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Threshold and rate-sensitivity of low-threshold thermal nociception

Barry G. Green^{1,2} and Carol Akirav¹

¹ The John B. Pierce Laboratory, 290 Congress Avenue, New Haven, CT, USA

² Department of Surgery (Otolaryngology), Yale University School of Medicine 333 Cedar Street, New Haven, CT, USA

Abstract

Previous studies have shown that sensations of burning, stinging or pricking can be evoked by warming or cooling the skin to innocuous temperatures (*low-threshold thermal nociception*; LTN) below the thresholds of cold- and heat-sensitive nociceptors. LTN implies that some primary afferent fibers classically defined as warm and cold fibers relay stimulation to the nociceptive system. We addressed this question in **humans** by determining if different adaptation temperatures (AT) and rates of temperature change would affect thermal sensation and LTN similarly. In exp. 1 subjects rated the intensity of warmth, cold and nociceptive sensations produced by increasing steps in temperature ($\pm 0.5^\circ\text{C}$ increments) from ATs of 35° , 33° , 31°C for cooling and 30° , 32° , 34°C for heating. Depending upon AT, thresholds for nociceptive and thermal sensations estimated from the rating data differed by as little as -1.0° for cooling and $+1.5^\circ$ for heating. Thresholds of thermal and nociceptive sensations shifted by similar amounts across the 3 ATs during cooling, whereas during heating the nociceptive threshold was significantly affected only between ATs of 32° and 34°C . In Exp. 2, increasing the rate of temperature change from $0.5^\circ/\text{sec}$ to $4.0^\circ/\text{sec}$ increased the intensity of thermal and nociceptive sensations significantly, but the effect was greatest for nociceptive sensations during heating. The results of both experiments are consistent with mediation of LTN by low-threshold thermoreceptors, although LTN caused by heating may depend on a subset of fibers that express less sensitive TRP-channels than those that serve sensations of warmth at the mildest temperatures.

Keywords

temperature; nociception; psychophysics; human

Introduction

Temperature and pain are generally considered to be separate sensory systems served by different receptors and afferent fibers. The sensitivities to warmth and cold are attributed to stimulation of specific warm fibers and cold fibers (Hensel and Iggo, 1971; Hensel and Zotterman, 1951; Hensel and Boman, 1960) that have thresholds in the range of normal resting skin temperatures (ca. 25° – 35°C), whereas nociceptive sensations such as burning, stinging or pricking are attributed to stimulation of temperature-sensitive nociceptors (Bessou and Perl, 1969; Iggo and Ogawa, 1971) that have high thresholds and respond to noxious heat or cold. However, recent data indicate that cooling the skin to temperatures as mild as 28° – 31°C (Green

and Schoen, 2005; Green and Pope, 2003) or heating it to just 37°C (Green and Akirav, 2007) can evoke nociceptive sensations of stinging, pricking or burning. These sensations, which have been called *low-threshold thermal nociception* (LTN), can be evoked from spots ($\approx 1\text{--}2\text{ mm}^2$) in hairy skin (Green et al., 2008) similar to classically defined ‘warm spots’ and ‘cold spots’. Indeed, in the latter study nociceptive sensations were evoked by the same mild temperatures (28° and 36°C) that were used to locate warm spots and cold spots. Based on these and other findings, it has been proposed that some afferent fibers typically identified as warm fibers and cold fibers relay stimulation to the nociceptive pathway in addition to, or instead of, the thermal pathways (Green, 2002;2004; Green and Schoen, 2007; Green et al., 2008; Yarnitsky, 2008; Belmonte et al., 2009).

