified using a modified silver-impregnation technique in tropical cattle (Amakiri *et al.* 1978). In a review on the morphology of cutaneous sensory receptors, Montagna (1977) claims that in adult human tissue, eFNEs have been predictably observed only in the eyelids, genitalia and the vestibule of the nose, while others note that eFNEs have been demonstrated in fetal lip, nipple and digit tissues (Breathnach, 1977). In contrast, others (Breathnach, 1977; Montagna, 1977; Winkelmann, 1988; Lever & Schaumburg-Lever, 1990) report that eFNEs occur rarely, if ever, in adult human skin. According to Lever (1990), attempts to resolve this issue using light microscopy have been indecisive because some techniques stain melanocytes and their dendrites in addition to FNEs. Also, cholinesterase staining cannot distinguish whether a FNE is in the epidermis or a dermal papilla. On the other hand, Arthur and Shelly (1959) used methylene blue stain and light microscopy to publish one of the few studies citing eFNEs in adult human skin. The authors describe the structure of eFNEs that terminated as superficially as the granular epidermal layer. In addition, they analyzed the distribution densities of eFNEs in various body parts. The cheek was found to be most densely innervated (8.5 nerve fibres/0.01 mm²) whereas the pad of the index digit was least densely innervated (0.5/0.01 mm²). More recently, Cauna (1980) observed eFNEs in human palmer skin with a density of 1-5/mm² using electron microscopy. Also in support of eFNEs, Tillman *et al.* (1995) calculated that receptor endings of C-mechanoheat nociceptors (CMHs) occur within human epidermis and dermis at a depth of 20-570µm with the use of a theoretical heat transfer model.

1.2.2. Dermal Innervation

The existence of dermal FNEs (dFNE) is less controversial than that of eFNEs. Many researchers report that networks of nerve fibres terminate in the dermis in association with hair follicles, dermal papillae, sebaceous glands, and blood vessels (Arthur & Shelley, 1959; Hensel

et al. 1974; Montagna, 1977; Cormack, 1987; Munger & Ide, 1988; Winkelmann, 1988; Terzis & Smith, 1990). Cauna (1980) observed dFNEs in approximately 60% of papillae, with a maximum of 5 endings/papilla leading to an estimate of up to 100 endings/mm². Montagna (1977) observed dermal nerve networks in both hairy and glabrous skin and found that the arrangement of these networks were similar except for the more orderly and structured pattern which surrounds the outer root sheath of hair follicles.

1.2.3. Vascular and Perivascular Innervation

Truex (1936) examined the sensory nerve terminations in the vasculature with pyridine-silver at least 46 days following ablation of feline sympathetic ganglia to ensure that no sympathetic nerve fibres remained. In the veins, afferent nerve fibres were only observed in the tunica adventitia where they followed a relatively straight course parallel to the vessel axis. In the arteries, the terminal endings of the tunica media consisted of unmyelinated fibres which ended freely between smooth muscle cells after a torturous spiral course. Innervation of the arterial tunica adventitia differed proximally compared to distally. While both were innervated by myelinated fibres which underwent extensive branching before terminating, thinner fibres with less extensive arborizations characterized distal innervation. The innervation of arterial perivascular tissue produced two main termination patterns: one made up of thin nerve fibres making thread-like terminations in fatty interspaces and another consisting of branches of coarser fibres ending either freely or in relation to small rounded cells.

Millen (1948) observed the innervation of small arteries without previous sympathectomy in the rabbit ear and stomach using methylene blue staining. He described several nerve plexuses supplying the small arteries: finely myelinated and unmyelinated fibres running parallel to the vessel axis in the tunica adventitia, a meshwork of fine fibres located between the tunica media and the tunica adventitia, and a plexus of very fine beads in the tunica media.

1.3. Structure of the Receptors

1.3.1. Cold Receptors

At the turn of the century, it was commonly believed that cold sensation was mediated by

end organs termed "Krause's end-bulbs" by von Frey (1896). In 1927, Dallenbach removed tissue directly beneath cold sensitive spots in human skin and stained the sections with methylene blue. Microscopic examination revealed numerous undifferentiated nerve-endings but failed to exhibit specialized end organs putting von Frey's conjecture into question. A similar preparation by Hensel et al. (1974) identified cold receptors as consisting of bundles of unmyelinated nerve arborizations with receptor branches that leave their Schwann cell ensheathment as they enter the basal lamina of the epidermis. Nerve terminals were observed to enlarge and to contain numerous mitochondria, glycogen particles, and a filamentous receptor matrix with vesicles of various sizes.

1.3.2. Nociceptors

In cutaneous nerve populations, $\geq 10\%$ of myelinated and $\geq 50\%$ of unmyelinated fibres are nociceptive with the actual percent distribution varying across species and body areas (Burgess & Perl, 1967; Kumazawa & Perl, 1977; Shea & Perl, 1985; Leem *et al.* 1993; Light & Perl, 1993). The structure of myelinated mechanical nociceptors has been described in cat hairy skin using light and electron microscopy by Kruger et al. (1981). Thinly myelinated fibres divide in the dermis below the sensitive area of the skin, lose their myelin sheaths, penetrate and finally terminate in the basal lamina of the epidermis surrounded by Schwann cell cytoplasm and keratinocytes. At the termination site, axons were observed to have large, dense core vesicles while the Schwann cell cytoplasm contained numerous pinocytotic vesicles (Kruger *et al.* 1981).

1.3.3. Free Nerve Endings

Cold and pain sensations are ultimately mediated by fine terminal branches of both thinly myelinated A δ -fibres and unmyelinated C-fibres. Whether "free" is the proper term to describe these terminal endings is debatable. The term has been in use at least as far back as 1889 (Koelliker, cited in Messlinger, 1996) to denote those endings not obviously enclosed by a specific end structure. The accuracy of this description is consistent with modern light and electron microscopy studies demonstrating that both A δ and C-fibres commonly only have Schwann cell cytoplasm associated with their terminal endings. Some researchers have proposed

alternate names such as: non-corporcular nerve endings, fine sensory endings (Messlinger, 1996) and penicillate endings (Cauna, 1980; Winkelmann, 1988). For the present purpose the term free nerve endings (FNEs) will be used, as in the past, to refer to the terminal branch endings of A δ and C-fibres that are devoid of encapsulation.

According to Cauna (1980) FNEs can be classified morphologically into three distinct types. **Open or coarse endings** contain between 1-4 axon terminals each 1-3 μ m in diameter and contain mitochondria, microvesicles and glycogen granules. **Beaded or varicose endings** have spindle-shaped segments approximately 2 μ m in diameter and 2-3 μ m in length and similarly contain mitochondria, microvesicles and glycogen granules. Finally, **plain or fine type endings** are extensive, ensheathed by Schwann-cells, and contain numerous cytoplasmic processes which extend distally along the main cell process. Their axon terminals are the smallest of the FNE types, being less than 1 μ in diameter, and they contain few organelles (Fig. 2). Plain or fine endings frequently follow the course of capillary loops and while most terminate subepidermally, others constitute the observed population of eFNEs (described in Section 1.2.1).

One limitation of these structural studies on FNEs is the lack of correlative analysis to link histological with physiological findings. This makes it difficult to ascertain whether the fibre is somatic or autonomic, whether it responds to thermal, mechanical or more than one modality, and whether it mainly responds to innocuous or noxious stimulation (Southwood, 1955). In their study of the structure of myelinated nociceptors in cat hairy skin, Kruger et al. (1981) did carry out physiological characterization before initiating staining. However, because only a sharp probe was used as the search stimulus, it is not known if the fibres responded exclusively to noxious mechanical stimulation or if they were polymodal nociceptors.

Taken together, histological studies of these terminal endings employing various staining techniques reveal that small myelinated and unmyelinated nerve fibres originate from a nerve plexus that runs parallel with the cutaneous blood vessels, branches upwards into the dermis and, depending on the preparation, into the epidermis. The tortuous, often branching course of these terminating fibres frequently overlaps with adjacent fibres. Near the actual termination site, A δ axons lose their myelinated sheaths and both A δ and C-fibres terminate unmyelinated, surrounded by a thin Schwann cell cytoplasm. This cytoplasm possesses a high concentration of

Figure 2: Plain-type Free Nerve Ending. A schematic representation of a terminal ending in human digital skin ensheathed by the processes of two Schwann cells. (A) Nerve terminal. (B) Terminal Schwann cell process. (C) Pre-terminal Schwann cell process.

Adapted from Cauna, 1980.

mitochondria in addition to glycogen particles as well as a filamentous receptor matrix containing numerous large, pinocytotic vesicles. The terminal fibre of nociceptors contains large, dense core vesicles (Poirier, 1968; Cauna, 1980; Kruger *et al.* 1981; Munger & Ide, 1988; Winkelmann, 1988).

1.4. Physiology of the Receptors Mediating Cold and Cold Pain Sensations

Cooling the skin from room temperature initially excites mainly cold receptors and evokes innocuous cold sensations. As the temperature approaches a level capable of causing tissue damage, nociceptors begin to respond and painfully cold sensations are perceived.

1.4.1. Cold Receptors

Fibres specifically responsive to cooling were first described by Zotterman (1936) in the feline tongue. Hensel and Zotterman (1951b, 1951c) subsequently demonstrated that these fibres were capable of sustained discharges to maintained cold temperatures. Later research demonstrated that the receptive fields (RFs) of cutaneous cold receptors consist either of a single small spot ≤ 1 mm in diameter (Iggo, 1969; Hensel & Iggo, 1971; Darian-Smith *et al.* 1973; Darian-Smith *et al.* 1973; Dykes, 1975; Sumino & Dubner, 1981), or multiple (2-8) spots separated from each other by relatively unresponsive regions (Kenshalo & Gallegos, 1967; Iggo, 1969; Duclaux & Kenshalo, 1973; Kenshalo & Duclaux, 1977; Sumino & Dubner, 1981). These multiple spot RFs suggest extensive branching in the terminal endings of cold fibres (described in Section 1.3.3.). Duclaux and Kenshalo (1973) showed that the activity in cold receptors with multiple RFs is similar whether one or all of their spots are stimulated. Additionally, their finding that warming one spot while keeping the others cool does not affect the fibre's response suggests that cold dominates the receptor's activity. Low-threshold cold receptors (LCRs) are very sensitive to small temperature decreases (0.5°C) from a neutral baseline ($30\text{-}35^{\circ}\text{C}$) (Zotterman, 1936; Hensel & Iggo, 1971; Light & Perl, 1993). These receptors are sensitive to the rate of cooling such that faster rates evoke higher frequencies of discharge than slower rates to the same temperature level (see Section 1.4.6. on cooling rate below). Their response also depends upon previously presented stimuli (Darian-Smith *et al.* 1973) and they are relatively

insensitive to innocuous mechanical stimulation (Kenshalo & Gallegos, 1967; Iggo, 1969; Hensel & Iggo, 1971; Darian-Smith *et al.* 1973; Sumino & Dubner, 1981). Most cold units show maximum static discharge rates between 20-28°C with an upper range of 35°C, which decreases as the temperature is raised or lowered, resulting in an inverted U-shaped response function (Hensel & Wurster, 1969; Iggo, 1969; Darian-Smith *et al.* 1973; Hensel, 1974; Pierau & Wurster, 1981; Sumino & Dubner, 1981; Shea & Perl, 1985; Light & Perl, 1993). Within this maximum sensitivity range, the peak discharge rate is ~6-14 imp/s (Hensel & Wurster, 1969; Darian-Smith *et al.* 1973; Dykes, 1975) and increases at a rate of 0.5imp/s/°C (Dykes, 1975). Conduction velocity measurements of the fibres subserving cold receptors have identified both A δ (Iggo, 1969; Darian-Smith *et al.* 1973; Duclaux & Kenshalo, 1973; Dykes, 1975; Sumino & Dubner, 1981) and C axons (Duclaux & Kenshalo, 1973; Nordin, 1990), although A δ cold receptors are more common.

Selective ischaemia or compression of A δ -fibres have been used by several groups to show that these fibres are critical for cold perception (Mackenzie *et al.* 1975; Fruhstorfer, 1984; Wahren *et al.* 1989; Yarnitsky & Ochoa, 1990b; Davis, 1998a). For example, as A δ -fibre blocks progress, the ability to discriminate between two innocuous cold stimuli becomes impaired. Also, subjects become less sensitive to cooling, as demonstrated by higher cold thresholds. Finally, once A δ -fibres are completely blocked subjects often do not report sensations of cold to innocuous cooling.

Because cold receptors may have identical ongoing discharge rates at more than one temperature, it has been argued that they are not capable of signalling absolute temperatures (Light & Perl, 1993). This potential ambiguity may be resolved by the bursting patterns of discharge which has been demonstrated in some afferents across species (Hensel & Wurster, 1969; Iggo, 1969; Iggo & Iggo, 1971; Darian-Smith *et al.* 1973; Duclaux & Kenshalo, 1973; Hensel, 1974; Dykes, 1975; Kenshalo & Duclaux, 1977; Pierau & Wurster, 1981; Sumino & Dubner, 1981; Davies *et al.* 1985; Shea & Perl, 1985). Iggo (1969) defined these bursts as groups of 2 or more impulses separated by an interval at least twice as long as the length of the burst itself. In a survey of A δ cold receptors in monkeys, Dykes (1975) found that decreasing temperatures increased several properties of cold-evoked bursts including: number of impulses

per burst, burst duration, and burst interval. These bursts changed their pattern of firing to temperature changes as small as 0.5°C and they generally disappeared when temperatures exceeded or fell below the receptor's maximum sensitivity range (Iggo, 1969; Dykes, 1975).

Cold-specific receptors are not the only receptors that can respond to cooling. Some slowly-adapting mechanoreceptors (SA-M) increase their response (Burton *et al.* 1972), whereas most warm receptors decrease their response (Iggo, 1969; Darian-Smith *et al.* 1973) to sudden cooling. Although these other fibres would appear to contribute to decreasing the ambiguity of cold receptor responses, Johnson and co-workers (1973) claim that the information they provide is insufficient to allow the discrimination between two cold stimuli. They claim that warm receptors can discriminate cold only at baseline temperatures above normal skin temperature and then only to small temperature changes (~2°C). In addition, they state the information SA-Ms provide to the CNS is of "nuisance value" only.

1.4.2. Nociceptors

To ascertain whether cutaneous nociceptors are involved in the experience of cold pain, researchers have looked at primary afferent fibre or dorsal root ganglia (DRG) neuron responses to noxious cold in several species and have used many different experimental parameters. Systematic studies on the effects of noxious cold stimuli have been reported in rats (Leem *et al.* 1993; Simone & Kajander, 1996; Simone & Kajander, 1997), cats (Iggo, 1959; Duclaux *et al.* 1980; LaMotte & Thalhammer, 1982; Saumet *et al.* 1985), monkeys (Iggo & Ogawa, 1971; Georgopoulos, 1976; Croze *et al.* 1976; LaMotte & Thalhammer, 1982), and humans (Campero *et al.* 1996). Peltier-type thermodes are the most common means of delivering noxious cold stimuli to the RF, ranging in size from 1mm² for stimulation of cat nose skin (Duclaux *et al.* 1980) to 8cm² for stimulation of the hairy skin of monkey arm and leg (Croze *et al.* 1976). Additionally, stimuli such as ice cubes or ice water (Iggo, 1959; Croze *et al.* 1976; LaMotte & Thalhammer, 1982; Saumet *et al.* 1985; Bester *et al.* 1994), cold metal probes (Duclaux *et al.* 1980; LaMotte & Thalhammer, 1982; Saumet *et al.* 1985), water-filled test tubes (Leem *et al.* 1993) and even liquid nitrogen (Campero *et al.* 1996) have been used to search for cold-sensitive RFs and to test primary afferent function. Other parameters that vary within and across studies

include: adapting temperature, cooling rate, step size, minimum temperature reached, and duration of the stimulus. For example, while Saumet *et al.* (1985) used cooling rates of 0.3-1.3°C/s, the cooling rate of Simone and Kajander's (1996) study was many times faster at 18°C/s. Table I summarizes these differing parameters in some selected studies of noxious cold stimulation.

Classifications of nociceptive primary afferents are based on conduction velocity (CV), degree of myelination, modality of stimulation evoking responses, and the functional response characteristics of the fibres (Meyer *et al.* 1994). The former two classification criteria can be used to identify two broad sub-classes of nociceptors: thinly myelinated A δ -fibres and unmyelinated C-fibres. In those nociceptors that are sensitive to cold, the latter two criteria can be used to identify two further sub-classes of A δ and C-nociceptors: high-threshold cold-specific receptors (HCR) and polymodal nociceptors (PMN).

1.4.2.a. Unmyelinated Nociceptors

Conduction velocities of unmyelinated (C-fibre) nociceptors are the slowest of cutaneous sensory fibres, spanning from $\approx$ 0.5-2.5m/s depending upon the species (Martin & Jessell, 1991b; Leem *et al.* 1993; Light & Perl, 1993). Most of these nociceptors have little or no resting discharge at room temperature as long as no previous injury or stimulation has occurred (Iggo, 1959; Bessou & Perl, 1969; Kumazawa & Perl, 1977; Campbell *et al.* 1989). Generally, C-fibres respond to noxious heat in the range of 42-56°C and increase their firing rate in a graded fashion when subjected to graded increases in temperature above their thresholds (Iggo, 1959; Campbell *et al.* 1989; Light & Perl, 1993).

C-fibres responsive to noxious cold stimuli have been identified by several groups (Iggo, 1959; Georgopoulos, 1976; LaMotte & Thalhammer, 1982; Handwerker *et al.* 1991b; Davis *et al.* 1993; Leem *et al.* 1993; Campero *et al.* 1996; Simone & Kajander, 1996) and, as mentioned previously, can be sub-classified physiologically either as those responsive almost exclusively to cold stimuli (C-HCRs), or those that are additionally excited by noxious stimuli such as heat and/or mechanical, and are therefore classified as C-polymodal nociceptors (C-PMNs). Iggo (1959) described C-HCRs in cats having maximum discharge frequencies of 10 imp/s in

Table I: Electrophysiological Primary Afferent Studies of Noxious Cold

Study	Species	Body Site	Cold Stimulus	AT (°C)	ΔT (°C)	Rate (°C/s)	Duration/ ITI (sec)	Min Temp (°C)
Iggo, 1959	cat	thigh, leg, foot	rods, ice water & T	n/a	n/a	n/a	n/a	0?
Geogopolous, 1974	monkey	hairy skin of hand	T (1mm ²) & ether	30	≤ 15	n/a	3-4/60	0
Croze et al. 1976	monkey	hairy skin leg, arm	ice	n/a	n/a	n/a	n/a	n/a
Duclaux et al. 1980	cat	nose	rod & T (1mm ²)	25-0	5	6.5	≥ 180	-5
LaMotte & Thalhammer, 1982	monkey, cat	wrist, hand, ankle, foot	rod, ice & T (1.4 cm ²)	15-32	1-2	n/a	5/25	2
Shea & Perl, 1985	rabbit	ear	ice water & T (3mm ²)	25	n/a	n/a	n/a	n/a
Saumet et al. 1985	cat	hairy skin	ice & T (6.5cm ²)	20	5	0.3-1.3	180/240	-40
Handwerker et al. 1991	rat	hind paw	ice	32	n/a	n/a	≤ 30	0?
Davis et al. 1993	monkey	fore and hind limbs	ice & ethyl chloride	n/a	n/a	n/a	n/a	0?
Leem et al. 1993	rat	foot	test tubes & T	32	5	4	20/120-300	0
Campero et al. 1996	human	ankle, knee, wrist	T (1cm ²) & liq Nitrogen	32	≤ 10	≥ 1	30	0
Simon & Kajandar, 1996	rat	hairy skin hindpaw	T (1cm ²)	32	2	5	10/180	-12
Simon & Kajandar, 1997	rat	hairy skin hindpaw	T (1cm ²)	32	2	5	10/180	-20

note: n/a = not available; AT = adapting temperature; ITI = inter-trial interval; T = thermode

response to ice in their RFs, but whose frequencies decreased to only 2–4 imp/s when the temperature was increased to 3°C. LaMotte and Thalhammer (1982) reported 3 C-HCRs, 2 that were unresponsive to heating up to 70°C. The total number of impulses elicited from these HCRs increased monotonically as the size of the temperature step increased. Handwerker et al. (1991) found that ~10% of mechanically-insensitive C-fibres were activated by ice placed in their RF. In contrast, Georgopolous's (1976) survey of 191 C-fibres subserving the glabrous skin of the hands of monkeys did not identify a single fibre that was responsive exclusively to noxious cold stimuli.