Electrophysiological support for this hypothesis has come from studies of spinal (Christensen and Perl, 1970; Price and Mayer, 1975; Khasabov et al., 2001; Zhang et al., 2006), trigeminal (Price et al., 1976; Zanotto et al., 2007) and thalamic (Bushnell et al., 1993; Hutchison et al., 1997) neurons that respond to innocuous cooling or warming as well as to nociceptive thermal, chemical, or mechanical stimuli. In addition, there is evidence that the capsaicin- and heat-sensitive channel TRPV1 can be co-expressed on DRG neurons with the putative cold receptor TRPM8 (McKemy et al., 2002; Abe et al., 2005; Xing et al., 2006; Okazawa et al., 2004; Hjerling-Lefler et al., 2007) and with the potential warm receptor TRPV3 (Smith et al., 2002), which has a heat threshold between 31°–39°C (Peier et al., 2002; Xu et al., 2002; Smith et al., 2002). Co-expression of high- and low-threshold channels could enable some primary afferent fibers that relay stimulation to the nociceptive pathway to respond to both innocuous and noxious temperatures. If so, low-threshold afferent fibers that converge on nociceptive spinothalamic tract (STT) neurons should have similar sensitivities and other response characteristics as fibers that project to cold- or warm-specific STT neurons (Dostrovsky and Craig, 1996; Andrew and Craig, 2001), and the psychophysical characteristics of LTN should not differ from those of warmth and cold.

The purpose of the present study was therefore to compare LTN and the perception of warmth and cold in terms of two basic psychophysical properties. In experiment 1, the thresholds for reporting thermal (warmth, cold) and nociceptive sensations (e.g., burning, stinging) were measured at different adapting (baseline) temperatures. In experiment 2, the effect of the rate of temperature change on the intensity of thermal and nociceptive sensations was compared. Of primary interest in experiment 1 was whether adapting temperature would affect thermal and nociceptive sensations equally, as would be expected if they were mediated by the same thermoreceptors. Similarly, the effect of rate of temperature change on the intensity of thermal and nociceptive sensations should be the same if LTN is mediated by a subset of thermoreceptors that differ only with respect to the STT neurons to which they project.

Methods

Subjects

Forty-three subjects (13 males, 30 females) served in experiment 1 and 36 subjects (14 males and 23 females) served in experiment 2, 21 of whom had served in experiment 1. All subjects were between 18 and 45 yrs of age and were paid for their participation. Recruitment was limited to non-smokers who were reported to be in good health and who had no history of injury or neurological disorders that might affect the cutaneous sensitivity of the right forearm. The research was carried out under a human subject research protocol approved by the Yale University human investigations committee, and all subjects gave **written** informed consent.

Thermal stimulation equipment

Temperature stimuli were produced by a 4×4 array of 0.64 cm^2 Peltier thermoelectric modules (referred to hereafter as the thermode) designed and built in the John B. Pierce Laboratory machine and electronics shop. The 16 modules are independently controlled and thermally isolated from one another. The ceramic surface of each module is covered with a copper plate ($8 \text{ mm} \times 8 \text{ mm} \times 0.96 \text{ mm}$), and temperature at the skin-module interface is monitored by a 40-gauge copper-constantin thermocouple epoxied into a 0.5 mm deep groove in the center of the plate. Stimulus parameters for each module are controlled via a LabView program that enables the experimenter to specify the base (adapting) temperature, rate of temperature change, target temperature, and dwell time at the target temperature.

Testing was conducted on the volar surface of the forearm. The subject sat in a modified dental chair with the right arm extended on a padded armrest. The thermode was mounted on a microscope stage that was attached to a floor-mounted positioning arm. At the beginning of each test block the thermode was adjusted against the forearm until all 16 modules made full contact with the skin. The positioning arm was then locked in place and any additional slight adjustments of the thermode's angle of contact or force against the skin were made via vernier controls on the microscope stage.

All thermal testing was conducted in a laminar-flow environmental chamber with air temperature and relative humidity controlled at 23°C and 30%, respectively.