C-PMNs are the most common unmyelinated nociceptor, making up between 30–90% of the cutaneous population in rats (Fleischer *et al.* 1983; Leem *et al.* 1993), cats (Bessou & Perl, 1969), rabbits (Shea & Perl, 1985), monkeys (Georgopoulos, 1976; Kumazawa & Perl, 1977) and humans. In monkeys, Georgopolous (1976) reported that ~45% of C-PMNs were cold-sensitive, most with thresholds between 0–10°C. LaMotte and Thalhammer (1982) found that approximately 60% of C-PMNs responded to ice. Davis et al. (1993) identified 45% of mechanically sensitive C-fibres that gave some response to cooling with ethyl chloride or ice. Samut et al. (1985) reported that whereas 30% of cat C-PMNs were responsive to ice presented in their RFs, 40% were activated by a –40°C stimulus. In the rat, Leem et al. (1993) found that ~10% of C-PMNs were responsive to ice water, while Simone and Kajander (1996) reported that 75% were responsive to temperatures $\leq 0^\circ\text{C}$. The marked differences in the properties of cold-sensitive C-PMNs found between these two rat studies is most likely due to the minimum temperature of the stimuli employed. In contrast, none of the C-PMNs identified in rabbits by Kumazawa and Perl (1977) were responsive to transient cooling pulses to a minimum temperature of 5°C. This total lack of findings cannot easily be explained by methodological differences and may be a species effect. These data indicate that it cannot be assumed that all C-PMNs respond to noxious cold stimulation. On the other hand, noxious cold search stimuli are rarely used to initially identify the receptors characterized in these studies. Therefore, a systematic selection bias may exist against noxious cold-sensitive units.

1.4.2.b. Myelinated Nociceptors

In a comprehensive survey of 345 A δ -nociceptors by Geogopolous (1976), no units were identified that responded exclusively to noxious cold stimuli (8°C and ice). On the other hand, 5 cold-specific A δ -nociceptors were identified in the monkey and cat by LaMotte and Thalhammer (1982). Their RFs were continuous and displayed a wide range of sizes (1-145mm²). Lowering the adapting temperature resulted in decreased thresholds for firing but had no effect on the dynamic responses to cooling steps. In addition, the total number of impulses increased monotonically with the size of the cooling step below threshold which was found to be 10-25°C for glabrous skin nociceptors and 19-25°C for hairy skin nociceptors. Ice placed on the nociceptor's RF caused a slight fatigue in its response to subsequent stimulations.

Geogopolous (1976) identified twice as many A δ -nociceptors responsive to mechanical, heat and cold stimuli (i.e. polymodal) than were responsive exclusively to mechanical and cold stimuli. These A δ -PMNs mainly displayed cold thresholds below 20°C, whereas those for heat were mostly above 50°C. Their RFs were usually less than 5mm², however some were found to be as large as 225mm². Spontaneous activity was not a prominent characteristic but, when it was observed, it consisted of discharge frequencies between 5-10 imp/s. Response to a test stimulus at 8°C resulted in continuous low frequency firing (10-50 imp in the first 10 s) that increased towards a plateau after several seconds. Afterdischarges and sensitization were noted occasionally. Bursting as a result of noxious cold stimulation was found in only two units (Georgopoulos, 1976). LaMotte and Thalhammer (1982) reported that A δ -PMNs responded with a low frequency discharge (6.5imp/s) to ice placed in their RF. Leem et al. (1993) identified A δ -nociceptors in the rat that responded to cold temperatures between 12-22°C as well as to noxious heat between 47-52°C. Simone & Kajander (1997) identified 9 A δ -nociceptors that responded to noxious mechanical, heat and cold stimuli in the rat hindlimb. While most of these units responded to a -12°C stimulus, all responded to a -18°C stimulus. The number of spikes evoked, the discharge rate, and the peak rate all increased monotonically as the temperature decreased.

1.4.3. Vascular Receptors

In 1930, Lewis described the "Hunting" reaction in which the temperature of the finger,

when immersed in water below approximately 18°C, initially falls but is subsequently followed by alternating periods of temperature increases and decreases. The pain induced by immersion disappeared as the finger temperature increased. It was later observed that cold, pain, numbness, and burning were related to decreases in blood flow and, therefore, to vasoconstriction (Abramson *et al.* 1966). Heating of the subjects' bodies, thereby inducing vasodilation, eliminated these symptoms and returned blood flow to almost control levels. Although these studies suggested that pain due to cold temperature was somehow related to cutaneous vasoconstriction, the mechanism for this effect was not discussed. Another important observation was made in 1951 by Hensel and Zotterman who found that infusion of cold water into the lingual artery caused activation of cold fibres.

The association between cold pain and the cutaneous vasculature was examined in more detail by Fruhstorfer and Lindholm (1983) who infused saline of varying temperatures into the empty veins of human volunteers. While infusion of saline below 26°C evoked sensations of cold only, saline below 20°C elicited sensations of both cold and pain. Pain tolerance was reached with saline of 5°C. The authors concluded that there are two separate types of vascular receptors: a cold-specific receptor and a nociceptor and that 'cold pain' may result from a concomitant activation of the two receptor types. In contrast to this hypothesis, a series of experiments by Klement and Arndt (1991) have argued that veins contain pain receptors only. In one series of elegant experiments the veins and cutaneous skin of human volunteers were anaesthetized, while measuring the psychophysical response to intravenous infusion of cold saline. During selective intravenous anaesthesia, cold saline infusion evoked sensations of innocuous cold only, whereas during selective cutaneous anaesthesia, only pain sensations were noted. These results indicate that nociceptors in the veins are responsible for the pain sensation elicited from venous cooling. In a subsequent study, Arndt and Klement (1991) found that regardless of the modality of venous stimulation (cold, heat, electrical, mechanical and osmotic), subjects felt pain at the site of stimulation and described its noxious quality as sharp, aching, and unpleasant. Furthermore, during selective cutaneous anaesthesia subjects could no longer differentiate between the various modalities of stimulation, with the exception of electrical stimulation, which indicated that human veins are innervated primarily by polymodal nociceptors which sense the noxiousness of

stimuli only. These results also suggested that cutaneous receptors may be involved in distinguishing the various modalities of noxious stimuli.

In a later study, Klement and Arndt (1992) examined the effect of applying ice water to the hand on intracutaneous and intravenous temperatures and evoked pain. Although cold-induced aching pain began after the sensation of numbness was felt, it persisted almost 100 seconds after the numbness had decreased following rewarming. Maximum pain intensity ratings were reported when the vein wall temperature reached 9°C. Both the threshold and the intensity of cold-induced pain were found to be more highly correlated with the intravenous compared to the intracutaneous temperature. In addition, while perivenous block resulted in a marked reduction in cold pain, intravenous block stopped it completely. These findings confirmed that the vein wall temperature was the critical determinant for cold pain sensation.

Further evidence that deep receptors are critical for cold pain sensation was demonstrated in a study by Kreh et al. (1984). This group used photoplethysmography to measure the relative blood flow to the finger in response to maintained cooling at 0°C and a thermocouple positioned 1-1.5mm below the surface of the skin to measure intracutaneous temperature. Although pain intensity was correlated with blood flow, the decisive parameter for predicting cold pain was intracutaneous temperature. The threshold for cold pain was found to correspond to an intracutaneous temperature of approximately 20°C. In addition, the increased pain intensity due to deep inspiration was less correlated with the resultant reflex vasoconstriction than with decreased intracutaneous temperature. Also, experimentally occluding blood flow using an inflated blood pressure cuff decreased intracutaneous temperatures and heat flux and increased pain ratings markedly. The authors concluded that because pain intensity was low or absent during initial cooling and vasodilation, even though the surface temperature was in the painful range, deep rather than superficial skin receptors are critical for cold pain perception.

1.4.4. The Involvement of the Sympathetic Nervous System in Cold and Cold Induced Pain Sensations

The SNS has been implicated in several important clinical pain syndromes. For example, blocking sympathetic discharge through surgical interruption, local anaesthetic or infusion of

blocking chemicals has been implicated in pain relief in some patients with: reflex sympathetic dystrophy syndrome (RSDS), causalgia, neuromas, rheumatoid arthritis, herpes zoster, Raynaud's disease, Sudeck's atrophy, and phantom limb pain (Jänig, 1985; Schott, 1986; Verrill, 1989; Roberts & Kramis, 1990; Jessell & Kelly, 1991; IASP Task Force on Taxonomy, 1994). Not all patients respond positively to this intervention and, for others that do respond, pain relief may not be long-term (Verrill, 1989). Furthermore, surgical sympathectomy in non-pain patients can itself cause localized symptoms of causalgic pain and hyperpathia in 18-25% of patients (Schott, 1986). In causalgia, the SNS has also been implicated due to the alteration in sweating and blood flow that often accompanies the characteristic persistent burning pain (Jänig, 1985; Schott, 1986; IASP Task Force on Taxonomy, 1994). Also, NE applied transcutaneously produces burning pain in causalgia (Jänig, 1985) and sympathetically maintained pain (Davis *et al.* 1991). Although sympathetic nerve stimulation is sufficient to whiten skin and decrease skin temperature by -0.5°C (Perl, 1994), its specific and necessary role in the abnormally cold skin found in some peripheral neuropathic syndromes was put into question in the rat model of Wakisaka *et al.* (1991). While a deficit in NE was found in the vasculature of some rats with abnormally cold paws, indicating SNS involvement, other rats displayed normal NE levels with abnormally hot paws or normal paws with abnormal NE levels. The authors concluded that sympathetic vasomotor activity was not necessarily involved in peripheral temperature abnormalities.

The SNS does not appear to play a significant role in pain generation in undamaged skin and nerve (Raja, 1995). For example, sympathetic nerve stimulation does not activate C-PMNs (Wiesenfeld-Hallin & Hallin, 1984; Sato & Perl, 1991; Perl, 1994) or AMHs (Roberts & Elardo, 1985) without prior sensitization, although it has been found to significantly lower the thermal threshold for C-PMNs in the rabbit pinna (Barasi & Lynn, 1998). On the other hand, Spray (1974b) reported that SNS activation and NE administration enhanced the sensitivity of frog cold receptors. This enhancement consisted of a wider range of thermal sensitivity as well as increased responses of the receptors to static and dynamic cold stimuli. In the trigeminal nucleus of rats, Davies (1984) showed that low-frequency sympathetic stimulation ($<5\text{Hz}$) caused enhancement, whereas high-frequency stimulation ($>10\text{Hz}$) caused suppression of cold-sensitive

neuron activity. Since suppression was also induced by bilateral carotid occlusion, Davies suggests that it may occur as a result of peripheral vasoconstriction due to anoxia.

Microelectrode recordings indicate an increase in muscle, but not skin, sympathetic nerve activity in response to painful cold stimulation (Fagius *et al.* 1989; Seals, 1990; Kregel *et al.* 1992). Kregel and co-workers (1992) measured increased muscle SNS activity from the cold pressor test only when there was a concomitant increase in perceived pain. The authors present several lines of evidence suggesting that the SNS activation by noxious cold was mediated by C-PMNs.

1.4.5. Physiological Differences Between Hairy and Glabrous Skin Thermal Receptors and Nociceptors

Identifying physiological differences between the receptors subserving hairy and glabrous skin in the studies mentioned above and in those concerned with non-cold stimuli is often problematic. Some researchers do not make specific distinctions between the receptors underlying hairy and glabrous skin while, in those that do, results may not specifically compare the two.

Comparisons of responses of hairy with glabrous skin receptors to innocuous cool and noxious cold are not abundant in the literature. This relative paucity of information may represent a lack of research on cold in general rather than a systematic failure to make such contrasts. In Perl's (1968) study of primate mechanoreceptors, only hairy skin receptors were found to be responsive to sudden cooling of their receptive fields. LaMotte and Thalhammer (1982) found no difference in the responsiveness of hairy and glabrous skin A δ -PMNs, C-PMNs or HCRs to noxious cold stimulation.

Findings on the differential effects of innocuous warm and noxious heat on hairy and glabrous skin receptors is mixed, with some groups (Campbell & Meyer, 1983; Treede *et al.* 1995) but not others (Fleischer *et al.* 1983; Lynn & Shakhaneh, 1988) finding differences between the two receptor types. Campbell and Meyer (1983) observed that hairy but not glabrous CMHs were sensitized to successive heat trials, which included a decrease in the thermal thresholds of hairy skin receptors. In addition, a 53°C burn caused sensitization in hairy skin

receptors and desensitization in glabrous skin receptors. It was also found that glabrous skin was significantly warmer than hairy skin upon initial measurement, although this evidently did not affect thermal thresholds which were equal for the two sites. Treede *et al.* (1995) identified a subtype of AMHs that was present in hairy skin but not glabrous skin nerves. The fibre subtype absent from glabrous skin nerves (Type II) displayed significantly shorter response and peak latencies, as well as lower heat thresholds and faster CVs than Type I fibres which were present in the nerves of both skin types. In contrast, similarities have been identified in the thresholds of hairy and glabrous skin receptors to innocuous (Fleischer *et al.* 1983) and noxious heat (Campbell & Meyer, 1983) stimuli. Furthermore, Fleisher *et al.* (1983) found no difference in the proportion of receptors that were sensitized, desensitized or unchanged to innocuous and noxious thermal stimuli. Similar responses between glabrous and hairy A δ -HTMs (Lynn & Shakhaneh, 1988) and CMHs (Treede *et al.* 1995) to supra-threshold heat stimulation have also been reported.

Okamoto *et al.* (1994) showed that innocuous thermal stimuli can effect the sudomotor and vasoconstrictor responses differentially in the hairy and glabrous skin sympathetic efferents of human volunteers. Whereas cooling from thermoneutral resulted in enhanced activity in both measures and in both skin types, warming increased activity in hairy skin but decreased activity in glabrous skin. Thus, thermal stimuli may cause differential sympathetic nerve activity depending upon the skin type.

As with both cold and heat described above, responses of glabrous and hairy skin receptors to mechanical stimuli have produced mixed results. Perl (1968) reported that the thresholds of slowly-adapting mechanoreceptors (SA-Ms) were higher in glabrous skin than in hairy skin, as was found for CMHs (Campbell & Meyer, 1983; Treede *et al.* 1995). On the other hand, no differences were found in mechanical thresholds for A δ -HTMs (Lynn & Shakhaneh, 1988) and both A δ and C-PMNs (LaMotte & Thalhammer, 1982). In addition, Perl (1968) found that hairy and glabrous HTMs displayed the same irregular 100imp/s response to a 20g pin prick.

CVs of hairy and glabrous primary afferents were not found to be significantly different in A δ -HTMs (Lynn & Shakhaneh, 1988) and CMHs (Campbell & Meyer, 1983). Conversely, higher CVs have been demonstrated for monkey glabrous skin cold-sensitive and heat-sensitive

A δ -nociceptors (Treede *et al.* 1995).

1.4.6. The Effect of Rate of Temperature Change on Cutaneous Receptor Physiology

(a) Cold

Hensel (1953) may have been the first to recognize the importance of the rate of cooling on the response of cold receptors, stating that "(t)he more slowly the temperature changes, the closer the receptor temperature approximates the surface temperature". He found that slower cooling rates led to smaller excitation overshoots in the response of lingual cold receptors. These overshoots are caused by jumps in the discharge frequencies of receptors due to sudden cooling which subsequently decline to new stable firing rates. More recently, researchers have used Peltier-type thermodes to better control the speed with which cooling stimuli are presented to the test area. For example, Molinari and Kenshalo (1977) recorded from cold-sensitive fibres in the feline trigeminal ganglia. They observed that increasing the cooling rate from $< 0.5^{\circ}\text{C/s}$ to 2°C/s resulted in increased mean discharge frequencies to dynamic temperature steps, peak discharge rates, and shorter latencies to peak discharge rate. Sumino and Dubner (1981) reported steeper stimulus-response functions and higher peak discharge rates for faster cooling rates in monkey facial A δ cold receptors. Kenshalo and Duclaux (1977) made similar observations in primate hairy and glabrous cutaneous limb receptors. Faster rates resulted in higher peak and average frequencies as well as cumulative impulses within the first 4 seconds of responding. Conversely, the total number of impulses recorded for the entire trial decreased with faster rates of cooling.

While these studies have not confirmed or refuted Hensel's hypothesis on the effect of cooling rate on temperature at the receptor, they do indicate that faster cooling rates cause cold receptors to become more active and, possibly, to more accurately encode the "coldness" of cutaneously applied stimuli. The above mentioned studies involved recordings of the responses of innocuous cold receptors to innocuous cold stimuli only. Similar studies using temperatures in the noxious range while recording from nociceptors do not seem to have been performed. Thus it is not known if faster cooling rates also cause cold-sensitive nociceptors to become more active.

(b) Heat

The effects of heating rate on the response thresholds of heat-sensitive nociceptors are somewhat controversial. While Croze *et al.* (1976) and Yarnitsky *et al.* (1992) found that the heating rate had no effect on primate and human C-PMN response thresholds respectively, faster heating rates were found to elevate thresholds in feline (Bessou & Perl, 1969) and primate C-PMNs (Tillman *et al.* 1995), and in both A δ and C heat-sensitive fibres in the rat (Yeomans & Proudfit, 1996). Kenshalo (1996) found that primate warm-sensitive fibres increase their thresholds only with very slow heating rates ($< 0.1^{\circ}\text{C/s}$), which suggests that adaptation may occur at very slow heating rates.

The responses of heat-sensitive fibres to supra-threshold heating is less controversial; faster rates have been found to increase mean discharge frequencies in rat A δ -fibres (Yeomans & Proudfit, 1996), feline C-PMNs (Bessou & Perl, 1969), primate warm fibres (Kenshalo, 1996), and human C-PMNs (Yarnitsky *et al.* 1992). Yeomans and Proudfit (1996) demonstrated that fast heating rates (6.5°C/s) predominately lead to responses in A δ -fibres, whereas slow heating rates (0.9°C/s) excited mostly C-fibres. Also, foot withdrawal responses, which occurred at lower temperatures with slower heating rates (Yeomans & Proudfit, 1996), were subsequently found to be mediated by C-fibres (Yeomans *et al.* 1996). Foot withdrawal responses occurring at higher temperatures from faster heating rates were found to be mediated by some other nociceptor, which the authors suggest were A δ -fibres (Yeomans *et al.* 1996). In humans, correlations between the reaction times for pain sensation and microneurographic recordings indicate that, at heating rates of 2°C/s , noxious heat messages are exclusively mediated by C-nociceptors (Yarnitsky *et al.* 1992). The differences found across studies may be due to the species tested or to methodological differences similar to those shown in Table I for studies on noxious cold. In addition, different anaesthetics were used and, in some studies, paralytic drugs were used across studies, both of which could effect the results obtained.

1.5. Function of the Receptor

Evidence suggests that cold receptor sensitivity is most likely due to a high surface area:volume ratio in conjunction with active ionic pumps in their terminal processes. The

numerous fine terminal arborizations of FNEs (described in Section 1.3.3. above) result in a huge surface area which allows a small movement of ions across the membrane along the temperature gradient across the membrane to produce significant depolarization (Spray, 1974a; Spray, 1986). A temperature-dependent sodium-potassium (Na-K) pump may mediate ion movement (Pierau *et al.* 1974) and the bursting discharge that occurs in some cold receptors (see Section 1.4.1.) may rely on a calcium-dependant pace maker (Schäfer *et al.* 1982).

Pierau and co-workers (1974) evaluated the role of temperature-dependent electrogenic Na-K pumps in the functioning of cold afferents. According to the authors, cooling of *Aplysia* neurons results in depolarization due to activation of these electrogenic pumps and a counterbalancing hyperpolarization due to passive kinetic forces which favour K entering the cell. Conversely, in mammalian neurons these kinetic forces favour the passive entry of Na into the cell thereby activating it. Thus, Pierau *et al.* (1970) suggest two possible mechanisms for cold transduction: the electrogenic Na pump and the differential membrane kinetics of K and Na. To test this hypothesis, they examined the effects of ouabain, an ATPase inhibitor, on the discharge rates of physiologically identified thermal afferents in the rat scrotum. At warm temperatures (38°C), ouabain increased the fibre's discharge frequency while at cool temperatures (22°C), ouabain had no effect. It was concluded that the electrogenic pump is active at warm temperatures which hyperpolarizes the cell and results in a low discharge frequency. At cooler temperatures the pump becomes inactivated allowing the passive membrane properties to take over, which highly favours Na entering the cell thereby depolarizing it. As described by Spray (1986), this electrogenic pump is very sensitive to extracellular K; as the K concentration around cold receptors increases, a gradual decline in static sensitivity and rise in dynamic response occurs. On the other hand, decreasing K concentrations cause increased static activity in cold receptors.

Schäfer (1982) demonstrated that the functioning of cold receptors also requires a proper balance of both calcium and potassium ions in the glabrous skin of the noses of cats. Intravenous calcium infusion resulted in decreased discharge rates to static temperatures, cumulative numbers of impulses to dynamic 5°C temperature drops, and cold bursts. I.V. infusion of the calcium chelator EDTA resulted in opposite effects as well as the appearance of bursting

responses in previously non-bursting cells. A possible mechanism to explain bursting, related to the mollusk pacemaker, is described in which oscillating low and high extracellular calcium concentrations would induce and terminate bursting respectively.

2. The Dorsal Horn and Spino-thalamic Tract

2.1. Anatomy of the Spinal Cord Areas Involved in Cold Pain Sensation and Transmission

The most common skin sites used to measure the effects of cutaneously applied innocuous and noxious cold stimuli are the limbs and the face. The cell bodies of the primary afferent fibres subserving cold receptors and nociceptors of the limbs reside in the DRG just dorso-lateral to the spinal cord. These neurons send projections peripherally to cutaneous terminals and centrally to the spinal cord grey matter. The central projections ascend and descend one or two spinal cord segments within the tract of Lissauer, terminating in the superficial dorsal horn (Fitzgerald, 1989; Martin & Jessell, 1991a; Spence & Mason, 1992b). The nucleus caudalis of the spinal trigeminal nucleus receives afferent input from orofacial structures and is analogous to the spinal dorsal horn (Fitzgerald, 1989; Dodd & Kelly, 1991).