General psychophysical procedure

In both experiments all subjects received practice in the psychophysical task prior to data collection. Individuals who had not previously participated in an intensity scaling experiment in this laboratory served in an additional practice session in which they were instructed in the use of the general version of the Labeled Magnitude Scale (gLMS; Bartoshuk et al., 2003; Green et al., 1996; Green et al., 1993) to rate the perceived intensity of thermal and nociceptive sensations. The gLMS is a vertically oriented scale on which intensity ratings are made relative to the location of intensity descriptors ('no sensation', 'barely detectable', 'weak', 'moderate', 'strong', 'very strong' and 'strongest imaginable') that are spaced along the scale according to their empirically determined semantic magnitudes (Green et al., 1993). Intensity ratings are made using a mouse to move a cursor to the location on the scale that matches the perceived intensity of the sensation. To give subjects experience using the gLMS to rate sensations of widely different intensity and quality, they were first asked to imagine and then rate the intensity of common thermal experiences (e.g., "the coolness of tap water"; "the heat from walking barefoot on hot sand"). Practice was then given rating actual thermal stimuli (30° , 28° , 26° , 24°C for cooling, 36° , 38° , 40° , 42°C for warming) delivered in pseudo-random order to the forearm via the thermode. The rate of temperature change and the dwell time were fixed at $\pm 3.0^\circ\text{C}/\text{sec}$ and 5.0 sec, respectively. For each stimulus subjects made independent ratings of the intensity of thermal (defined as warmth, cold, or heat) and nociceptive sensations (defined as burning, stinging, or pricking) on separate gLMS scales that were displayed sequentially on the computer monitor.

To reacquaint returning subjects with the rating task, individuals who had prior experience using the gLMS received practice with the same set of sample thermal stimuli at the beginning of the first experimental session.

Experiment 1: Thermal and nociceptive sensations as a function of temperature

This experiment was designed to obtain data on the effect of adapting temperature (AT) on perception of nociceptive and thermal sensations. Of particular interest was the difference in

temperatures at which thermal and nociceptive sensations were first perceived, and whether these temperatures would be similarly affected by changes in AT.

The psychophysical procedure called for subjects to use the gLMS to rate the perceived intensity of thermal and nociceptive sensations produced when thermode temperature was incrementally changed from an AT by a fixed amount ($\Delta T = \pm 0.5^\circ\text{C}$) on successive trials (i.e., an ascending intensity series). Subjects also reported the sensation qualities they perceived by selecting descriptors from the pick list. Each series of trials began with the thermode being placed on the forearm and set to one of 6 ATs (35° , 33° , 31°C for cooling; 30° , 32° , 34°C for heating) for 5-min. At the end of this adaptation period testing began with a blank trial in which thermode temperature did not change (i.e., $\Delta T = 0$). This trial insured that adaptation was complete and the AT was perceived to be thermally neutral. On subsequent trials thermode temperature was progressively increased to a maximum of 38°C for heating, or progressively decreased to a minimum of 28°C for cooling. The number of ΔT s (and thus trials) in a given intensity series depended on the difference between the AT and the ending temperature, with the number ranging from as few as 7 ΔT 's (cooling from AT = 31° to 28°C) to as many as 17 ΔT s (warming from AT = 30° to 38°C).

The stimuli were delivered at 60-sec inter-stimulus intervals (ISIs) on two rows (8 modules) of the thermode, and the rows that were stimulated were alternated across trials. The resulting 2-min interval between successive stimulations on the same skin area, together with the use of ascending intensity series, were designed to minimize the possibility that thermal adaptation would affect perception of the weakest stimuli. For all stimuli the rate of temperature change was $\pm 4^\circ/\text{sec}$ and the dwell time at the target temperature was 4 sec.

Two ATs, one warm and one cold, were tested in each session, with half of the subjects starting with warm temperatures and half with cold. After testing at the first AT was completed the thermode was moved to an adjacent area of the forearm and another 5-min adaptation period was begun at the new AT. Subjects served in 3 testing sessions, and ATs were tested in 3 different orders such that each one was presented first, second and third in the series an equal number of times across subjects.

Experiment 2: The effect of rate of temperature change on nociceptive and thermal sensations

This experiment was designed to determine whether varying the rate of heating or cooling at innocuous temperatures affects nociceptive and thermal sensations similarly. Similar effects would support the hypothesis that the two qualities of sensation are mediated by the same thermal transduction mechanisms (e.g., TRP channels) and afferent fibers.