A variety of methods have been used to identify the specific laminae containing innocuous and noxious cold sensitive neurons including: microlesions (Burton, 1975; Craig & Kniffki, 1985; Hutchison *et al.* 1995; Dostrovsky & Craig, 1996), staining with c-fos (Strassman *et al.* 1993), dyes (Christensen & Perl, 1970; Dostrovsky & Hellon, 1978) or plant lectin (Sugiura *et al.* 1986), and estimating depth with a microdrive (Dostrovsky & Hellon, 1978; Davies *et al.* 1985). These studies indicate that low-threshold cold neurons are found mainly in Lamina I and II (Christensen & Perl, 1970; Dostrovsky & Hellon, 1978; Craig & Kniffki, 1985; Davies *et al.* 1985; Strassman *et al.* 1993; Hutchison *et al.* 1995; Dostrovsky & Craig, 1996) whereas thermal nociceptive neurons may additionally be found in deeper layers, including laminae III-IV in guinea pigs (Sugiura *et al.* 1986), laminae V-VIII in the cat (Burton, 1975), and laminae IV-V in monkey (Surmeier *et al.* 1986b; Surmeier *et al.* 1986a).

At least five major spinal cord pathways have been identified in conveying nociceptive information to supra-spinal areas: the spinothalamic tract (STT), the spinoreticular tract, the spinomesencephalic tract, the spinocervical tract, and the postsynaptic dorsal column tract

(Willis, 1989; Jessell & Kelly, 1991). The origin, termination, and relative importance of these nociceptive tracts differ across species (Willis, 1989; Willis & Westlund, 1997). The STT is the most prominent of these pathways, carrying information from nociceptive-specific (NS) and wide dynamic range (WDR) neurons from Lamina I and IV-VIII to the thalamus. These fibres decussate at the spinal segment where they originate, ascend within the anterolateral tract, and terminate in two major subdivisions of the thalamus. The medial thalamus mainly receives input from Laminae VII and VIII, while the lateral thalamus primarily receives its input from NS and WDR neurons of Laminae I and V (Willis, 1989; Jessell & Kelly, 1991; Craig & Dostrovsky, 1997; Willis & Westlund, 1997). Although most of these STT cells project to the contralateral thalamus, 10-15% project ipsilaterally (Craig & Dostrovsky, 1997; Willis & Westlund, 1997). Evidence also indicates that the STT projections are organized somatotopically with lower body areas travelling more laterally than upper body areas (Craig & Dostrovsky, 1997; Willis & Westlund, 1997).

2.2. Physiology of Spinal Cord Neurons Involved in Cold and Cold Induced Pain

2.2.1. Innocuous Cold

Responses of spinal and medullary dorsal horn neurons to innocuous cold stimulation will be combined for the purpose of generalizability. The RFs of dorsal horn neurons are typically substantially larger than those of peripheral primary afferents, with estimates ranging from $>20\text{mm}^2$ (Burton, 1975) to $>10\text{cm}^2$ (Iggo & Ramsey, 1976) to a maximum of one quarter of the entire monkey's hind paw (Dostrovsky & Craig, 1996) or half the cat's face (Dostrovsky & Hellon, 1978). This magnification of RF implies substantial convergence of peripheral afferents onto dorsal horn neurons. Dorsal horn "cold" neurons generally have background activity that is decreased or stopped entirely by warm stimulation (Christensen & Perl, 1970; Burton, 1975; Iggo & Ramsey, 1976; Dostrovsky & Hellon, 1978; Craig & Kniffki, 1985; Davies *et al.* 1985; Dostrovsky & Craig, 1996). Interestingly, Davies *et al.* (1985) showed a cessation of background activity following ganglionectomy, suggesting that peripheral input is required for this ongoing activity. Like cutaneous cold receptors, dorsal horn neurons are capable of responding to very small changes in temperature, some as small as 0.5°C (Poulos & Lende, 1970) and often display

an inverted U-shaped function peaking in the range of 20-30°C (Dostrovsky & Hellon, 1978; Davies *et al.* 1985; Hutchison *et al.* 1995). Generally, dorsal horn neurons increase their discharge frequency as the size of dynamic temperature steps increases (Poulos & Lende, 1970; Burton, 1975). Bursting discharge patterns, which are common in cutaneous cold receptors, are generally not found in spinal cord neurons (Dostrovsky & Hellon, 1978; Davies *et al.* 1985; Dostrovsky & Craig, 1996), but were identified by Christensen and Perl (1970) as occurring with an instantaneous frequency of 100-200Hz. According to Dostrovsky and Hellon (1978), this lack of bursting is likely due to desynchronization of primary afferent input due to convergence on spinal cord neurons. The only study to systematically investigate how cooling rate influences neural activity (Davies *et al.* 1985) reported that increasing cooling rates resulted in a steady increase in the peak firing rate of medullary dorsal horn neurons.

2.2.2. Noxious Thermal

Very few studies of spinal cord and medullary dorsal horn neurons have employed cold stimuli that would be considered painful in human psychophysical studies. Because those studies that did use temperatures under approximately 15°C typically used an innocuous cold search stimulus to identify sensitive neurons, the lack of noxious cold responsive neurons may be due to a selection bias. Kenshalo *et al.* (1982) identified dorsal horn neurons that significantly increased their peak response to temperature steps from 20°C to 5°C but not from 20°C to 15°C or 10°C, indicating the possibility that they could distinguish between levels of noxious cold. Davies *et al.* (1985) used brass rods cooled to 0°C as search stimuli and found that activities in all the neurons that they identified approached zero as the temperature was lowered to 10°C. Craig and Kniffki (1985) characterized heat-pinch-cold (HPC) cells that responded equally well to noxious pinch, heat, and cold. Since ice held against the cell's RF was the only cold stimulus used, it cannot be inferred whether these HPC neurons respond preferentially to noxious or innocuous cold stimuli. More recently, Craig and Bushnell (1994), characterized HPC cells in the cat that responded to an innocuous cold (20°C) stimulus. Although Sugiura *et al.* (1986) stained neurons from cold nociceptors responsive to marked cooling below 15°C, they did not specify their response properties.

Noxious heat has been found to be an adequate stimulus for some or all dorsal horn neurons responsive mainly to cold stimulation in their RFs by some groups (Christensen & Perl, 1970; Burton, 1975; Kenshalo, Jr. *et al.* 1982; Craig & Kniffki, 1985; Hutchison *et al.* 1995) but not by others (Poulos & Lende, 1970). Further, prior noxious heat stimulation sensitizes some neurons, leading to increased background discharges (Hämäläinen *et al.* 1985) and responses to cold in previously unresponsive neurons (Christensen & Perl, 1970). Conversely, Burton (1975) found cold neurons that decreased their response to cold after heat stimulation above 42°C. Studies have also shown that some cold neurons respond to innocuous (Hutchison *et al.* 1997) and noxious (Burton, 1975; Sugiura *et al.* 1986) mechanical stimulation.

2.3. Differences in Spinal Cord Inputs from Hairy and Glabrous Skin Sites

Although specific comparisons of the properties of dorsal horn neurons receiving inputs from glabrous and hairy skin sites are rare, two studies have specifically looked at these differences for heat stimulation (Surmeier *et al.* 1986b; Surmeier *et al.* 1986a). While no differences in thermal thresholds were found for laminae IV-V STT neurons, neurons with inputs from hairy skin sites displayed significantly lower thresholds to noxious heat (Surmeier *et al.* 1986a). In addition, the long latency subclass of neurons with hairy skin inputs displayed shorter onset and peak latencies to noxious heat stimuli (Surmeier *et al.* 1986b). However, higher mean discharge rates for the entire population of STT neurons were found for neurons with glabrous skin inputs for stimuli >49°C (Surmeier *et al.* 1986a).

3. Supra-spinal Areas Involved in Cold and Cold Pain Sensation

3.1. Thalamus

There has been extensive testing of thalamic nuclei with noxious heat stimuli in animal studies. Research in the monkey suggests that lateral ventroposterior thalamic neurons, with their small RFs, are well suited for signalling spatial information whereas medial thalamic neurons, with their large bilateral RFs and sensitivity to the attentional state of the animal, may provide other intensity and affective components of pain (Bushnell & Duncan, 1989; Apkarian & Shi, 1994).

Physiological studies of the thalamic areas involved in processing noxious cold input suffer from the same methodological problems as those discussed for the peripheral and spinal processing and relay areas. Noxious cold search stimuli are almost never used and, when noxious cold stimuli are used to test identified neurons, often only ice or cold probes are used which do not accurately differentiate between innocuous and noxious-related activity.

Of the several hundred medial ventrobasal (VB) neurons studied by Poulos and Benjamin (1968) in the monkey, only 15 responded specifically to thermal stimulation while another 20 responded additionally to mechanical stimulation (T+M). All of these thermal-sensitive neurons responded to innocuous cooling, with a peak rate of activity around 29°C. Although T+M neurons generally did not give sustained responses to constant cold stimuli, they did increase their response linearly as the magnitude of the cooling stimulus increased, and thus were assumed to be capable of providing precise information about cooling. Burton et al. (1970) found only T+M neurons in the dorsal VB of the monkey using a 16°C search stimulus in peripheral glabrous skin. Many of these neurons displayed similar peak frequencies as the cold-sensitive primary afferent and dorsal horn neurons described above (17-19°C), however others were described with peaks at higher temperatures and still others which did not reach their peak discharge to the lowest temperature tested (15°C). Lesions sparing only the dorsal columns abolished all thermal responses suggesting that STT input was vital for the functioning of these neurons. Bursts to cooling between 37-32°C were described in 61% of the warm cells identified in the rostral VB of rats by Jahns et al. (1975). These bursts consisted of between 2-8 impulses which increased in frequency as the temperature decreased. Eighty percent of the cold neurons characterized in the VB of cats by Auen et al. (1980) displayed ipsilateral RFs in the facial region that ranged in size from 1-30 mm². The authors theorize that the ipsilateral nature of these RFs indicates a direct projection to the thalamus via the ventral principal trigeminal nucleus. Craig et al. (1994) found that almost all of the primate neurons identified in the posterior aspect of the ventromedial nucleus (VMpo) were specifically responsive to noxious or thermal stimuli, including an ice cube. This thermal-specific area was also identified by Craig et al. (1994) in the human thalamus. Lenz et al. (1993a) recorded from the ventral caudal nucleus (Vc), which is analogous to the monkey VB, in patients undergoing stereotactic surgery for treatment of chronic

pain. Although neurons were found which responded to the presentation of innocuous and noxious cold, heat and mechanical stimuli peripherally, the largest initial response occurred with the noxious cold stimulus. Davis *et al.* (1997) recorded from neurons in the VMpo of 3 patients which responded both to innocuous cold and noxious heat stimuli presented cutaneously.

Perceptually, cold sensation has been elicited by microstimulation in the Vc (Lenz *et al.* 1993b) and VMpo (Davis *et al.* 1997) in humans. The perception of cold was found to increase in intensity (i.e. became colder) concomitantly with an increase in the intensity of stimulation in VMpo but did not become painful (Davis *et al.* 1997). The projected fields in Lenz's (1993b) study were large and often encompassed more than one body area, indicating extensive convergence in this area of peripheral inputs.

3.2. Imaging Studies and Clinical Reports

Improvements in both the spatial and temporal resolution of modern brain imaging techniques, make possible visualization of the brain regions with increased blood flow in response to painful thermal stimulation. Although several studies have analyzed brain activations from noxious heat stimuli, few have used noxious cold (see Table II). In addition, while studies of noxious heat employ either a laser or a thermode to deliver stimuli to the skin, the studies of noxious cold have mostly used a circulating water bath and are only now beginning to use thermodes (e.g. Davis *et al.* 1998). Until research accumulates with cold stimuli delivered via thermodes, the correlation between the peripheral studies outlined above (which almost exclusively use thermodes) with human brain mapping studies will remain contentious.

Table II shows the major brain areas that have been found to become activated following noxious thermal stimulation in the periphery. The brain areas most commonly activated in response to noxious heat stimulation contralaterally are: thalamus (Jones *et al.* 1991; Casey *et al.* 1994; Coghill *et al.* 1994; Vogt *et al.* 1996; Svensson *et al.* 1997; Davis *et al.* 1998b), primary somatosensory cortex (SI) (Talbot *et al.* 1991; Casey *et al.* 1994; Coghill *et al.* 1994; Rainville *et al.* 1997; Svensson *et al.* 1997), secondary somatosensory cortex (SII) (Talbot *et al.* 1991; Casey *et al.* 1994; Coghill *et al.* 1994; Casey *et al.* 1996; Craig *et al.* 1996; Rainville *et al.* 1997; Svensson *et al.* 1997; Xu *et al.* 1997; Davis *et al.* 1998b), anterior cingulate cortex (ACC) (Jones

Table II: Brain Imaging Studies of Noxious Thermal Stimulation

Study	Method	Sub #	Area	Stimuli	ACC	SI	SII	Thal	Lenti	Insula	Midb	CB	Front
Talbot et al. 1991	PET	8	volar forearm	heat	c	c	c						
Jones et al. 1991	PET	6	DLH	heat	c			c	c				
Casey et al. 1994	PET	9	volar forearm	heat		c	b	b		c	i	i	
Piero et al. 1994	SPECT	7	hand	cold		c							c
Casey et al. 1996	PET	9	volar forearm	1. heat, 2. cold	1. c 2. b	2. c	1. c	1. c 2. i	1. c 2. i	1. c 2. b	1. i	1. b 2. i	1. i 2. i
Vogt et al. 1996	PET	7	DLH	heat	b i			b		b	b		c
Svensson et al. 1997	PET	10	volar forearm	heat	c	c	c	c	i	b	b	i	
Rainville et al. 1997	PET	n/a	hand	1. heat 2. 1aVAS	1. c 2. b	1. c 2. c	1. c 2. c						
Xu et al. 1997	PET	6	DLH	heat			b	b	i	b		b	c
Davis et al. 1998b	fMRI	12	TE	1. heat 2. cold			1. c 2. c	1. b 2. b		1. b 2. b			
Davis et al. 1998c	fMRI	6	TE	1. heat 2. cold	1. c 2. c								

note: Sub # = number of subjects; ACC = anterior cingulate cortex; SI = primary somatosensory cortex; SII = secondary somatosensory cortex; Thal = thalamus; Lenti = lentiform nucleus; Midb = midbrain; CB = cerebellum; Front = frontal cortex; PET = positron emission tomography; SPECT = single photon emission computerized tomography; fMRI = functional magnetic resonance imaging; DLH = dorso-lateral hand; TE = thenar eminence; heat = noxious heat; cold = noxious cold; c = contralateral; i = ipsilateral; b = bilateral; l = decreased activation; 1aVAS = scan where affect was decreased from hypnosis

et al. 1991; Talbot *et al.* 1991; Casey *et al.* 1996; Craig *et al.* 1996; Rainville *et al.* 1997; Svensson *et al.* 1997; Davis *et al.* 1998c), and insular cortex (Casey *et al.* 1994; Coghill *et al.* 1994; Casey *et al.* 1996; Craig *et al.* 1996; Vogt *et al.* 1996; Svensson *et al.* 1997; Xu *et al.* 1997; Davis *et al.* 1998b). Contralateral activation from noxious cold was localized in SI, lentiform nucleus, insula, and premotor cortex by Casey *et al.* (1996) and in SI, frontal and temporal cortices by Piero *et al.* (1994). Functional MRI has also been used to localize contralateral activation of SII, thalamus, insular cortex and the ACC in response to noxious contact cold (Davis *et al.* 1998b, 1998c). Craig and colleagues (1996) used 20°C and 5°C stimuli to examine the cerebral activation in response to innocuous and noxious cold respectively. While SII and insular cortex were activated by both stimuli, the ACC was activated for the noxious cold stimulus only. This differential activation of ACC was also found for noxious heat stimuli. These findings led the authors to suggest that the ACC is involved in the motivational, emotional aspects of pain. In contrast, because the insular cortex was activated by all innocuous and noxious stimuli, it was assigned the role of monitoring the general physiological state of body tissues (Craig *et al.* 1996).

Interestingly, ipsilateral activations have also been noted from noxious heat stimulation in: SII (Casey *et al.* 1994; Xu *et al.* 1997; Davis *et al.* 1998b), thalamus (Casey *et al.* 1994; Casey *et al.* 1996; Vogt *et al.* 1996; Xu *et al.* 1997), lentiform nucleus (Svensson *et al.* 1997), insula (Vogt *et al.* 1996; Svensson *et al.* 1997; Xu *et al.* 1997; Davis *et al.* 1998b), midbrain (Casey *et al.* 1994; Casey *et al.* 1996), cerebellum (Casey *et al.* 1994; Casey *et al.* 1996; Svensson *et al.* 1997; Xu *et al.* 1997), and frontal cortex (Casey *et al.* 1996). Casey *et al.* (1996) noted ipsilateral activation from noxious cold in: ACC, thalamus, insula, cerebellum, and frontal cortex, while Piero *et al.* (1994) only found ipsilateral activation in the temporal cortex. Davis *et al.* (1998b) identified ipsilateral activation in thalamus and insular cortex in response to noxious contact cold stimulation.

Davis and coworkers (1994) completed several thermal tests on a patient undergoing cingulotomy and capsulotomy for treatment of schizoaffective disorder. Surgery resulted in an increased heat threshold and ratings to supra-threshold heat concomitant with increased cold and pain plus decreased tolerance to the cold pressor test. Treatment of an obsessive-compulsive

patient by surgical lesions of the anterior capsule resulted in significantly decreased intensity and affective responses to noxious heat stimulation and increased pain and decreased tolerance to the cold pressor test (Talbot *et al.* 1995). These effects were no longer present 6 months post-operatively.

(II) **Psychophysics**

1. Introduction and History

Psychophysics arose as a specific branch of experimental psychology which challenged the notions of early empiricists, such as John Locke, who proposed that humans were born with a "tabula rasa" or blank mind upon which subsequent sensory experience was built (Martin, 1991). Psychophysics focussed on the relationship between a stimulus' physical characteristics and the sensory experiences that it evoked, and attempted to find mathematical expressions to define how all humans experience sensory stimuli. Ernst Weber described the just noticeable difference (JND) between two sensory stimuli using the formula $\Delta S = K \cdot S$; where ΔS is the minimum difference in strength needed to distinguish two stimuli, S is the reference stimulus and K is a modality specific constant (Goldstein, 1989; Martin, 1991). Gustav Theodore Fechner published perhaps the first psychophysical text, *Elemente der Psychophysic*, in 1860 in which he extended Weber's Law to describe the relationship between the intensity of a stimulus and its perceived magnitude. He used the equation $I = K \log(S/S_0)$; where I is the subjective intensity, S_0 is the threshold and S the supra-threshold stimulus used as a test. This equation introduced the concept that equal stimulus *ratios* resulted in equal *differences* in sensation (Stevens, 1967; Goldstein, 1989; Kruger, 1989; Martin, 1991; Borg, 1994). S.S. Stevens argued that since equal stimulus *ratios* produce equal sensation *ratios*, subjective experience should increase as a power rather than a logarithm. He derived the formula $\psi = k\phi^\beta$ to explain the fact that natural stimuli can vary vastly in intensity. In this formula ψ is the sensation, ϕ is the stimulus, k is a constant and β is the exponent of the power function (Stevens, 1967; Stevens, 1970).

In the 1940's, Woolf and his co-workers began to use psychophysical methods to assess and measure physical pain. From this point, research into the underlying aspects of the pain

experience proceeded slowly, but began to gather speed as the methods for delivering noxious stimuli improved, and as an interest in the neural mechanisms of pain increased (Borg, 1994). The development of psychophysics was helped immensely by the development of more quantifiable measures of pain from indirect or global measures of pain (such as pain tolerance) to direct magnitude scaling responses of supra-threshold stimuli. Further improvements in measures of pain have occurred because of the recognition that pain is a multi-dimensional experience. Thus, rating scales have been created for both the intensity and the affective (or emotional) aspects of pain.

2. Threshold Measurements

Sensory thresholds are a central concept in psychophysical testing of pain and temperature perception. All threshold methods ask subjects to identify a point on a continuum of increasing intensity at which either a thermal sensation or pain is felt. Several different techniques exist for this measure including: method of limits, method of levels, method of constant stimuli, staircase, and forced-choice. The main differentiating factor is whether or not the reaction time of the subject is included in the measurement of the threshold. In the method of constant stimuli, sub- and supra-threshold stimuli are presented to subjects and they indicate whether or not the stimulus was felt. Threshold is taken as the intensity for which detection occurs in 50% of trials (Goldstein, 1989). The method of levels involves presenting a stimulus and then making fine adjustments in its intensity until the exact threshold is obtained (Yarnitsky & Ochoa, 1990a). In the forced-choice method, subjects must decide in which of two successive trials the stimulus was actually presented (Claus *et al.* 1990).

2.1. The Method of Limits

A modern version of the method of limits (MOL) is similar to the classical psychophysical method of constant stimuli, except that the order in which stimuli are presented is not random. MOL is also conceptually similar to the Marstock method described by Fruhstorfer *et al.* (1976). In MOL, the subject reverses the direction of the temperature ramp (i.e. cooling or warming) of the thermode, usually by depressing a switch, whenever thermal or pain thresholds

are reached. The thermode temperature then returns to a baseline, and an inter-trial interval elapses before the initiation of the subsequent threshold measurement (Kenshalo *et al.* 1961; Fruhstorfer *et al.* 1976; Claus *et al.* 1990; Yarnitsky & Ochoa, 1990a; Yarnitsky & Ochoa, 1991).

The main advantage of MOL is that the operator is not directly involved during the measurement process. This allows it to be a more objective assessment of threshold (Fruhstorfer *et al.* 1976). In addition, the test can be completed quickly and easily by most subjects, thereby reducing the effects of attention or education (Fruhstorfer *et al.* 1976; Yarnitsky & Ochoa, 1990a). Hilz and colleagues (Claus *et al.* 1990; Hilz *et al.* 1995) found no significant difference between MOL and forced-choice in the retest reliability nor in the variability. Finally, although MOL cannot give the absolute thresholds due to its dependence on the subject's reaction time, comparisons between skin sites are still valid. This is especially true given that Pertovaara and Kojo (1985) found that minor fluctuations in conduction distance do not greatly affect threshold values in their comparisons of the thenar eminence and forearm.

However, there are also disadvantages to the MOL. First, threshold values vary based on the reaction time between sensation and switch depression (see Section 2.2. on rate below), thus providing an estimate rather than an absolute threshold value. As opposed to the research reported above, other studies have shown high levels of variability between and/or within subjects for the MOL which may decrease its ability to discriminate abnormal findings (Fruhstorfer *et al.* 1976; Hilz *et al.* 1995). Further, low repeatability has also been demonstrated which has been attributed to the effects of training or learning which may improve subsequent determinations (Yarnitsky & Sprecher, 1994).

Methods and results of studies examining cold and cold-induced pain thresholds are shown in Tables III and IV. In these tables, the term "limits" includes studies which used methods where a decreasing temperature is halted and reversed towards baseline when subjects make the desired response.

2.2. The Effect of Rate on Psychophysical Threshold

Cold threshold testing in human subjects has demonstrated both a reduction (Kenshalo *et*

Table III: Psychophysical Studies of Cold Thresholds

Study	Sub #	Site	Method	Thermode size (cm ²)	Cooling Rate (°C/s)	AT (°C)	Results Th (°C)	Comments ΔT (°C)
Kenshalo et al. 1961	4	fore	MOL	6	2.5?	27-45	0.4	Male ~ 1 Th (AT $\geq$ 30°C)
Kenshalo, 1966	4	fore	MOL	14.44	≤ 2	30-40	1.25	1 @ menses, 1 @ ovulation
Pertovaara & Kojo, 1985	8	TE, fore	MOL	12.5	1.4-3.9	n/a	n/a	1 rate ~ 1 Th; no effect of conduction distance
Claus et al. 1987	42	volar wrist	MOL	12.5	1-2.8	35	1.25	1 rate ~ 1 Th; no age effect
Fowler et al. 1987	209	DLH	Lev	7.5	1	n/a	≤ 1	no age effect
Strian et al. 1989	11	TE	MOL	6	0.5-1	n/a	1.14	no diurnal effect
Wahren et al. 1989	20	DLH	MOL	12.5	0.5-2	32	30.9	
Yarnitsky & Ochoa, 1990a	19	TE	MOL	12.5	2	32	30.5	
Yarnitsky & Ochoa, 1991	15	TE	MOL, Lev	12.5	2.4, 4.5, 6.6	32	MOL=1.5-3.2	Lev=no rate effect; MOL=1 rate ~ 1 Th
Verdugo & Ochoa, 1992	61	TE, hypo	MOL	12.5	n/a	32	TE=30.6; Hypo=30	
Greenspan et al. 1993	32	TE, fore	Stair	n/a	≤ 8	34	TE=1.5, fore=3	
Dyck et al. 1993	8	DLH	Forced Choice	2.9, 10	0.5, 1.5, 4	n/a	0.2-0.53	1 rate ~ 1 Th; no effect thermode size
Yarnitsky & Sprecher, 1994	106	TE	MOL, Lev, Stair	12.5	1	32	MOL=30.2; Lev=31.2; Stair=31.5	
Hilz et al. 1995	20	volar fore	MOL	12.5	n/a	30, 35	30-1.6; 35-2.0	1 repeatability w/AT = 30

note: n/a = not available; Sub # = number of subjects; AT = adapting temperature; ΔT = change in temperature; |Th| = absolute threshold; Th = threshold; DLH = dorso-lateral hand; TE = thenar eminence; hypo = hypthenar; fore = forearm; Lev = method of levels; MOL = method of limits; Stair = staircase method

Table IV: Psychophysical Studies of Cold-Induced Pain Thresholds

Study	Sub #	Site	Method	Thermode size (cm ²)	Cooling Rate (°C/s)	AT (°C)	Results Threshold (°C)	Comments
Fruhstorfer et al. 1976	26	TE	Marstock	12.5	1-1.5	30	14 (n=1)	1 rate ~ 1 Th
Croze & Duclaux, 1978	17	TE	MOL	6.5	0.3-2	20	0.3,0.8,1.4°C/s~ 11.6,10.3, 9.6°C	1 rate ~ 1 Th
Machet-Pietropaoli & Chery-Croze, 1979	5	n/a	MOL	0.25-8	1	20	1, 3.5, 4.9 cm ² -3,4.4, 5.3 °C	1 area ~ 1 Th
Pertovaara & Kojo, 1985	8	TE, forearm	MOL	12.5	1.4-3.9	n/a	n/a	1 rate ~ 1 Th; no effect of CD
Wahren et al. 1989	20	DLH	MOL	12.5	0.5-2	32	19.1±5.8	
Yarnitsky & Ochoa, 1990a	19	TE	MOL	12.5	4	32	13.4±1.2	
Verdugo & Ochoa, 1992	61	TE, hypo	MOL	12.5	n/a	32	TE=12.3±.6; Hypo=9±1	
Mengel et al. 1993	12	Teeth	n/a	15.9mm ²	n/a	n/a	1st pain (sharp)=14.2; 2nd pain (dull, burn) = 5	
Meh & Denislic, 1994	150	TE, Ventral Forearm	Marstock	12.5	1	30	TE=23.7, Forearm=24.6	No diff M/F
Morin & Bushnell, 1998	8	TE	iVAS	9	5	32	-9	

note: Sub # = number of subjects; AT = adapting temperature; Th = threshold; |Th| = absolute threshold; MOL = method of limits; iVAS = intensity visual analogue scale; TE = thenar eminence; hypo = hypthenar; M/F = male/female; CD = conduction distance

al. 1968; Claus *et al.* 1987) and an elevation (Pertovaara & Kojo, 1985; Yarnitsky & Ochoa, 1991) of thresholds in response to faster cooling rates with MOL. In addition, an elevation in threshold was also found in response to faster cooling rates using the forced-choice paradigm (Dyck *et al.* 1993).

Püter (1922), reported lower JNDs for slower rates of temperature change in the noxious cold range. Elevated cold-evoked pain thresholds in response to faster cooling rates have also been found (Fruhstorfer *et al.* 1976; Croze & Duclaux, 1978; Pertovaara & Kojo, 1985). Fruhstorfer *et al.* (1976) noted that cooling rates above 0.5°C/s resulted in higher deviations of pain thresholds from their absolute values, which were previously determined with a reaction-time exclusive method. Similar results were found with larger ranges of cooling rates by Croze and Duclaux (1978) (0.3-2°C/s) and by Pertovaara and Kojo (1985) (1.4-3.9°C/s). While all of these studies tested thresholds on the thenar eminence, Pertovaara and Kojo (1985) additionally tested the hairy skin of the forearm.

Although Croze and Duclaux (1978) observed that faster heating rates resulted in higher heat pain thresholds, Yarnitsky and Ochoa (1990a) found the opposite results for measurements with the reaction time exclusive method of levels and no effect of heating rate at all on MOL determinations. As expected, MOL gave higher threshold values compared to the method of levels, and these differences became more pronounced with faster heating rates. Davies *et al.* (1983) found no effect of heating rate on thermal thresholds at facial skin sites.

The most likely explanation for increased thresholds at faster rates of temperature change is a reaction time artefact (Croze & Duclaux, 1978; Yarnitsky & Ochoa, 1990a; Yarnitsky & Ochoa, 1991). Although the reaction time of subjects to a thermal stimulus is presumably equal regardless of the rate of change, a faster rate will result in a larger absolute change in the thermode temperature over this fixed time, resulting in an artificially high threshold. Yarnitsky and Ochoa (1991) provide evidence in support of this hypothesis by additionally testing the effect of the rate of temperature change with the reaction-time exclusive method of levels. Thresholds determined with this method were not affected by altering the rate, and the difference between values measured with the two methods increased as the rate increased.

2.3. Effect of Skin Type on Psychophysical Thresholds

Few studies have specifically compared cold or cold-induced pain thresholds between hairy and glabrous skin sites. Greenspan *et al.* (1993) reported significantly higher cold thresholds for the thenar eminence (glabrous skin) compared to the dorsolateral forearm (hairy skin), although no values were given. Meh and Denislic (1994) found higher thresholds in the thenar eminence (-23.6°C) compared to the forearm (-24.6) but no specific across site analysis was completed. Pertovaara and Kojo (1985) examined cold thresholds in the thenar eminence and the forearm and, although they did not report the actual threshold values nor make specific comparisons across sites, inspection of their summary graph (see Pertovaara and Kojo, 1985 Fig. 1) seems to indicate higher thresholds for the thenar eminence. Threshold comparisons across studies is problematic because of differences in the experimental parameters used (see Tables III and VI). For example, initial comparisons of cold thresholds measured in the thenar eminence in one study (Yarnitsky & Ochoa, 1990b) with those in the forearm in another (Wahren *et al.* 1989) seem to indicate that hairy skin is more sensitive. Upon further inspection it can be seen that the former study employed a cooling rate twice that of the latter which artificially increases threshold values (see previous section).

In modalities other than cold, studies have demonstrated increased sensitivity (i.e. lower thresholds) for hairy skin sites to the following stimuli: warm (Pertovaara & Kojo, 1985), noxious heat (Casey *et al.* 1987; Pertovaara *et al.* 1988; Meh & Denislic, 1994; Pertovaara *et al.* 1996), and noxious mechanical (Casey *et al.* 1987). On the other hand, other studies have shown that hairy skin is less sensitive to innocuous touch (Hämäläinen & Järvilehto, 1981) and warm (Stevens & Chou, 1998), as well as to two-point threshold discrimination (Holden, 1966). No difference in sensitivity between skin types has also been reported in response to innocuous warmth (Campbell & LaMotte, 1983) and noxious heat (Pertovaara & Kojo, 1985).

3. Supra-threshold Measurements

3.1. Rating Scales

Pain is a sensory experience that can vary along several dimensions, however it is often only expressed in terms of two main components. First is a sensory-discriminative component

which describes pain in terms of quality, intensity, duration, and locus. Second is the motivational, hedonic or affective component which expresses the degree of discomfort or unpleasantness of the pain sensation (Gracely *et al.* 1978a). Various techniques are used to tease out the sometimes subtle differences between these two dimensions including: Visual Analogue Scales (VAS), Verbal Descriptor Scales (VDS), and Cross-Modality Matching (CMM). With CMM, subjects estimate the perceptual magnitude of a given stimulus by adjusting one of several possible modalities including: line length, brightness of a light, or duration of a tone (Gracely, 1989). In VDS, subjects chose from a list of words which most accurately describes the pain felt. These words, which may describe both intensity and unpleasant pain sensations, are given numerical values for later analysis (Duncan *et al.* 1989).

3.2. The Visual Analogue Scale

VAS requires that subjects give a quantitative estimate of the magnitude of the pain they experience by marking a point along a line containing verbal anchors such as 'no pain' and 'the most pain imaginable' (Price *et al.* 1983; Chapman *et al.* 1985; Duncan *et al.* 1989; Price *et al.* 1989). If the results of VAS ratings are fitted to power functions, then the scale becomes what Stevens considered to be a form of magnitude estimation (Chapman *et al.* 1985). In addition, Price and co-workers (1983) showed that similar power function exponents were obtained from VAS and CMM estimations of stimulus intensity. From this finding they concluded not only that VAS is a form of CMM, but that it can overcome the limitations of other CMMs by allowing separate ratings for different dimensions of pain sensation. Thus, the versatility and adaptability of the VAS make it well suited clinically for pain measurement in patients and for experimentally induced pain in both patients and control subjects.

An important question in analyzing VAS ratings is whether the measurements obtained are of the interval or the ratio type. A ratio scale allows a percent change to be given to clinical or experimental manipulations. Price *et al.* (1983) asked subjects to choose a level of heat that was twice as intense as a 43°C standard stimulus and then compared the observed value to a predicted value obtained by fitting previous data to stimulus-response functions. The high coincidence of predicted and observed values suggest that the scale is indeed of the ratio type.

The VAS has a number of advantages: it is quick and simple, few instructions are needed, and no sophisticated vocabulary is needed (Price *et al.* 1983; Chapman *et al.* 1985; Langley & Sheppard, 1985; Duncan *et al.* 1989; Levy *et al.* 1989; Claus *et al.* 1990; Hilz *et al.* 1995). This allows for cross-cultural comparisons due to minimal language translation difficulties. In addition, results have shown low between-subject variability and high between-session reliability (Price *et al.* 1983).

One disadvantage of VAS is that the scale is closed. Since subjects are forced to compress a wide range of psychological experiences into an artificially small continuum, they may spread their responses over the entire scale regardless of the magnitude of the actual sensations felt (Chapman *et al.* 1985). Duncan *et al.* (1989) cautions that a VAS with a restricted maximum could result in a ceiling effect which could conceal real differences that may occur near the extremes of the scale. As a result they often use unbounded scales (Rainville *et al.* 1992).

3.3. The Dimensions of the Pain Experience: Intensity and Affect

It is generally accepted that pain can be distinguished by its sensory-discriminative component (intensity) and its motivational-emotional component (affect) (Gracely *et al.* 1978a; Gracely *et al.* 1978b; Gracely & Wolskee, 1983; Price *et al.* 1983; Melzack, 1984). These dimensions of pain can be separated with the use of separate VAS scales for affect (aVAS) and for intensity (iVAS) with verbal anchors specific to the dimension of pain being studied.

Price *et al.* (1983) found that while chronic lower back pain patients initially reported aVAS:iVAS ratios ≥ 1 when rating their pain state, treatment with trans-cutaneous electrical nerve stimulation (TENS) lowered the ratio to <1 (i.e. the treatment relatively lowered pain affect). Price *et al.* (1986) tested the effects of I.V. administration of the opioid-agonist fentanyl on clinical and experimental pain in similar pain patients. Fentanyl treatment lowered aVAS and iVAS ratings to clinical pain and to heat pulses between 45-51 °C, however in both cases aVAS was relatively more affected by the drug. In contrast, Gracely *et al.* (1979) found a selective decrease in intensity with a similar dose of fentanyl although they used VDS rather than VAS. The superiority of VDS over VAS was also found for distinguishing intensity and affect of

experimental heat by Duncan et al. (1989).

In what is certainly one of the most interesting studies differentiating pain intensity and affect, Price et al. (1987) found relatively higher aVAS ratings in cancer patients and relatively lower aVAS ratings in perinatal women. When these perinatal women were asked to focus on the imminent birth of the child, aVAS but not iVAS was lowered. This group has also used contact heat stimuli to show that aVAS ratings are systematically lower than iVAS ratings (Price *et al.* 1989).

Rainville et al. (1992) used unbounded VAS-type scales to measure pain affect and intensity to several modalities of experimentally induced pain. They found that whereas cold pressor and ischaemic block elicited equal amounts of pain affect and intensity, contact heat (45.5-50°C) and electrical stimulation led to significantly higher iVAS ratings. Results from this lab also demonstrate that hypnotic suggestion can selectively reduce aVAS ratings (Rainville *et al.* 1997)

Explanations of why a dissociation may occur between the intensity and affective dimensions of pain were offered by Price et al. (1987) and Duncan et al. (1989). They suggest that when there is a possible threat to one's health or life (i.e. pain due to cancer), a selective increase in affect should result. In contrast, affective pain ratings should be selectively reduced if this perceived threat is removed or if the pain can be associated with a positive outcome (such as child birth). This predicts that experimentally-induced pain would result in lower aVAS ratings if subjects are assured at the outset that they will receive no permanent damage from the stimuli and that they are free to withdraw from the experiment at any time.

Although some studies have examined these differences for the cold-pressor test, continuous affect and intensity ratings due to noxious yet tolerable cold stimulation have not been evaluated.

3.4. Experimentally Derived Power Functions

Several studies have fit psychophysically derived ratings of innocuous and noxious thermal stimuli to Stevens-type power function equations. For example, exponents of 1 for cold and 1.5 for warmth have been reported for innocuous stimuli delivered to the arm with a 3.14cm²

thermode (Stevens, 1967; Stevens, 1970). Marks and Stevens (1972) found exponents between 1.2-1.7 for thermal magnitude estimates of whole body cooling of different durations. Although longer durations of cooling led to higher absolute cold ratings, shorter durations were associated with higher exponents. Exponents for heat induced pain have been found to be -1 for determinations on both the forehead and the forearm with a heat lamp (Adair *et al.* 1968)

Few studies have fit the intensity evoked from noxious cold stimuli to power functions. Chéry-Croze (1983) used a 7cm² Peltier thermode to deliver stimuli between 20-0°C to the thenar eminence. Results of ratings with a VAS-type scale were plotted as a power function with an exponent of 1 indicating a linear relationship between pain and temperature. Arndt and Klement (1991) reported a mean exponent of 3.0 for the pain intensity evoked from the injection of cold saline into the veins of human volunteers. For noxious heat stimulation, Price *et al.* (1983) asked subjects to separately rate the intensity and affect of the pain experienced. Mean exponents for VAS ratings with intensity and affective anchors were 2.1 and 3.8 respectively. In a later study, this group found exponents of 3.3-4.2 and 5.0-5.8 for ratings of noxious heat with iVAS and aVAS respectively (Price *et al.* 1989).

3.5. The Effect of Rate on Supra-threshold Ratings

No studies were found which examined the effect of cooling rate on supra-threshold pain ratings. In contrast, Kenshalo and Duclaux (1977) report unpublished observations of Molinari who found that faster cooling rates did not affect the peak estimate of the intensity for innocuous cooling steps between 1-5°C. Conversely, faster cooling rates were reported to decrease the latency of the peak cold ratings. This result is not surprising considering that slower rates result in an increased time interval before the temperature step is completed. Davies *et al.* (1983) reported the faster rates of temperature increase or decrease resulted in stronger sensations to a 5°C step. In the noxious range of heat simulation, Yarnitsky *et al.* (1992) found that faster heating rates from 0.3 to 6°C/s evoked higher pain magnitude estimates and peak pain ratings.

Thus it can be seen that a distinct deficit exists in the literature on the effects of temperature rate on supra-threshold pain ratings in human subjects.

3.6. The Effect of Skin Type on Supra-threshold Ratings of Cold Pain

Although results from studies using innocuous cool stimuli are consistent with heat data in showing that hairy skin thresholds occur at higher temperatures (Johnson *et al.* 1973; Greenspan *et al.* 1993; Meh & Denislic, 1994), systematic comparisons between skin types in response to noxious cold stimulation have not been done in humans.

4. Intraneural Microstimulation of Primary Afferent in Humans

Intraneural microstimulation of primary afferents in awake human subjects is a powerful tool that can be used to examine the perceptual consequences resulting from the activation of specific sensory channels. In many cases the sensations evoked from primary afferent stimulation mirror those produced from natural stimuli. However, a selection bias may occur as single fibres are often chosen for study only if their stimulation leads to conscious perception (Torebjörk & Ochoa, 1980). For instance, it has been shown that low intensity activation of single C-afferents does not necessarily lead to perception (Torebjörk & Hallin, 1974). Therefore, while it may be concluded that fibres of a certain sub-type can elicit a particular sensation, it cannot be assumed that all fibres of that sub-type will act accordingly. In addition, many fibres of the same sub-type as well as fibres of different sub-types may travel together within the same axon bundle (Breathnach, 1977; Kruger *et al.* 1981; Messlinger, 1996). This makes the isolation of a single fibre or even a single sub-type problematic (Torebjörk & Ochoa, 1980; Handwerker, 1987; Ochoa & Torebjörk, 1989; Handwerker & Kobal, 1993).

4.1. A-Fibre Stimulation

According to Light and Perl (1993), stimulation of myelinated nociceptive fibres in awake human volunteers results in sharp, stinging pain referred to the nociceptors' RF. Mention of focal pricking, sharp or stinging pain in response to direct A δ -fibre microstimulation has been made in several papers (Torebjörk & Ochoa, 1980; Schady *et al.* 1983; Torebjörk *et al.* 1984; Ochoa & Torebjörk, 1989; Torebjörk, 1992; Ochoa, 1993). Konietzny *et al.* (1981) isolated only two fibres with CVs in the A δ range. In both of these fibres, low intensity stimulation elicited innocuous mechanical sensations of tap while increasing the intensity led to painful pricking

sensations that were described as similar to that produced by a needle. In addition, the reports of pricking pain in Ochoa and Torebjörk's (1989) study of C-fibre microstimulation were attributed to unintended activation of A δ -fibres.

Studies of selective fibre blocks in human volunteers adds evidence in favour of the role of A δ -fibre in signalling sharp and pricking pain. Torebjörk and Hallin (1973) found similar sensations to nerve stimulations during selective C-fibre blocks with lidocaine. The sensations elicited from electrical stimulation of the skin were mainly innocuous tapping for low intensity stimulation and sharp pain for high intensity stimulation. MacKenzie et al. (1975) observed that pressure blocks of A δ -fibres decreases the sharp sensations evoked from pin prick implying that A δ -fibres were necessary for these sensations.

4.2. C-Fibre Stimulation

Intraneural microstimulation of C-fibres in awake human volunteers results, generally, in dull pain at low frequencies and burning pain at higher frequencies (Torebjörk & Hallin, 1973). While only one-third of subjects reported burning to C-fibre stimulation at 1 Hz in Handwerker et al.'s (1991a) study, all subjects reported this sensation when the frequency was increased to 10-50 Hz. Konietzny et al. (1981) noted that C-PMN stimulation at 3-4 Hz elicited pricking followed by burning sensations on successive trials in one subject. Ochoa and Torebjörk (1989) reported that pricking pain projected to punctate skin areas while dull or burning pain projected to significantly larger skin area. In this study pain occurred during stimulations between 3-30 Hz and dropped off at higher stimulation frequencies, which they suggest may have been due to excitation failure. Studies also show that sensitization can occur in C-PMNs around one minute after intraneural stimulation and can last for several hours. This sensitization consists of decreased threshold to activation coupled with an increased background discharge and an increased response to noxious stimulation (Saumet *et al.* 1985; Light & Perl, 1993). Other C-PMNs show the opposite effect of desensitization after repeated stimulation (Georgopoulos, 1976).

5. Paradoxical Heat Sensations

Paradoxical heat (PH) is a phenomenon whereby sensations of heat or burning are evoked by cold stimulation of the skin. Paradoxical cold may also occur whereby cold sensations are evoked from heating, however this phenomenon was not specifically examined in this thesis.

5.1. Human Psychophysical Studies

In human psychophysical studies, PH sensations have been elicited with the use of three distinct experimental manipulations: (1) Spatial positioning of warm and cold stimuli, (2) Blocking of A δ -myelinated but not C-unmyelinated fibres, and (3) Temporal presentation of warm and cold stimuli.

(1) Juxtaposed warm and cold stimuli on hand region of normal subjects (Green, 1977; Craig & Bushnell, 1994) can produce PH. Green (1977) applied three 21.5mm² thermodes to adjacent fingers and varied the temperatures of the outer two relative to the inner one. PH sensations were reported when the outer thermodes were maintained at 44°C and the inner one varied in temperature between 10-40°C. The greatest incidence of PH (~ 30% of trials) occurred with the sequence 44°C-21°C-44°C. Craig and Bushnell (1994) employed a 28cm² thermode consisting of 15 bars, the temperatures of which could be set to warm (40°C), cold (20°C), or alternating warm/cold. The latter alternating sequence, known as the Thunberg or Thermal Grill, resulted in PH sensations in all but one of eleven subjects. This PH pain was rated as 12.5 out of 50, and was described qualitatively as hotter than the warm sequence, less cold than the cold sequence and often as stinging, smarting or burning.

(2) Cooling during compression or ischaemic blocks, which selectively blocks A δ -fibres, has also been reported to induce PH sensations (Fruhstorfer, 1984; Wahren *et al.* 1989; Yarnitsky & Ochoa, 1990b; Davis, 1998a). In Fruhstorfer's study (1984), ~50% of subjects reported cold-evoked icy, stinging, burning, and hot sensations. In addition, the block increased cold thresholds at the thenar eminence from 28.4 to 25°C. Wahren *et al.* (1989) found a similar effect on cold thresholds due to compression block of the superior radial nerve. Cold thresholds increased from 30.9°C before the block to 27.5°C after the block concomitant with decreased cold pain thresholds from 19.1°C to 22.8°C. Qualitatively, pain caused by descending temperature ramps

changed from cold before the block to burning with an increased unpleasant component during the block. PH sensations were not quantified in this study. Yarnitsky and Ochoa (1990b) used ischaemic blocks to confirm both the increase in cold threshold and the decrease in pain threshold reported in the previous two studies. After 20 min of block the quality of the initial sensations induced by temperature decreases were hot or burning in ~60% of subjects. Furthermore, pain sensations changed qualitatively at 20 min from cold to aching, hot or burning in ~70% of subjects, and ~60% of subjects felt hot or burning sensations to application of ice at this point during the block. In Davis' study (1998a), cold ramps during ischaemic blocks evoked PH in ~50% of subjects.

(3) Cold and warm stimuli presented successively have been found to sometimes induce PH sensations (Hämäläinen *et al.* 1982; Greenspan *et al.* 1993; Hansen *et al.* 1996; Davis, 1998a). Hämäläinen *et al.* (1982) noted PH sensation in 10% of trials during which subjects maintained thermal neutrality by depressing a switch and thus reversing the direction of temperature change when either warm or cold was felt. However, a single prior test of heat pain threshold increased reports of PH to 30% while two tests increased reports to 41%. A subsequent experiment found that several factors decreased the threshold at which PH sensations occurred: slower cooling rates, testing of hairy skin compared to glabrous skin and preheating. In Greenspan *et al.*'s (1993) study, 3/32 subjects did not report a sensation of cold to an 8°C step at 4°C/s with the multiple-staircase paradigm. When smaller temperature steps were used, these non-responders reported PH sensations during 30-100% of trials while responders only reported these sensations 5-25% of the time. Because this study was also testing warm and heat pain thresholds, it is assumed that preheating occurred before many cool threshold determinations. Hansen *et al.* (1996) observed PH sensations in 12% of normal subjects which occurred exclusively during thermal sensory limen testing of the dorsal foot region. Lastly, 2/16 subjects reported PH sensations in Davis's study (1998a) of noxious cold. Although warm and heat pain thresholds were not tested, the baseline temperature used was 35.5°C which may be slightly warm.

In addition to experiments on normal subjects, several studies have reported PH sensations in different patient populations. In the paper described above, Fruhstorfer (1984)

noted unpublished observations of patients with polyneuropathy, 10% of whom displayed similar PH sensations to normal subjects undergoing ischaemic blocks. Kashiwara and Yabuki (1987) described two patients with acute idiopathic polyneuritis, possibly caused by the Guillain-Barré virus, who felt burning sensations when cold objects were placed in their mouths. Ochoa and Yarnitsky (1994) describe 12 polyneuropathy patients with normal cool thresholds proximally but with abnormal thresholds accompanied by PH sensations distally. Half of the patients with multiple sclerosis without signs of peripheral damage were found by Hansen et al. (1996) to have PH sensations during thermal sensory limen testing. These sensations occurred more frequently in the foot and were independent of abnormalities in cold threshold.

5.2. *Proposed Mechanisms*

Clearly, cold stimuli can elicit sensations of heat or burning in control subjects and in certain patient populations. The question of why this odd phenomenon occurs can only be sufficiently answered when the mechanism of how it works is understood. Several mechanisms have been proposed to account for PH sensations to cooling: (1) Green (1977) explains his observations of "synthetic heat" as the dominance of warmth over cold in establishing the intensive aspects of sensation. (2) Härmäläinen et al. (1982) suggest that PH may be a function of cutaneous receptors that respond to both heating and cooling, such as polymodal nociceptors and, in addition, to "some central interaction". (3) Fruhstorfer (1984) proposes that A δ -fibres normally have an inhibitory effect upon unmyelinated afferents, which suppresses an allodynic response to innocuous cool and which is released by A δ blocks. He also suggests that the size of Härmäläinen et al.'s thermode was too small to activate a sufficient amount of A δ -cold receptors necessary to inhibit unmyelinated low threshold cold receptors. The question of why activation of these low threshold cold receptors would result in heat and burning sensations was not addressed. (4) Wahren et al. (1989) combine the ideas of Härmäläinen et al. and Fruhstorfer concerning both PH and the concomitant decrease in cold pain threshold from A-fibre block. They suggest that cold stimulation of A δ -fibres normally inhibits unmyelinated fibre input centrally. A δ -fibre blocks release this inhibition, thereby altering the central decoding process of temperature stimulation. (5) Yarnitsky and Ochoa's (1990b) explanation is conceptually similar

to that proposed by both Fruhstorfer and Wahren *et al.*, and highlights the concept of a central 'gating' of C-nociceptors by cold-specific A δ -afferents. (6) Greenspan *et al.* (1993) make several suggestions including the possibility that warm stimuli suppress the activity of the receptors that result in the perception of cold, thereby allowing the activity arriving from other cold-sensitive nociceptors to predominate. (7) Craig and Bushnell's (1994) hypothesis states that warm stimuli decrease the activity in cold-specific fibres leading to a "central unmasking" of cold-activated C-PMN channel activity. Hansen *et al.* (1996) build upon this hypothesis, suggesting that the normal inhibition of C-PMNs originates from a supraspinal area such as the thalamus.

5.3. Experimental Evidence

Electrophysiological studies have provided support for possible mechanism(s) involved in eliciting sensations of PH. One question that must be answered is whether there are heat-sensitive cutaneous receptors that are also capable of responding to innocuous cold stimuli. One C-fibre was identified whose heat threshold was 33°C by Iggo (1959). He also observed the odd heat-sensitive fibre that responded with irregular, low-frequency firing (5imp/sec) to cooling of the skin. If such innocuous cold and heat-sensitive fibres existed in abundance in human nerves, they could transmit ambiguous messages centrally resulting in PH to cooling. Stronger evidence comes from studies on PMNs, which are traditionally thought to be activated solely by noxious stimuli. In his survey of monkey A δ -PMNs, Georgopolous (1976) found that while most cold-sensitive fibres had cold thresholds <20°C, some had thresholds as high as 30°C. C-PMNs responsive to innocuous cool without preheating were reported in 87 of 112 monkey fibres by LaMotte and Thalhammer (1982) but in only 2 fibres in humans by Campero *et al.* (1996). In both these cases the afferents only responded to temperatures below 25°C. Intraneural stimulation of C-fibres in awake human subjects suggests that activity induced in these fibres can evoke the burning sensation characteristic of PH (Konietzny *et al.* 1981; Ochoa & Torebjörk, 1989; Handwerker *et al.* 1991a). If A-fibres normally inhibit the activity in C-nociceptors in response to innocuous cold, then it seems possible that after A δ blocks by ischaemia, compression, pre-heating, or juxtaposed warm and cold, C-fibre activity could then produce PH sensations to cooling.

Bessou and Perl (1969) describe C-PMNs in the cat which were unresponsive to cooling even down to -10°C , but which became sensitized by noxious heat and subsequently responded to cooling in the range of $40-20^{\circ}\text{C}$. This result was confirmed in rabbits (Shea & Perl, 1985) and in primates (Croze *et al.* 1976; Kumazawa & Perl, 1977), although Croze *et al.* (1976) did not test conduction velocities, resulting in some uncertainty as to whether recordings were from C or A δ -PMNs. Thus, it seems that Härmäläinen's hypothesis that PMNs were sensitized to preheating is plausible.

Craig and Bushnell (1994) provide evidence for their explanation of the thermal grill illusion using a scaled-down grill on cat glabrous skin while simultaneously recording from lamina I dorsal horn neurons. COLD cells were excited by all-cold bars, slightly inhibited by alternating warm/cold bars, and strongly inhibited by all warm bars. The responses of HPC neurons, on the other hand, did not differ significantly when comparing the all-cold with alternating paradigms. Thus, the relative pattern of HPC/COLD neuron activity is shifted in favour of HPC in response to the thermal grill, a relation that resembles responses to moderate heat and noxious cold stimuli. Furthermore, the thermal grill results in cerebral activation, measured with PET, that more closely resembles that of noxious cold rather than innocuous warm or cold stimulation (Craig *et al.* 1996). Therefore, the relative pattern of noxious cold-like activation to interlaced warm and cold bars is preserved up to the level of the cortex.

Section 3. Aims and Objectives

Psychophysical responses to noxious cold stimulation of the skin have not been extensively studied in normal human subjects. Continuous pain ratings with the visual analogue scale is an important method to assess these responses. As the use of continuous pain ratings increases, several important issues remain to be resolved about the parameters with which stimuli are delivered: the type of skin stimulated, the rate with which the stimulus temperature decreases, and the dimension of the pain rated by subjects.

Based on previous studies of noxious heat and the limited studies on noxious cold, the following hypotheses were generated to investigate the effect of skin type, cooling rate and rating scale (dependent variables) on pain (independent variable):

- (1) Noxious cold stimuli applied to hairy skin will result in increased psychophysical measures of pain compared to glabrous skin.
- (2) Supra-threshold noxious cold stimuli delivered at fast cooling rates will result in higher psychophysical ratings of pain compared to stimuli delivered at slower cooling rates.
- (3) The intensity component of cold-induced pain sensations will be greater than the affective component.

Section 4. Materials and Methods

4.1. Subjects

Subjects were recruited from a posted advertisement and from staff and students at The Toronto Hospital. A pre-session telephone interview was carried out to explain the main themes of the experiment and to screen out potential subjects with a history of neurological disorders that could effect thermal perception (e.g. diabetes). An additional inclusion criterion was that subjects were between the ages of 18-40.

On the day of the experiment, subjects were seated comfortably in a quiet, temperature controlled room (mean 22.5°C; 21-24°C). Each subject gave informed consent to the experimental procedures that were approved by the University of Toronto Human Subjects Review Committee. Subjects were informed that they could withdraw from the study at anytime without penalty. Subjects were paid \$20 for participating in the experiment which lasted ~2 hours.

Several practice trials were run at both sites of stimulation. Practice trials introduced subjects to supra-threshold temperatures and ensured that subjects felt pain to the coldest temperatures tested.

During the initial threshold determinations and practice trials which preceded supra-threshold cold stimulation, two subjects (one male, one female) did not report pain to the minimum cold stimulus and were excluded from further testing. In addition, excluded from the study were one male subject who reported painful sensations to the neutral temperature, and another male subject who misunderstood rating instructions for the supra-threshold experiment.

The number of subjects included for each experiment were as follows:

- (I) cold and cold-induced pain threshold determinations - 21 subjects; 10 females, 11 males; ages 21-40; 17 right-handed, 4 left-handed.
- (II) supra-threshold cold stimulation - 19 subjects, 10 females, 9 males; ages 21-40; 16 right-handed, 3 left-handed.
- (III) timing of paradoxical heat sensations - 7 subjects, 2 females, 5 males; ages 19-35; all right-handed.

4.2. Apparatus

Cold stimuli were delivered via a Peltier-type thermal stimulator (TSA-2001, Medoc, Israel). The two sites of stimulation chosen for this experiment were the thenar eminence (glabrous skin) and the dorso-lateral hand (hairy skin). The 3 X 3 cm thermode head was fastened to the test site with a velcro strap. The thermode is shown in the inset of Fig. 4.

A computerized visual analog scale (VAS) (Davis *et al.* 1995) was used to obtain psychophysical ratings of pain intensity and unpleasantness. Subjects moved a linear, mouse-driven scale displayed on a computer monitor from 0 to 100 to register their pain rating with the non-tested hand. Because each unit of this scale was divided into thirds, the entire 100 point scale consisted of 300 sub-units. Subjects were familiarized with the difference between pain intensity and unpleasantness (affect) using a set of instructions similar to those used by Price *et al.* (1983). Verbal anchors were placed at quarterly intervals to aid subjects in deciding the most accurate measure of their pain. Anchors for the intensity scale were: 0=not painful, 25=slightly intense, 50=moderately intense, 75=very intense and 100=extremely intense. Anchors for the affect scale were: 0=not unpleasant, 25=slightly, 50=moderately, 75=very and 100=extremely unpleasant (Fig. 3). Ratings were sampled at 1Hz and saved for off-line analysis.

4.3. Experiment I: Threshold determinations

Cold and cold-induced pain thresholds were determined by the method of limits as follows: The thermode was maintained at 34°C until the subject indicated a thermally neutral sensation. The temperature was then decreased at 1.5°C/s until the subject pressed the response button indicating the onset of either cold or pain. The temperature returned to baseline immediately following the button press. Five consecutive trials for cold threshold were followed by five trials for cold-induced pain. The inter-trial interval was 10-15s for cool trials and 15-20s for pain trials. After thresholds were determined for one skin site, the above procedures were repeated for the other skin site. The instruction set that subjects read before threshold determinations is shown in Appendix Ai. Additional simplified instruction sets were placed in front of subjects during cold and cold-induced pain threshold determinations to ensure that the desired sensation was being attended to. These instruction sets are shown in Appendix A ii & iii

Figure 3: Visual analogue scales. A schematic representation of the intensity (A) and affect (B) scale used for supra-threshold cold stimulation in Experiment II. Verbal anchors are at 25% intervals.

A

B

Figure 4: (A) Schematic representations of cold stimuli for Experiment II. Three cooling rates were used to descend from 34°C to 2°C. For all trials, the temperature then remained at 2°C for 20 seconds after which time it ascended back to 34°C at a rate of 9°C/s. (B) The MEDOC thermode used for all experiments. The thermode consists of a 4cm² Peltier-type thermode attached to tubing which brings cold water to and from the head. The device is fastened to the test site with a velcro strap. Note that the position of the thermode is slightly medial to that used for trials on the thenar eminence.

for cold and cold-induced pain thresholds respectively.

4.4. Experiment II: Supra-threshold Stimulation

The effect of prolonged painful stimuli delivered at different cooling rates was determined as follows: In each trial the thermode temperature descended from a baseline of 34°C at one of three cooling rates to 2°C which was then maintained for 20s. Rates of cooling were: 0.5, 1 and 2°C/s and the rewarming rate was 9°C/s (see Fig.4a). Stimuli were delivered to the non-dominant hand to assess pain intensity and to the dominant hand to assess pain affect. Instructions for differentiating between pain intensity and affect are included in Appendix B i. Instructions for the supra-threshold cold stimulation experiment are included in Appendix B ii.

The order with which stimulation site and ramp rate were tested was randomized. The inter-trial interval was 3 minutes during which time the thermode was maintained at 34°C. After all three ramp rates were tested on one skin site the thermode was moved to the second site and the experiment was repeated. Once all rates were tested on both sites, the thermode was moved to the dominant hand and the trials repeated with the affect scale. These trials are shown graphically in Fig. 5. After the six intensity trials, subjects were asked to fill out a modified 78 word McGill Pain Questionnaire (Melzack, 1975). They were instructed to mark any and all words describing the pain that they felt during the previous trial. A sample MPQ is given in Appendix C.

4.5. Experiment III: Timing of Paradoxical Heat Sensations

To determine when during cooling trials PH sensations occurred, Experiment III was conducted in a separate session from the previous two experiments. The protocol with which cold stimuli were delivered to the two skin sites in this experiment was identical to that of Experiment II. However, instead of continuously rating with a VAS scale, subjects were given a list of words describing thermal sensations and were asked to indicate verbally whenever any of the three warmth/heat related sensations were felt. This list consisted of the following words: cool, cold (painful), freezing (very painful), warm, hot (painful), and burning (very painful). Subjects were told that some or all of these sensations might not necessarily be felt.

Figure 5: Branching diagram depicting the trials for Experiment II. Two rating scales (intensity and affect), two skin sites (glabrous and hairy), and three cooling rates (0.5, 1, and 2 °C/s) were used.

4.6. Data analysis

Cold and cold-induced pain thresholds, measured via MOL, were defined as the mean of all five trials.

Several indices of pain were used to analyze pain ratings in Experiment II:

- (1) *Onset of Pain*, or pain threshold, was defined as the first time subjects rated ≥ 1 with the intensity or affect VAS. A threshold value of ≥ 1 controlled for unintentional mouse movements between trials or when the subject was signalled that the trial was about to begin.
- (2) *Total pain* was calculated as the sum of pain ratings from the point when the thermode reached 2°C until pain ratings returned to <1 . Because ratings were acquired at 1 Hz, this sum represents the integral of the pain rating curve. Total pain was not calculated from the beginning of the experiment in order to minimize biases in trials with slower rates which had longer durations. All trials are identical in length from when 2°C is reached until the trial ends.
- (3) *Peak pain rating* was defined as the maximum rating reached during a trial.
- (4) *Latency to peak pain* was calculated as the interval of time from 2°C until the maximum rating was made. As for total pain, this index was analyzed from 2°C rather than from the beginning of trials because of the increased length of trials with slower cooling rates.
- (5) *Persistence of pain* was calculated as the time interval with ratings ≥ 1 once the thermode had returned to neutral temperature (i.e. baseline). Ratings of several subjects returned to 0 during the rewarming phase of the experiment. In these cases, a negative time value was assigned corresponding to the length of the interval before baseline was reached.
- (6) *Pain Ratios*, calculated as hairy pain rating/ glabrous pain rating at discrete points, allowed an examination of the differences between pain ratings at the two skin sites. These ratios were analyzed separately for the three phases of the experiment: (i) *Cooling Phase* from the time point when both hairy and glabrous pain ratings were ≥ 1 until the target temperature (2°C) was reached. (ii) *Static Phase* in which the thermode temperature was maintained at 2°C for 20s. (iii) *Rewarming Phase* from when the thermode temperature increased at 9°C/s from 2°C towards baseline until either hairy or glabrous pain ratings were <1 .
- (7) *Data modelling* was completed for mean ratings during the cooling phase using the method of least squares. Various functions were modelled and compared in a trial-and-error fashion, with

SigmaPlot, to find the one with the least amount of variability (i.e. the highest r^2 value).

Data from trials at a cooling rate of 2°C/s were excluded from several indices examining the cooling phase of the experiment since the temperature decrease with this rate was non-linear. Because this non-linearity differed across subjects and skin sites, off-line correlation of rating with an exact temperature during cooling was imprecise.

MPQ data were analyzed using the three indices outlined by Melzack (1984). (a) the total number of words chosen (NWC) were summed on a per trial basis. (b) the pain rating index total (PRIT) was calculated by assigning an ascending value from 1 for each word within a sub-class and then summing the highest values of the words chosen within each sub-class. (c) the present pain index (PPI) was a single word which subjects chose from a list of six words of increasing value from "no pain" to "excruciating" as a representation of the maximum pain felt during a trial. An additional measure of the percent of subjects who chose a particular word was used to analyze the group as a whole.

4.7. Statistical Analysis

Before analysis, the Kolmogorov-Smirnov test were used to establish whether the data were normally distributed. Data were considered to come from a normal distribution if the P-value was less than 0.05. For normally distributed data, two-tailed paired t-tests were used to compare two within-group factors, while two-tailed t-tests were used to compare two between-subject factors. Repeated measures analysis of variance (ANOVA) was used to compare more than two within-group factors. The null hypothesis that no difference existed between groups was rejected for P-values of less than 0.05. For post-hoc comparisons of two factors, the Student-Newman-Keuls method was used. Post-hoc contrast analyses were used to test significant differences between more than two factors. For repeated contrasts, a Bonferroni correction was used in which the P-value needed to obtain a significant difference between factors was divided by the number of contrasts. All normally distributed values are presented as means $\pm$ the standard error of the mean (SEM).

For data which failed the normality test, non-parametric descriptive and inferential statistical methods were used. Wilcoxon signed ranks tests were used to compare two related

factors. The Friedman test was used to compare more than two related factors. Chi-square tests were used to compare groups where the data consisted of proportions of subjects. In all cases, the null hypothesis that no difference existed between groups was rejected for P-values of less than 0.05. As for the normally distributed data, a Bonferonni correction was used to obtain a more conservative P-value when more than one post-hoc test was required. Non-normally distributed data are presented as medians $\pm$ the 25% and 75% quartiles.

Section 5. Results

5.1. Experiment I: Threshold Determinations

Cold and pain thresholds were measured bilaterally in 9 subjects to determine the effect of laterality. No significant differences were found between dominant and non-dominant hands at the hairy skin site for either threshold measurement ($P>0.1$). Therefore, data from the dominant hand only was used as a single threshold value for each subject. Both cold and pain threshold data was found to be normally distributed.

5.1.1. Cold Thresholds

Seventeen of 21 subjects (90%) detected a change from neutral to cold at higher temperatures during stimulation of hairy skin compared to glabrous skin. An example of one subject's raw MOL data are shown in Fig. 6A&B. The mean decrease in temperature required for this subject to respond to cold from neutral baseline ($-\Delta T$) was 0.9°C for the DLH, and 1.7°C for the thenar eminence. For all subjects combined, the mean $-\Delta T$ was 1.5 ± 0.2 for hairy skin and 2.3 ± 0.2 for glabrous skin. These values were found to be significantly different ($P<0.005$). Mean absolute temperature thresholds were 32.5°C for hairy skin compared to 31.7°C for glabrous skin. Cold threshold data for all subjects combined are shown graphically in Figure 7 and Table V. Males and females did not differ significantly for cold threshold measurements at the glabrous or hairy skin sites ($P>0.05$).

5.1.2. Pain Thresholds

Pain thresholds, like cold thresholds, differed according to skin type in that 15/21 subjects (71%) detected painful sensations at higher temperatures for hairy skin stimulations. Of those subjects who displayed the reverse effect, i.e. lower pain thresholds for glabrous skin, 50% responded similarly for cold thresholds. Data from one subject are shown in Fig. 6A&B. The mean $-\Delta T$ was 11.6 for hairy skin and 20.6 for glabrous skin. For all subjects combined, the mean $-\Delta T$ was significantly lower for hairy skin ($19.8\pm1.9^{\circ}\text{C}$) compared to glabrous skin ($23.5\pm1.4^{\circ}\text{C}$) ($P<0.05$). These values correspond to absolute temperatures of 14.2°C and 10.5°C

Figure 6: The method of limits. Raw data from threshold determinations of cold and cold-induced pain from one subject. (A) Measurements from the glabrous skin site (thenar eminence). (B) Measurement from the hairy skin site (dorso-lateral hand). Trials began at a baseline temperature of 34°C. The height of each bars represents the thermode temperature for one trial and the number beneath each bar represents the absolute threshold (°C) for that trial. Del = mean decrease from baseline of 5 trials (°C); Rslt = mean absolute threshold of 5 trials (°C); Var = variance of 5 trials; Rate = 1.5°C/s.

A. Thenar Eminence

Mod COLD SEN. COLD PAIN
Del 1.7 20.6
Rslt 32.3 13.4
Var 0.0 9.0
Norm
Rate 1.5 1.5

B. Dorso-lateral Hand

Mod COLD SEN. COLD PAIN
Del 0.9 11.6
Rslt 33.1 22.4
Var 0.0 0.4
Norm
Rate 1.5 1.5

Figure 7: Threshold values for glabrous skin (filled circles) and hairy skin (open circles). Data are mean values $\pm$ S.E.M. (A) Cool thresholds. (B) Cold-induced pain thresholds comparing measurements made with the intensity VAS to those made with the method of limits. (C) Cold-induced pain thresholds comparing measurements made with the affect VAS to those made with the method of limits.

* $P < 0.05$, ** $P < 0.005$.

Table V: Cold Thresholds

	Glabrous Skin			Hairy Skin		
	Males (n=9)	Females (n=10)	Pooled	Males (n=9)	Females (n=10)	Pooled
Mean (°C)	31.7	31.6	31.7 **	32.9	32.1	32.5
Median (°C)	32.1	31.8	31.8	33.1	33.1	33.1
SEM (°C)	0.2	0.3	0.2	0.1	0.2	0.2
Range (°C)	30.4-32.3	30.0-32.6	30-32.6	31.9-33.2	30.9-33.0	30.9-33.2

note: SEM = standard error of the mean

** = $P < 0.005$, significant difference between hairy and glabrous skin sites

for hairy and glabrous skin respectively. The right-hand side of Figure 7 B&C and Table VI display these values. No effect of sex on pain thresholds was found.

5.2. *Experiment II: Supra-threshold Stimulation*

5.2.1. *Time Course Analysis*

Figures 8 & 9 show the time course of mean pain intensity and pain affect ratings for hairy and glabrous skin stimulations at the three cooling rates. Pain ratings increased monotonically with decreasing temperature, reaching a maximum at approximately the end of the static 2°C phase. Generally, onset of pain during hairy skin stimulations occurred earlier than during glabrous skin stimulations. However, the slope of pain ratings at supra-threshold temperatures often was steeper for glabrous skin such that pain could ultimately surpass hairy skin ratings during the static 2°C phase (Fig. 8B, 9B&C). Upon rewarming of the skin, pain ratings decreased but did not return to zero until several seconds after the thermode had regained baseline temperature. During the rewarming phase, hairy skin ratings were consistently higher than glabrous skin ratings. Further analysis of the onset, peak, and persistence of pain ratings follow.

Figures 10&11 show examples of raw pain intensity and pain affect ratings with a superimposed temperature trace for two different subjects. Although the general pattern of ratings described above are apparent for pain ratings at all cooling rates and both scales, several departures from this pattern are evident. For example, in Figures 10B and 11B, pain ratings for glabrous skin trials begin sooner. Also, in Figure 10C and 11A, pain ratings are higher for glabrous skin trials than for hairy skin trials during the rewarming phase.

5.2.2. *Onset of Pain*

Pain thresholds were defined as the temperature at which VAS ratings were ≥ 1 . Pain thresholds did not significantly differ when either the intensity or the affect scales were used. Two-way repeated measures ANOVAs were completed separately for pain threshold data for the intensity and the affect scales. There was an overall effect of skin site and cooling rate on ratings for both of these dimensions of pain ($P < 0.05$). Post-hoc analysis showed that mean pain

Table VI: Cold-Induced Pain Thresholds

Site:		Glabrous Skin					Hairy Skin				
Scale/Method:		Intensity		Affect		MOL	Intensity		Affect		MOL
Cooling Rate (°C/s):		0.5	1	0.5	1	1.5	0.5	1	0.5	1	1.5
Mean (°C)	Male	14.7	11.4	18.8 *	12.3	10.6	20.5	17.6	18.4	17.8	15.7
	Female	14.9	12.3	12	8.4	10.5	19.8	16.8	18.6	12.1	12.9
	Pooled	14.8 †	11.9 †	15.4	9.1 †	10.5 †	20.1	17.2	18.5	14.9	14.2
Median (°C)	Male	12	6	19	13	13	20.5	19	21	20	17.1
	Female	11	8	9.5	4	11.4	18.5	20	20.5	9	11.2
	Pooled	12	8	16.8	7.5	12.3	19.8	19.5	20.8	14	11.8
Range (°C)	Male	6-24	2-22	6.5-28.5	2-14	2.4-19.5	8.5-28.5	4-27	2.5-27	7-25	2.9-27.1
	Female	7.5-27	2-30	2-23.5	3.5-13	1.2-17.4	11-30.5	3-27	8-25.5	4-31	4.9-28.4
	Pooled	3.5-27	2-30	2-28.5	3.5-13	1.2-19.5	8.5-30.5	3-27	2.5-28.5	4-31	2.9-28.4
SEM (°C)	Male	1.4	2.1	1.5	1.8	2.2	1.4	1.8	2.1	1.6	3.4
	Female	2.2	2.4	1.7	2.1	1.9	1.7	2.1	1.4	2.1	2.2
	Pooled	1.8	2.2	1.8	2.0	1.4	1.5	1.9	1.7	1.9	1.9

note: SEM = standard error of the mean

* = $P < 0.05$, significant difference between sexes

† = $P < 0.05$, significant difference between skin sites

Figure 8: Time course analyses of cold-evoked pain measured with the intensity VAS. Data are plotted as mean rating $\pm$ SEM for glabrous skin (closed circles) and hairy skin (open circles). Superimposed on each plot is a theoretical temperature trace (thick line) assuming that descents were linear at the three cooling rates used. Each trial began at a baseline temperature of 34°C. The thermode temperature then descended to 2°C where it remained for 20s, and rewarmed at +9°C/s back to 34°C. (A) Cooling rate of 0.5°C/s, ratings displayed at 0.33 Hz. (B) Cooling rate of 1°C/s, ratings displayed at 0.5 Hz. (C) Cooling rate of 2°C/s, ratings displayed at 0.5 Hz.

Figure 9: Time course analyses of cold-evoked pain measured with the affect VAS. Data are plotted as in Figure 2. (A) Cooling rate of 0.5°C/s , ratings displayed at 0.33 Hz. (B) Cooling rate of 1°C/s , ratings displayed at 0.5 Hz. (C) Cooling rate of 2°C/s , ratings displayed at 0.5 Hz.

Figure 10: Example of raw pain intensity data from one subject. Data are plotted as in Figure 8.

Figure 11: Example of raw pain affect data from one subject. Data are plotted as in Figure 9.

A **B** **C**

thresholds were significantly higher for the hairy skin site regardless of scale or cooling rate ($P < 0.05$). The only exception was for pain thresholds measured with the affect scale at a rate of 0.5°C/s . These results paralleled threshold measurements determined with the method of limits (see right-hand side of Fig. 7B&C). Post-hoc analysis on the effect of cooling rate revealed the following significant differences for the intensity scale: $0.5^{\circ}\text{C/s} > 1^{\circ}\text{C/s}$ and $0.5^{\circ}\text{C/s} > \text{MOL}$ ($P < 0.05$). For the affect scale, post-hoc analysis revealed a significantly higher pain threshold only for determinations at 0.5°C/s compared to 1°C/s ($P < 0.01$). All of these differences are shown in Figure 7B&C and Table VI.

When analyzed for the effect of sex on the onset of pain, females responded at significantly higher temperatures than males only for determinations on the glabrous skin site at a rate of 0.5°C/s using the affect rating scale (see Table VI, $P < 0.05$).

5.2.3. Data Modelling

Pain ratings for the cooling phase of the experiments at 0.5°C/s and 1°C/s were modelled mathematically using the method of least squares. A comparison of correlation coefficients for mean ratings with linear, quadratic and exponential models revealed that r^2 values were highest ($r^2 = 0.98\text{--}0.99$) with the exponential model. Therefore, data were modelled to the function $y = ax^b$ where the exponent b is the "power" of the function. The mean exponent for pain evoked at the glabrous skin site was higher (3.0) than for pain evoked at the hairy skin site (2.2). These results are shown graphically in Fig. 12. As is customary in plotting power functions, both the ordinate and the abscissa are scaled logarithmically to obtain straight line curves whose slope represents the exponent of the function. This figure illustrates several important points noted in the time course analysis section above. First, mean pain thresholds for hairy skin occur at higher absolute temperatures than for glabrous skin. Second, pain evoked at the glabrous skin site increases at a faster rate and thus has a higher exponent than at the hairy skin site. Third, pain at glabrous skin begins to approach pain at hairy skin near the end of the cooling phase.

Power functions were also fitted to each subject's raw ratings during the cooling phase of trials with cooling rates of 0.5°C/s and 1°C/s . Similar trends were found in the modelling of raw data. Three-way repeated measures ANOVA revealed a significant effect of skin type only;

Figure 12: Power functions fitted to mean VAS ratings during the cooling phase for glabrous skin (thick lines) and hairy skin (thin lines) trials. Pain Intensity is plotted for rates of 0.5°C/s (A) and 1°C/s (B); pain affect is plotted for rates of 0.5°C/s (C) and 1°C/s (D). Data is plotted in log-log coordinates so that the slope of the line represents the exponent of the power function.

glabrous skin exponents were significantly higher compared to hairy skin exponents ($P<0.05$). The mean overall exponent for trials at glabrous skin was 4.0 ± 0.4 while that for trials at hairy skin was 3.6 ± 0.4 . Post-hoc comparisons indicated that the mean exponent was always higher for glabrous skin trials compared to hairy skin trials for both scales and rates ($P<0.05$).

5.2.4. Total Pain

Three-way repeated measures ANOVA revealed no significant effect of skin type or rating scale on the amount of pain reported from 2°C to the end of the experiment. In contrast, total amount of pain was significantly dependant on ramp rate; total pain increased as the cooling rate decreased ($P<0.001$) (Fig. 13A). The sex of the subjects was not found to significantly affect the total amount of pain.

5.2.5. Persistence of Pain

The median amount of time that subjects continued to rate pain once the thermode returned to neutral temperature at the end of trials was 9 seconds for ratings with the affect scale and 10 seconds for ratings with the intensity scale. These differences approached significance ($P=0.052$). No significant difference was found in the persistence of pain ratings when comparing hairy and glabrous skin sites. A significant effect of cooling rate was found, wherein pain persisted longer after trials at 0.5°C/s than at 1°C/s (10 vs. 9 seconds, $P<0.01$). No significant difference was found in the persistence of pain ratings when ratings at 2°C/s were compared to ratings at 0.5 or 1°C/s . The effects of cooling rate on the persistence of pain ratings is shown in Figure 13B. In addition, no effect of sex on the persistence of pain ratings was found.

5.2.6. Peak Pain Rating

Peak pain magnitude and latency are shown in Figure 14. Three-way repeated measures ANOVA of the maximum pain reported by subjects revealed a significant effect of cooling rate only; slower cooling rates resulted in higher peak pain ratings ($P<0.001$). When collapsed across site and scale, mean peak pain scores were 79.0 ± 4.2 , 74.3 ± 3.9 , and 67.3 ± 4.0 for cooling rates of 0.5 , 1 and 2°C/s respectively (Fig. 14A).

Figure 13: (A) Total Pain comparisons of cooling rates for data collapsed over scale and site. Mean $\pm$ SEM integrated pain ratings were calculated for the period starting when the thermode temperature reached 2°C until pain were <1 . (B) Persistence of pain comparisons of cooling rates for data collapsed over scale and site. Data are mean $\pm$ SEM time that pain ratings were ≥ 1 after the thermode temperature returned to 34°C.

* $p < 0.05$, ** $p < 0.005$.

A **B**

Figure 14: (A) Peak pain ratings. Mean $\pm$ SEM collapsed over scale and site. (A) Maximum pain rating. (B) Latency from the onset of the static 2°C phase until the maximum rating was reported. * $p < 0.05$, ** $p < 0.01$.

A **B**

Latencies to peak pain ratings also revealed an effect of cooling rate only; peak pain ratings occurred earliest during 0.5°C/s trials ($P < 0.01$) (Fig. 14B). No significant difference was found for latencies to peak pain ratings for cooling rates at 1 and 2°C/s. When data was collapsed across site and scale, mean latencies to peak pain were 16.7 ± 2.4 , 20.4 ± 1.7 , and 21.1 ± 1.4 seconds for 0.5, 1 and 2°C/s cooling rates respectively. Since the static 2°C phase was 20 sec long, these values indicate that the mean peak pain ratings occurred near the end of this phase.

5.2.7. Pain Ratios

Pain ratios were calculated to facilitate detecting differences between skin sites over time. Fig. 15 illustrates that hairy/labrous pain ratios were >1 for the entire cooling phase of the experiment, indicating that cooling of hairy skin consistently evoked greater pain compared to labrous skin.

As shown in Fig. 16, pain evoked by hairy skin stimulation during the static 2°C phase was generally higher compared to labrous skin stimulation regardless of cooling rate. Pain ratios for all three cooling rates tended to approach unity by the end of the 20s static 2°C phase, consistent with data presented in Figures 8&9 showing that mean pain for the two sites equalize during this phase.

Figure 17 shows pain ratios for the rewarming phase of the experiment. While pain evoked from hairy and labrous stimulation is almost identical during rewarming, once the thermode returned to 34°C subjects rated pain as relatively more intense and unpleasant during hairy skin trials. Note the equalization of ratings occurring at approximately 13 sec after return to baseline for pain intensity ratings and 15 sec for pain affect ratings.

5.2.8. The Quality of Cold-Evoked Pain

The MPQ was filled out a total of 6 times by each of 19 subjects. Figure 18 shows that a wide variety of words was chosen after trials on both skin types. χ^2 analysis revealed that the distribution of the words most often chosen by subjects differed significantly across skin sites ($P < 0.05$). The words that were most frequently chosen by subjects included: 'cold' (79%), 'freezing' (68%), 'sharp' (79%), 'burning' (63%), 'tingling' (63%), 'aching' (58%), and 'pricking'

Figure 15: Pain ratios calculated as hairy pain rating divided by glabrous pain ratings for the cooling phase of Experiment II. (A) Intensity scale. (B) Affect scale. Data are mean pain ratios $\pm$ SEM for 0.5°C/s (closed circles) and 1°C/s (open circles). Only temperature points where both hairy and glabrous ratings were ≥ 1 for a particular subject are included. Therefore the number of subjects contributing to each point varies. To equalize the number of rating points, 0.5°C/s trials were sampled at 0.5 Hz, while 1°C/s trials were sampled at 1 Hz. Data from trials at 2°C/s are not included because of the non-linearity of the descending temperature.

A **B**

Figure 16: Pain ratings for the static 2°C phase of Experiment II. (A) Intensity scale. (B) Affect scale. Data are mean pain ratios $\pm$ SEM for all subjects at cooling rates of 0.5°C/s (closed circles, thick line), 1°C/s (open circles), and 2°C/s (grey triangles, dashed line). All data were sampled at 1 Hz.

Figure 17: Pain ratios for the the rewarming and return to baseline phases of Experiment II. Data are mean pain ratios $\pm$ SEM for pain intensity (closed circles) and pain affect (open circles) collapsed over site and rate. Note that since pain persisted for different amounts of time across subjects, data near the right-hand side of the graph consists of decreasing numbers of subjects. For example, the final point on the graph consists of ~20% of subjects.

Figure 18: McGill Pain Questionnaire (MPQ). The MPQ was filled out 6 times by each of 19 subjects. The 15 words most often chosen are shown for trials following glabrous skin (A) and hairy skin (B) stimulation. Data are the percentage of subjects who chose a particular word.

(58%). Interestingly, 'radiating' was chosen by 37% of subjects following glabrous skin trials but was never chosen following hairy skin trials. 'Cold' and 'freezing' were most frequently chosen for the slowest cooling rate. Also, as shown in Figure 19A, the frequency with which 'pricking' was chosen increased, while 'sharp' decreased as the cooling rate increased. 'Burning' was chosen most frequently at a rate of 1°C/s and 'aching' was chosen less frequently as the rate increased. However, χ^2 analysis revealed no significant effect of rate on these distributions.

Reports of PH sensations with the MPQ occurred for 39.5% of stimulation trials even though thermal stimuli never exceeded 34°C throughout the experiment. 'Hot', 'burning', 'scalding', or 'searing' were chosen at least once by 12 of 19 (63%) subjects, independent of the cooling rate or skin site tested. For all six trials together, 50% of males and 78% of females chose at least one PH word.

Although there was a consistently higher NWC following glabrous skin trials and trials with slower cooling rates, these differences did not reach significance (Fig.19B). The highest NWC occurred following trials at 0.5°C/s on the glabrous site (120 words), while the lowest NWC occurred after trials at 2°C/s on the hairy site (95 words). Analysis of the effect of site on the NWC also revealed no significant effect. NWC data for males vs. females was not normally distributed and, therefore, non-parametric analysis was performed. The median NWC value for males was 5, which was significantly lower than the median value for females, which was 6 ($P<0.0001$). These data are shown in Figure 19C.

Two-way repeated measures ANOVA on PRIT data showed a significant effect of cooling rate ($P<0.05$) while differences between skin sites approached significance ($P=0.053$). As shown in Figure 20A, post-hoc analysis revealed a significant difference between PRIT scores at cooling rates of 0.5 and 2°C/s only ($P<0.05$). Collapsing across rate, mean PRIT scores were 13.4 ± 1.0 for glabrous skin trials and 11 ± 1.4 for hairy skin trials. Analysis of PPI scores displayed a significant effect of cooling rate only. Although the Friedman test revealed an overall effect of cooling rate ($P<0.05$), post-hoc comparisons were deemed non-significant because of the conservative P-value used for multiple testing (Fig. 20B).

Figure 19: McGill Pain Questionnaire. (A) Mean number of words per trial, collapsed across site, for word associated with A δ - (pricking, sharp) and C- (burning, aching) fibre activation. (B) Mean number of words chosen (NWC) $\pm$ SEM per form after glabrous (dark bar) and hairy (light bar) trials. (C) Median NWC $\pm$ 25 and 75% quartiles per trial, collapsed across rate and site, for male (dark square) and female (light square) subjects.

Figure 20: McGill Pain Questionnaire. (A) Pain Rating Index Total (PRIT). Mean PRIT scores $\pm$ SEM for forms filled out following glabrous skin (filled circles) and hairy skin (open circles) trials. (B) Present Pain Index (PPI). Median PPI scores $\pm$ 25 and 75% quartiles for forms filled out following glabrous skin (filled circles) and hairy skin (open circles) trials.

* = $P < 0.05$.

5.3. Experiment III: Timing of Paradoxical Heat Sensations

In Experiment III the timing of paradoxical heat sensations (PH) was examined in more detail. Sensations of painful 'hot' or 'burning' occurred at least once for 74% of trials. Painful PH sensations occurred in 85%, 71.4%, and 40% of trials at cooling rates of 0.5, 1, and 2 °C/s respectively (Fig. 21A). As Figure 21B illustrates, painful PH sensations occurred most often during the cooling and static 2 °C phases, while innocuous PH sensations occurred most often at the return to neutral. Of the total reports of 'painful hot', 52%, 10%, 10%, and 28% occurred during the cooling, the static 2 °C, the rewarming, and the baseline phases respectively. The corresponding percentages for sensations of 'burning' were 63%, 29%, 5%, and 3%. When 'warm' was reported, it occurred predominantly when the temperature returned to baseline (77%) (Fig. 21B). χ^2 analysis revealed that all of these distributions differed significantly from the expected distributions ($P < 0.001$).

Figure 21: Experiment III: Paradoxical Heat Sensations. (A) Incidence of cold-evoked painful heat or burning sensations collapsed across skin site. (B) Phase of the experiment in which cold-evoked innocuous warm, painful heat, and painful burning sensations were elicited.

Section 6. Discussion

This is the first study to use continuous VAS ratings of pain intensity and affect to assess the effect of cooling rate in hairy and glabrous skin. Several important findings are apparent. First, continuous VAS ratings can be used to measure pain thresholds. Similar trends across sites and rates were apparent in comparing this threshold method with the more traditional method of limits. Second, all indices of pain were increased for slower ramp rates. Third, while hairy skin displayed lower thresholds to initial cooling, pain indices were equal and sometimes higher for glabrous skin stimulations. This indicates that glabrous skin may be more sensitive once threshold has been exceeded. Fourth, the intensity and affective indices of cold pain did not differ significantly. Finally, a high proportion of subjects reported cold-evoked paradoxical painful heat sensations, most often during the initial cooling phase of the experiment.

6.1. Methodological Issues

Two computers were coordinated for each block of supra-threshold trials; one to deliver the cold stimuli and one to measure the subject's VAS response. Pilot trials indicated that the two computers were possibly out of synch by approximately ± 1 -2 seconds. Pre-analysis tests were conducted on the effects of this and larger possible missynchronizations (3-4 seconds) of stimulus temperature and rating. The raw pain ratings of several randomly chosen subjects were shifted by various amounts and the effect upon time-sensitive pain indices (onset, latency to peak pain, persistence of pain) was analyzed. This analysis indicated only a small effect, which did not change the direction of the results or their significance.

6.2. The VAS as a Measure of Pain Thresholds

The VAS is generally used as a supra-threshold rather than as a threshold measurement tool. Contrary to this, the VAS was successfully used in this study for both purposes. Since subjects were clearly instructed to move the mouse-driven VAS scale above zero only when pain was first experienced, it is felt that the first movement of the mouse is an accurate measure of pain threshold. In addition, since the movement of a mouse is conceptually and physically similar to pressing a button, comparisons between VAS and MOL are valid. Both the intensity

and the affect VAS scales proved to be sensitive in discriminating between thresholds at the two sites of stimulation. Further, VAS thresholds were higher with faster rates of temperature change as has been found with other reaction-time inclusive threshold methods (Fruhstorfer *et al.* 1976; Croze & Duclaux, 1978; Pertovaara & Kojo, 1985).

The between-subject variability was comparable to, but sometimes higher than, that measured with MOL (see Fig. 7). This may have been due to the fact that the MOL values comprising this figure are the arithmetic mean of five determinations while the VAS was completed only once per subject per site and rate. It is concluded that the use of the first rating as the threshold during continuous rating with VAS is valid and can significantly decrease the duration of experiments, thereby reducing the fatigue in patients and allowing repeated testing in subjects.

6.3. The Effect of Cooling Rate on Psychophysical Measurements

The cooling rate was found to be a significant factor in both the threshold and supra-threshold measures of pain. In general, faster rates of cooling increased thresholds and decreased supra-threshold indices of pain.

6.3.1. Threshold Measurements

The present finding that increased cold pain thresholds occurred when faster cooling rates were used (i.e. occurred at lower temperatures) has been reported previously by Croze & Duclaux (1978). Several possibilities may explain these findings. First, a reaction time artefact may have artificially increased threshold values. Both MOL, used to measure cold and pain thresholds, and VAS, which was only used to measure pain thresholds, require subjects to make conscious decisions between very similar stimuli. For example, in the pain threshold assessment, subjects must judge between what is merely 'very cold' and what is 'painful' and then make the appropriate response. The time required for conduction along peripheral and central pathways which transmit information concerning cold to the brain areas involved in decision making, the decision making process itself, and the conduction along the descending motor pathways involved in the response is assumed to be identical regardless of cooling rate. Therefore, a faster

rate will result in a larger absolute change in the thermode temperature during this fixed time. This will result in artificially inflated threshold values with faster rates of temperature change (Croze & Duclaux, 1978; Yarnitsky & Ochoa, 1990a; Yarnitsky & Ochoa, 1991).

Another possibility is that different cooling rates activate different primary afferents with different conduction velocities. Yarnitsky et al. (1992) found that C-heat nociceptors increased their firing rate with faster heating rates and that at a rate of 2°C/s , which was the fastest rate used in the present study, heat pain was mediated exclusively by C-fibres. Parallel studies using cold stimuli have apparently not been performed. However, since cold messages are transmitted by both A δ and C-fibres (see Section 2), a relative shift towards C-fibre activity with faster cooling rates would increase the overall transmission time and, therefore, increase threshold values. Because this is the opposite to what was found in the present study, it seems unlikely that faster rates of cooling result in a shift towards C-fibre activity as was found for heat.

To calculate whether a reaction-time artefact could explain the rate-dependent threshold differences, several assumptions were made. First, the central processing time and the resultant motor response to the stimulus were estimated at ~ 200 ms (Campbell and LaMotte, 1983). Second, the distance from the test site to C7 was estimated at ~ 0.8 m. Third, estimates for peripheral conduction velocities for C-fibres was 0.5m/s , and for A δ -fibres was 10m/s . Using these estimates, the reaction time for an exclusively C-fibre response would be ~ 1.8 sec, while that for an exclusively A δ -fibre response would be ~ 0.3 sec. From the data given in Table IV, these reaction time estimates suggest that the absolute thresholds become more similar with the C-fibre estimate than with the A δ -fibre estimate. For example, absolute thresholds for the glabrous skin become 15.7 , 13.7 , and 13.2°C for measurements with an estimate of C-fibre activity at 0.5 , 1 , and 1.5°C/s respectively, whereas these values are 15.0 , 12.2 , and 11.0 for measurements with an estimate of A δ -activity. Therefore, even if the noxious cold stimuli activated exclusively C-fibres, which is unlikely based on previous studies, the rate-dependent threshold differences could not be entirely explained by a reaction-time artefact.

Although reaction-time inclusive methods do not give absolute threshold values, relative threshold values can still be used for comparisons of within-subject factors. Therefore, the comparisons between skin sites as well as those between the MOL and the VAS methods of

threshold determination should not have been significantly affected by reaction time artefacts.

6.3.2. *Supra-threshold Measurements*

Psychophysical ratings in supra-threshold experiments were also significantly affected by cooling rate. Faster cooling rates resulted in decreased values for all psychophysical indices of pain. In addition, the MPQ indicated that more pain was experienced for trials with slower cooling rates.

Few, if any, studies have looked at the effects of cooling rate on psychophysical pain ratings or nociceptor activity. In contrast, several studies have investigated these effects in the innocuous range for cold. Faster cooling rates have been shown to increase several properties of cold receptors including: mean discharge rate (Dubner *et al.* 1975; Kenshalo & Duclaux, 1977; Molinari & Kenshalo, 1977), maximum rate (Kenshalo & Duclaux, 1977; Davies *et al.* 1983), cumulative impulses within the first 4 seconds of firing (Kenshalo & Duclaux, 1977), and the range over which there is a linear relationship between cold temperature and discharge frequency (Dubner *et al.* 1975). Also, dorsal horn neurons show higher peak firing rates for faster cooling rates (Davies *et al.* 1985). Davies and co-workers (1983) completed one of the few studies testing the effects of increased cooling rate on the sensations evoked in human subjects, although the temperatures used were exclusively in the innocuous cold range. They found that stronger sensations of innocuous cold were elicited with faster cooling rates for a 5°C temperature step.

Similarly, faster heating rates increase the mean discharge rates in rat A δ -fibres (Yeomans & Proudfit, 1996), feline C-PMNs (Bessou & Perl, 1969), monkey warm fibres (Kenshalo, 1996), and human C-PMNs (Yarnitsky *et al.* 1992). In addition, work by Yeomans and Proudfit (1996a, 1996b) suggest that A δ -fibres are primarily activated by faster heating rates while C-fibres are mainly excited by slower rates. Yarnitsky *et al.* (1992) also found higher peak magnitude estimates of pain from fast heating rates in human subjects.

The results of these studies on innocuous warm and cold and noxious heat suggest that rapid temperature changes result in more intense activation of temperature-sensitive primary afferents and in increased pain sensations. Nevertheless, the present results suggest the exact opposite hypothesis for noxious cold. The data suggest that slower cooling rates increase activity

of cold-sensitive nociceptors which evoke higher psychophysical ratings. Several possibilities could explain the present results. First, because slower cooling rates result in longer trials there may be a greater summation of cold at the level of the nociceptor. An additive effect of increased exposure to cold could result in more intense activation of primary afferents which could then translate into more intense pain sensations. However, if this were so, it could also be hypothesized that a similar bias towards slower rates of heating should cause increased activity in heat nociceptors and, therefore, increased perceptual responses. The studies mentioned above on noxious heat suggest that this effect does not in fact occur. Since there have not been similar studies conducted on cold nociceptors, the extent of this effect cannot be resolved at the present time. However, even if present, this bias should have been minimized in indices of peak pain latency and total pain which were calculated from the point when the thermode temperature reached 2°C. In addition, previous studies have not looked at whether there is a physical difference in the intracutaneous build-up, storage, and dissipation of thermal energy in response to heating compared to cooling. Thus it cannot be assumed that the studies on the effects of heating rate of physiological and psychophysical parameters are comparable to the present study.

Results from MFQ analysis also indicate the possibility that A δ and C-nociceptors may have been preferentially activated by high and low rates respectively. As illustrated in Fig. 19, more subjects reported 'pricking' sensations for faster cooling rates, while 'aching' was reported more often for slower cooling rates. Previous studies have indicated that intraneural stimulation of A δ -fibres elicits mainly sensations of pricking, while stimulation of C-fibres often elicits 'aching' sensations (Torebjörk & Hallin, 1973; Torebjörk & Ochoa, 1980; Konietzny *et al.* 1981; Ochoa & Torebjörk, 1989; Torebjörk, 1992).

Finally, the increased pain reported for slower cooling rates may have been due to central effects at the level of the spinal cord or higher. Because the effect of ramp rate on spinal cord and supraspinal neurons has not been studied, it cannot be predicted whether the present results can be explained in terms of higher order processing of thermal information.

6.4. The Effect of Skin Type on Psychophysical Measurements

This study demonstrated significant differences between cold and pain sensations due to

stimuli applied to hairy and glabrous skin sites. Generally, pain ratings for stimulation of glabrous skin began later, then increased at a faster rate to reach equal and often higher maximum pain ratings during maintained noxious cold stimulation. Pain ratings for the two sites were mostly equal initially during rewarming, however glabrous skin pain ratings decreased at a faster rate once the thermode had returned to baseline. MPQ measures were also higher following cold stimulation of glabrous skin.

Possible explanations for the differences found in the sensitivity of hairy and glabrous skin include: (a) Anatomic differences between the skin types; (b) Physiological differences in the functioning of peripheral and central pathways encoding noxious cold and contributing to conscious perception.

(a) There is evidence that hairy and glabrous skin differ in composition, sympathetic innervation and, possibly, the location and density of receptors mediating noxious cold sensation. In general, glabrous skin is five-fold thicker than hairy skin, consisting mainly of more keratinous layers in the stratum corneum of the epidermis (Whitton & Everall, 1973; Cormack, 1987). This thicker superficial layer may be due to a relatively increased and extended use of glabrous skin areas, causing increased callousing. Additionally, the epidermis of hairy skin displays a thinner stratum spinosum, a discontinuous stratum granulosum, and an absence of the stratum lucidum (Cormack, 1987). These differences may affect the rate with which cold passes through the skin before reaching underlying cold receptors and nociceptors. In support of this hypothesis, no difference was found between skin types for peak pain ratings, indicating that a 20 second tonic cold stimulus may be sufficient to allow temperatures at the level of hairy and glabrous cold-sensitive nociceptors to become equal. Indeed, Treede et al. (1995) placed a thermocouple 450 μ m below the surface of hairy skin, demonstrating a levelling off of intracutaneous temperature within 6 seconds to stimulation with a CO₂ laser. Thicker skin in the glabrous site is also a likely explanation for the delayed responses found to initial cooling, and thus the higher cold and pain thresholds measured. However, if skin thickness was the sole factor involved, ratings should have increased at similar rates once the threshold had been exceeded. In fact, power function analysis revealed that glabrous skin ratings rose significantly faster once threshold was surpassed.

Fleisher et al. (1983) observed that rat cold-sensitive C-fibres were significantly more common in the nerves supplying glabrous compared to hairy skin sites. Although it is questionable whether the glabrous skin of the tail and the hairy skin of the hindpaw are comparable with the thenar eminence and the DLH, this study raises the interesting supposition that there may be differences in the peripheral innervation densities of the nerves subserving the two skin types. Indeed, Hensel (1982) reported a higher density of cold spots on the DLH compared to the thenar eminence. In contrast, Stevens (1989) found a significantly lower two-point threshold in the thenar eminence compared to the forearm suggesting that, at least for mechanoreceptors, the thenar eminence may be more densely innervated.

Treede et al. (1995) found an absence of type II AMH receptors in monkey glabrous skin which have the characteristic short response and peak latencies of first pain sensations in response to heat stimulation. This result may explain the absence of first pain sensations from noxious heat stimulation of glabrous skin. Although a similar deficit in afferent types has yet to be identified for noxious cold sensitive fibres, the present study suggests that this is a possibility.

Sympathetic innervation and/or activation may also differ between skin types. Okamoto et al. (1994) found that whereas warm stimulation resulted in increased skin sympathetic nerve activity and vasoconstriction in both skin types, sudomotor activity (bursts of sweat) increased in glabrous skin and decreased in hairy skin. Conversely, both measures of sympathetic activation were raised to innocuous cooling. Campbell and LaMotte (1983) observed that primate glabrous skin tended to be warmer than hairy skin prior to testing. Since skin temperature is at least partly determined by sympathetically mediated vasodilation or vasoconstriction, this finding indicates that sympathetic activity may differ between skin types. If there was an increased initial skin temperature in glabrous skin more cooling would be required to reach a temperature equal to that of hairy skin. In the present study, the thermode was placed on the skin and the baseline temperature was maintained for several minutes before threshold determinations began. However, since the actual skin temperature was not measured, the possibility that the glabrous skin was initially warmer cannot be entirely ruled out. Perceptually, artificially induced sweating has been shown to lower cold thresholds exclusively for the thenar eminence (Hilz *et al.* 1992), indicating that sympathetic activity may produce different psychophysical affects depending on

the skin type stimulated. This “cold sweat” may result from secretion of the sympathetically innervated eccrine sweat glands, which are more abundant in glabrous skin sites (Cormack, 1987; Lever & Schaumburg-Lever, 1990). Although this effect has not been studied for cold-induced pain thresholds, it can be hypothesized that increased sweating in glabrous skin may affect the rate with which cold passes through the skin thereby increasing threshold measurements. On the other hand, Hagbarth et al. (1972) provide physiological evidence that the sympathetic innervation of hairy and glabrous skin is similar. They found no differences in either the spontaneous or the evoked discharges in sympathetic fascicles supplying the two skin sites.

(b) There may also be physiological differences in how cold information is encoded, transmitted, and processed from peripheral receptors to supraspinal brain areas in the two skin types. There are only a few primary afferent or perceptual studies in humans that have systematically looked at physiological differences between skin types in response to cold stimuli. The data from the present experiment are consistent with those of Greenspan and co-workers (1993) who reported that cold thresholds were significantly higher in the thenar eminence compared to the forearm. However, no differences in cold threshold were found between these same two sites in a recent study by Stevens and Chou (1998). Perl (1968) found that hairy but not glabrous SA-Ms responded to cooling of the skin as has also been shown in C-mechanoreceptors (Leem *et al.* 1993). If these afferents contributed to the perception of cold, they could account for the higher sensitivity of glabrous skin. Conversely, Johnson et al. (1973) claim that the information relayed from these thermal sensitive mechanoreceptors is of “nuisance value” only. Finally, LaMotte and Thalhammer (1982) found no differences in the responses of A δ and C-PMNs to noxious cold reaching 2°C, suggesting that these nociceptors process cold similarly.

Several studies have compared the perceptual and physiological differences between the two skin types in response to non-cold stimulation. Human heat pain thresholds have been found to be relatively lower in hairy skin (Pertovaara *et al.* 1988; Pertovaara *et al.* 1996), as have detection latencies for supra-threshold heat stimuli (Campbell & LaMotte, 1983). These studies parallel the results from thresholds in this study, however they also indicate that fibres subserving hairy skin sites are more sensitive to supra-threshold stimuli. Electrophysiological

recording studies in monkey STT neurons indicate that while neurons subserving hairy skin sites display shorter onset and peak latencies to heat stimulation, neurons subserving glabrous skin sites demonstrate higher mean rates of discharge to heat stimuli above 49°C (Surmeier *et al.* 1986b; Surmeier *et al.* 1986a). These findings are in agreement with the present findings of increased sensitivity of hairy skin for threshold temperatures and increased sensitivity of glabrous skin for supra-threshold cold stimulation.

Studies have shown that primate hairy skin CMH nociceptors have significantly lower mechanical thresholds compared to glabrous skin CMHs (Campbell & Meyer, 1983; Treede *et al.* 1995). In human subjects, lower thresholds for hairy compared to glabrous skin have also been found in response to innocuous (Hämäläinen *et al.* 1985) and noxious (Casey *et al.* 1987) mechanical stimulation. In contrast, laminae IV & V neurons with inputs from glabrous skin sites show significantly increased responses to pinch and squeeze (Surmeier *et al.* 1986a). The lower thresholds for hairy skin and the greater response for supra-threshold stimulation in glabrous skin is consistent with the results found presently for cold.

Systematic examinations of the conduction velocities of primary afferents subserving hairy and glabrous skin sites have also been examined. Studies report faster conduction velocities for both human glabrous skin AMHs (Treede *et al.* 1995) and monkey cold-sensitive afferents (Iggo, 1969). It is possible that the primary afferents subserving glabrous skin sites evolved faster conduction velocities to compensate for the increased thickness of the skin overlying them. Thus, although it might require more time for stimuli to pass through glabrous skin to reach the underlying receptors, once these receptors are activated they could transmit their messages to spinal and supraspinal areas at faster rates leading to faster responses. Conversely, other studies found no differences between skin types in the response of CMHs to heat (Campbell & Meyer, 1983; Fleischer *et al.* 1983; Treede *et al.* 1995) and mechanical stimuli (LaMotte & Thalhammer, 1982), or in conduction velocity measurement in A δ -HTMs (Lynn & Shakhaneh, 1988), CMHs (Campbell & Meyer, 1983), and LTMs (Kakuda, 1992).

These reports indicate that the properties of the receptors, primary afferents and spinal cord neurons subserving hairy and glabrous skin sites may differ in how they process cutaneously applied stimuli. If such differences could be demonstrated for the receptors

mediating noxious cold sensation, there would be strong evidence to support a physiological explanation for the results found in this study.

The limitations in this study and in previous work on noxious cold do not allow a definitive explanation for the differences observed between the two skin sites. Nevertheless a hypothesis may be generated based on the studies mentioned above. The higher thermal and pain thresholds from cold stimulation measured may be due to anatomical differences between the skin sites such as increased thickness of the epidermis, decreased density of cold receptors, and differential sympathetic innervation. Once threshold has been exceeded, an increased sensitivity of the fibres mediating noxious cold sensation may account for the faster rate with which glabrous ratings increase as well as the increase in the quality of the pain evoked from stimulation. Further studies are needed to measure the intra-cutaneous temperature of both hairy and glabrous cold nociceptors in order to confirm this hypothesis or offer alternative ones.

One such possibility could be a difference in how cold messages from hairy and glabrous skin receptors are processed in the CNS. Threshold and supra-threshold psychophysical measurements may be affected by subtle differences in the strength of connections between periphery and cortical areas or differential cortical magnifications of peripheral input.

6.5. Power Functions

Exponents of power functions during the cooling phase of Experiment II were higher for the glabrous skin site regardless of the cooling rate or the VAS scale used. These power functions were fit with high correlation coefficients both to raw data for all subjects separately and to mean data for all subjects combined. While the finding of higher exponents for glabrous skin compared to hairy skin trials was maintained for the two determinations, exponent values were smaller for the method employing the mean of all subjects. Examination of individual subjects' exponents revealed that the reason for this discrepancy was due to extreme outliers. For instance, exponents as large as 11.9 were found for a subject who began rating near the end of the cooling phase. Because exponents calculated with mean values reduce the effect of outliers, these may be a more accurate way to measure group data.

Few studies have fit noxious cold data to power functions. Chéry-Croze (1983) found a

mean exponent of 1 for intensity ratings in response to randomly presented stimuli from 20 to 0°C. In contrast, Arndt and Klement (1991) reported a mean exponent of 3.0 for continuous pain intensity ratings elicited from the infusion of noxious cold water into the veins. In response to noxious heat stimulation, Price et al. (1983) obtained exponents of 2.1 and 3.8 for pain ratings with intensity and affect scales respectively. It is not immediately apparent why the exponent values found in the present study more closely approximate those from venous infusion and heat stimulation rather than cold stimulation. Chéry-Croze used a larger thermode but a faster cooling rate than was used in the present experiment. One obvious difference in Chéry-Croze's study is that pain ratings were not taken continuously but, rather, a single number was given by subjects at the end of each trial. The use of continuous rating methods may produce an additive effect on pain ratings. In these methods, the preceding stimuli may influence the ratings coming afterwards, thereby increasing the rate with which pain ratings increase.

6.6. The Intensity vs. The Affect of Cold-Evoked Pain

No difference was found in this study between pain indices for ratings with the intensity VAS compared to ratings with the affect VAS. These findings are in keeping with previous research employing static noxious cold stimuli (Morin & Bushnell, 1998) and the cold pressor test (Rainville et al. 1997).

Price et al. (1987) and Duncan et al. (1989) suggest that a selective increase in affective ratings should occur when a threat to one's health or life is perceived. This theory is used to explain the relative increase in affective ratings for cancer patients (Price et al. 1987). Conversely, a positive outcome or a lack of perceived threat should decrease affective but not intensity ratings. This explains the relative decrease in affect ratings when perinatal women focus on the birth of their child (Price et al. 1987). It has also been used to explain the relatively lower affect ratings for experimental stimulation with heat or electrical stimuli (Rainville et al. 1992) when subjects are assured that they can escape for the stimuli at any time. Similarly, since in this study subjects were informed that they could stop the experiment at any time, it was predicted that aVAS ratings would be relatively lower than iVAS ratings. The results from the present experiment suggest that there is no relatively lowered affect in response to contact noxious cold

stimulation.

6.7. Quality of Cold-Evoked Pain

Although stimulation of the skin with heat primarily elicits sensations of 'burning', noxious cold stimulation generally evokes a wider variety of qualitative pain descriptors (Morin *et al.* 1994; Morin & Bushnell, 1998). Results from this study demonstrate that subjects respond to noxious cold stimulation not only by choosing words such as 'cold' and 'freezing' but by choosing a myriad of other pain related words. This finding may be biased by the educational background of the subjects in the present study. Most subjects were either students at the University of Toronto or employees of the Toronto Hospital and therefore may have been more educated than the general population.

Previous studies have demonstrated that certain qualitative descriptors are related to the type of primary afferent activated by a particular stimulus. Evidence from studies employing intraneural microstimulation or specific fibre blocks suggest that sensations of 'sharpness' and 'pricking' are mediated by A δ -fibres, whereas sensations of 'burning' and 'aching' are mediated by C-fibres (Mackenzie *et al.* 1975; Torebjörk & Ochoa, 1980; Konietzny *et al.* 1981; Fruhstorfer, 1984; Ochoa & Torebjörk, 1989; Wahren *et al.* 1989; Yarnitsky & Ochoa, 1990b; Handwerker *et al.* 1991a; Mengel *et al.* 1993). In this experiment, noxious cold stimuli typically elicited qualitative descriptors suggestive of activation of both fibre types (Fig. 19). This finding is in agreement with studies in several species that demonstrates both A δ (Georgopoulos, 1976; LaMotte & Thalhammer, 1982; Leem *et al.* 1993; Simone & Kajander, 1996; Simone & Kajander, 1997) and C-fibre (Iggo, 1959; Georgopoulos, 1976; LaMotte & Thalhammer, 1982; Leem *et al.* 1993; Campero *et al.* 1996; Simone & Kajander, 1996) activation during noxious cold stimulation of the skin.

6.8. Paradoxical Heat Sensations

A high proportion of subjects chose words representing sensations of heat when filling out the MPQ after stimulation with noxious cold. In Experiment III, it was shown that paradoxical sensations occurred most often during the cooling and static 2°C phases regardless of

stimulus rate or site. This result was unexpected considering that stimuli never exceeded 34°C and that subjects were specifically told that the object of the experiment was to test their reactions to cold stimuli. There was no mention of possible heat stimuli being given in the instructions or during the pre-experiment interview.

Reports of PH sensations to cooling are quite common following A-fibre blocks (Mackenzie *et al.* 1975; Fruhstorfer, 1984; Wahren *et al.* 1989; Yarnitsky & Ochoa, 1990b; Ochoa & Yarnitsky, 1994; Davis, 1998a), preheating of the test site (Chery-Croze & Duclaux, 1980; Hämäläinen *et al.* 1982; Greenspan *et al.* 1993; Hansen *et al.* 1996), and presenting spatially alternating warm and cold stimuli (Jahns, 1975; Green, 1977). Conversely, PH sensations during cooling without previous heating of the test site occurred in only ~10% of trials in Hämäläinen *et al.*'s (1982) study and only 2 subjects by Davis (1998a). Several explanations are possible for the high incidence of paradoxical heat responses observed in this study. The most likely explanation based on physiological evidence is that moderate cooling of the skin activates polymodal nociceptors in addition to cold-specific receptors. Innocuous cooling has been found to activate both A δ -PMNs (Georgopoulos, 1976) and C-PMNs (LaMotte & Thalhammer, 1982; Campero *et al.* 1996). Since intraneural microstimulation in awake humans often evokes the burning sensation characteristic of noxious heat but never sensations of noxious cold (Torebjörk & Ochoa, 1980; Konietzny *et al.* 1981; Ochoa & Torebjörk, 1989; Handwerker *et al.* 1991a; Torebjörk, 1992), it is possible that activation of PMNs by cooling may result in sensations of burning being perceived. One problem with identifying such receptors arises because most studies do not employ cold stimuli or use non-specific stimuli such as ether, ethanol, or ice when testing heat-sensitive afferents.

Furthermore, as suggested by Fruhstorfer (1984) to account for Hämäläinen *et al.*'s (1982) PH responses, the thermode used in this study may not have been large enough to recruit sufficient A δ -fibres to inhibit C-nociceptive fibre output. There is no objective evidence in the literature to prove or disprove this hypothesis. However, as described above, MPQ analysis demonstrated that a high proportion of subjects chose words commonly associated with activation of both A δ and C cold-sensitive afferents. This indicates that A δ -afferents were indeed activated to a large extent.

Research by Franz and Iggo (1968) suggests that A δ -fibres are blocked at higher temperature than C-fibres in response to cooling of feline skin. A selective blocking of A δ -fibres during the cooling phase of this experiment may have reduced their inhibitory activity upon C-fibres centrally and thereby increased the relative contribution of C-fibres towards perception. Since C-fibre activity results mainly in sensations of burning, as was described above, sensations of painful PH to cooling would predominate. This would also explain the low incidence of painful PH reported during the rewarming phase of the experiment when A δ -fibres would have been active (Fig. 21).

Finally, a hypothesis that has not been previously suggested is that the juxtaposition of the cold thermode beside relatively warmer skin may create a Thurnberg grill-type arrangement. This alternating warm, cold, warm arrangement could produce PH through central mechanisms as outlined by Craig and Bushnell (1994) and described in Section 2.

6.9. What is Cold Pain?

Near the turn of the century, Alrutz (1898) proposed that the sensation of 'hot' was, in fact, a combination of the activation of warm and cold specific channels. In contrast, it is possible that 'cold pain' results from the activation of the cold specific channel combined with a paradoxical activation in the noxious heat channel which give rise to sensations of innocuous cold and burning pain respectively. Several lines of evidence support this theory. First, intraneural microstimulation of primary afferents in awake human subjects often elicit painful sensations but never sensations of 'painful cold' (Torebjörk & Ochoa, 1980; Konietzny *et al.* 1981; Schady *et al.* 1983; Torebjörk *et al.* 1984; Ochoa & Torebjörk, 1989; Handwerker *et al.* 1991a; Torebjörk, 1992; Ochoa, 1993). Second, studies of the effects of infusion of noxious cold saline into the veins of human subjects during selective anaesthetic blocks of veins and cutaneous tissues indicate that the 'cold' and 'pain' aspects of cold pain can be differentially inhibited (Arndt & Klement, 1991; Klement & Arndt, 1991). Selective anaesthesia of the veins results in sensations of 'cold' only for venous infusion of noxious cold saline. In contrast, when the cutaneous tissue was selectively anaesthetised, noxious cold infusion results in sensations of 'pain' only, devoid of a specific quality. These findings indicate that the 'cold pain' sensations

felt from infusion of noxious cold saline into the veins is a combination of modality specific cold receptors intracutaneously and modality non-specific nociceptors in the vein walls. Third, interventions such as selective A δ -blocks (Fruhstorfer, 1984; Wahren *et al.* 1989; Yarnitsky & Ochoa, 1990b; Davis, 1998a), juxtaposed warm and cold stimuli (Green, 1977; Craig & Bushnell, 1994), and preheating (Hämäläinen *et al.* 1982; Greenspan *et al.* 1993; Hansen *et al.* 1996; Davis, 1998a) can result in painful heat sensations to innocuous cooling. More importantly, results from the present study and from previous studies (Hämäläinen *et al.* 1982; Davis, 1998a) suggest that these sensations can also be evoked from cooling without any intervention. That sensations of heat can be elicited so readily by cooling the skin indicates that nociceptors that give rise to sensations of heat pain may be activated by cooling under normal circumstances.

Therefore, these separate lines of evidence suggest that during cooling of the skin, both cold receptors that give rise to sensations of 'cold' and nociceptors that give rise to sensations of 'painful heat' are activated. The relative contribution of these two cold-sensitive channels in determining the sensations felt to cooling may be altered by peripheral or central factors, such as reciprocal inhibition. If both channels are highly active, sensations of 'cold pain' will predominate. However, if the activity of the cold channel is somehow decreased, then painful paradoxical 'heat' sensations will predominate.

Section 7. Summary and Conclusions

Several important findings have resulted from the experiments that comprise this thesis:

(1) Cold thresholds, measured with the method of limits, were lower (i.e. occurred at higher absolute temperatures) for the hairy skin of the dorso-lateral hand compared to the glabrous skin of the thenar eminence.

(2) A similar pattern was evident for cold induced pain thresholds with MOL at 1.5°C/s and with intensity and affect VAS scales at 0.5 and 1°C/s .

(3) Exponents for supra-threshold ratings fit to power functions were larger for the glabrous skin site than the hairy skin site regardless of cooling rate or dimension of pain measured. The former two findings support hypothesis 1, stated in Section 3, which predicted that hairy skin would be found to be more sensitive compared with glabrous skin. In contrast, the third finding suggests that glabrous skin may be more sensitive to cooling above the threshold for pain. The differences in threshold found for hairy and glabrous sites are likely the result of a thicker epidermis in glabrous skin, a higher density of cold receptors in hairy skin, and/or other anatomical dissimilarities between the two skin types. On the other hand, the equal and often increased sensitivity of glabrous skin above threshold is most probably due to physiological differences in the neural circuitry involved in encoding and processing noxious cold stimuli.

(4) All pain indices were higher for slower cooling rates. Therefore hypothesis 2, which predicted that faster cooling rates would result in higher psychophysical ratings of pain, must be rejected. Hypothesis 2 was derived from previous research on the effects of changing temperature rates on the psychophysical and electrophysiological responses to innocuous cold and innocuous and noxious heat stimulation. These studies overwhelmingly found that faster rates of temperature change increased the response to thermal stimulation. On the other hand, this study suggests the opposite effect for noxious cold stimulation. However, it cannot presently be determined whether these results were due to the longer duration of trials with slower cooling rates or whether there is a fundamental difference in how noxious cold is processed in the nervous system. These results predict that electrophysiological recordings from noxious cold-sensitive afferents should show an increased response to slower cooling rates or an increased

recruitment of afferents. Further studies will determine whether this prediction is correct.

(5) No significant differences were found in the pain indices for pain ratings of intensity and affect. Thus hypothesis 3, which predicted a relatively reduced pain affect, must also be rejected.

(6) A substantial proportion of subjects chose words representing paradoxical heat with the McGill Pain Questionnaire.

(7) Painful heat sensations occurred most often during the cooling phase, while innocuous warm sensations mainly occurred during the rewarming phase. These results show that even in the absence of A δ -blocks, paradoxical heat responses can be readily elicited in normal subjects.

As was demonstrated in the literature review (Section 2), very little is known about how noxious cold is encoded and processed from the peripheral receptors to the brain areas involved in conscious perception. This thesis was an initial step towards filling in the gaps in our present scientific knowledge towards understanding what 'cold pain' really is.

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Appendix A: Instruction Sets for Experiment I - Cold and Cold-Induced Pain Thresholds

(i) Thresholds

Part I - Cool Sensation

With the thermal stimulator attached to your hand, you will be asked to press a button IMMEDIATELY when the temperature felt changes from neutral to cool. Once you press the button, the temperature will return to neutral, some amount of time will elapse, and the test will be repeated. This will be done 5 times.

Part II - Cold Pain

In this section of the experiment, you will press the button IMMEDIATELY when the sensation that you feel becomes **painful**. One way to distinguish between cold and pain is to concentrate on whether you feel any painful sensation other than the cold sensation - for example painful pricking, burning, aching, or some other pain quality.

(ii)

COOL THRESHOLD

**PRESS THE BUTTON IMMEDIATELY
WHEN THE TEMPERATURE CHANGES
FROM NEUTRAL TO COOL.**

(iii)

PAIN THRESHOLD

**PRESS THE BUTTON IMMEDIATELY
WHEN YOU FEEL PAIN:
E.G. PRICKING, BURNING
ACHING, ETC.**

Appendix B: Instruction sets for Experiment II - Supra-threshold Cold Stimulation

(i) INTENSITY VS UNPLEASANTNESS

In this experiment we ask you to evaluate the pain sensation felt when we vary the temperature on your skin. In evaluating the painfulness of the cold, we would like you to consider two separate aspects of the pain. First is the **intensity** of the pain sensation. That is how strong is the burning, pricking, aching, or other pain sensation. Second is the “**unpleasantness**” of the pain. That is, how much does the pain bother you.

As an example of the distinction between intensity and unpleasantness, imagine yourself at a concert for your favourite rock (or other) band. Although the volume of the music may be quite high, you are enjoying yourself and will not be very bothered by it. Now exchange the band with your least favourite one. Although the volume (**intensity**) remains the same, it is now very irritating (**unpleasant**) to you and you may want to put your hands over our ears. Thus, pain can be perceived as being intense, and not very unpleasant, or it could be unpleasant without being intense.

As a further example, imagine that you have an abdominal pain. If you have just finished eating a huge meal, and think that the pain is related to indigestion, you may not perceive the pain as very unpleasant. If, on the other hand, you have just been diagnosed with cancer of the intestine, an abdominal pain of the same intensity may be very unpleasant to you.

(ii) EXPLANATION OF THE EXPERIMENT

You will be asked to evaluate either the **intensity** or the **unpleasantness** of the stimuli over time, during various levels of cold stimuli presented to one of two sites on your hand,

Please note that the experimental trials may last slightly longer than the practice trials, and that each trial may not necessarily be the same as the last.

The scale that you will use asks you to evaluate the “PAIN” that you feel, not the “COLD”. You may use any part of the scale, so do not feel obligated to jump between verbal descriptors.

Also, keep in mind that you are free to withdraw from the experiment at any time without penalty.

Intensity

In the first part of the experiment, you will be asked to rate the **intensity** of the cold stimulus. With your free hand, you will move a mouse which raises or lowers the scale on the computer screen from a value of 0 “No Pain” to a value of 100 “Extremely Intense” Pain.

“No Pain” for cold means that, although you may feel a cold sensation, this feeling is not painful.

To help define “Pain”, please keep in mind that a cold sensation without pricking, burning, aching, or some other quality of pain should not be regarded as painful.

Unpleasantness

The second part of the experiment is almost identical to the first except that now you will be asked to rate the **unpleasantness** of the cold. As with the intensity, you will use the mouse to rate the cold from a value of 0 “Not Unpleasant” to a value of 100 “Extremely Unpleasant”.

It is also important to keep in mind that although the stimuli may feel quite cold, we have chosen stimuli that will not damage your skin in any way. In addition, please remember that you are free to stop the trial at any point without penalty.

Patient's name: _____				ID _____
Test site _____				Date _____
SP	CH	ME	VH	other: _____

A) How strong is your pain?

- (0) no pain
- (1) mild
- (2) discomforting
- (3) distressing
- (4) horrible
- (5) excruciating

B) What does your pain feel like?

Please circle the words which describe your pain:

1) flickering
quivering
pulsing
throbbing
beating
pounding	2) jumping
flashing
shooting	3) pricking
boring
drilling
stabbing
lancinating	4) sharp
cutting
lacerating
5) pinching
pressing
gnawing
cramping
crushing	6) tugging
pulling
wrenching	7) hot
burning
scalding
searing	8) tingling
itchy
smarting
stinging
9) dull
sore
hurting
aching
heavy	10) tender
taut
rasping
splitting	11) tiring
exhausting	12) sickening
suffocating
13) fearful
frightful
terrifying	14) punishing
gruelling
cruel
vicious
killing	15) wretched
blinding	16) annoying
troublesome
miserable
intense
unbearable
17) spreading
radiating
penetrating
piercing	18) tight
numb
drawing
squeezing
tearing	19) cool
cold
freezing	20) nagging
nauseating
agonizing
dreadful
torturing