The subjects' task was to rate the perceived intensities of thermal and nociceptive sensations produced by two rates of heating and cooling: $\pm 0.5^\circ$ and $4.0^\circ\text{C}/\text{sec}$. The test temperatures used were 34° , 36° and 38°C for warming and 32° , 30° , 28°C for cooling. The warming stimuli were initiated from an AT of 30°C and the cooling stimuli from an AT of 35°C . Based on data obtained in experiment 1 (see below), the two mildest test temperatures were expected to evoke only thermal sensations whereas the two stronger temperatures were expected to also evoke LTN in some individuals. The temperatures were presented in pseudo-random sequence with warm and cool temperatures presented on alternate trials with the ramp rate varying independently. The stimuli were presented at an ISI interval of 60 sec to single rows of the thermode. Each row was set to the appropriate AT for 5 min prior to the beginning of testing (i.e., rows 1 and 3 were set to 30° , rows 2 and 4 were set to 35°C). This procedure created a 4-min ISI between stimulation of a given area of skin. The longer ISI helped to avoid thermal adaptation because the stimuli were presented in random sequence, causing stronger stimuli to sometimes precede weaker ones on the same site. After each stimulus had been presented

twice the subject was given a brief break as the thermode was moved to an adjacent skin site to begin another 5-min adaptation period. At the end of that period a second block of trials was presented with a different stimulus order. The experiment therefore comprised just a single test session in which two replicate ratings were collected for each stimulus.

Data Analyses

In experiment 1, *perceptual thresholds* for thermal and nociceptive sensations were estimated from ratings of perceived intensity. The threshold criterion was arbitrarily defined as the first temperature in an ascending series that received a mean rating greater than 'barely detectable' on the gLMS. This method and criterion were expected to lead to higher threshold estimates than have been obtained using sensitive psychophysical methods designed specifically to measure the minimum threshold for detection (e.g., Kenshalo et al., 1961; Yarnitsky and Ochoa, 1991). However, such a task could not be used with confidence to measure detection thresholds for nociceptive sensations, which are almost always accompanied by thermal sensations. Accordingly, we refer to these data as perceptual thresholds rather than detection thresholds, with the understanding that they represent conservative estimates of sensitivity.

In both experiments intensity ratings were averaged across replicates within subject and then converted to $\log_{10}$ to normalize the distribution of ratings across subjects. All statistical analyses were based on the logged data. Main effects and interactions were determined using repeated measures ANOVAs, and specific contrasts were evaluated using the Tukey HSD test. Because in experiment 1 the maximum cold and warm temperatures were fixed, the number of ΔT 's that could be tested was different for each AT. As a result, analyses of the perceived intensity of temperature sensations were based on the first 7 ΔT 's for cooling and the first 9 ΔT 's for heating, which were the maximum numbers of ΔT 's that could be tested from AT's of 31°C and 34°C, respectively. In addition, because for some AT's ratings of nociceptive sensations for the smallest ΔT 's were uniformly zero (i.e., 'no sensation'), the associated variables had no variance and could not be included in ANOVAs. In these cases the analyses included the lowest ΔT 's for which non-zero ratings were made.

Results

Experiment 1: Perception of nociceptive sensations as a function of adaptation temperature

Because not all subjects experience LTN (Green et al., 2008; Green and Schoen, 2005), estimates of the threshold for nociceptive sensations based on data from all subjects would bias the results toward lower temperatures for cooling and higher temperatures for heating. The analysis therefore included data only from subjects who reported at least 'barely detectable' burning, stinging, or pricking at 28° during cooling (AT = 31°) or at 38°C during heating (AT = 34°C). These criteria identified 28 subjects (65% of those tested) who perceived cold LTN and 32 subjects (74.4%) who perceived heat LTN. The log mean ratings for these two groups of subjects for both thermal and nociceptive sensations are graphed in Fig. 1. The top two graphs (a & b) contain ratings of thermal sensations and the bottom two graphs (c & d) contain ratings of nociceptive sensations, with cooling stimuli on the left and heating stimuli on the right. A dashed horizontal line in each graph indicates 'barely detectable' on the gLMS, the criterion intensity level that was used to compare perceptual thresholds across sensation quality and AT. Estimated temperatures of the perceptual thresholds are shown in Table 1. These are conservative estimates, in that the threshold for a particular quality was assumed to be the temperature at which the log-mean intensity rating for that quality first reached or exceeded 'barely detectable' on the gLMS.

Looking first at the ratings for thermal sensations, the lowest perceptual thresholds for cooling and heating were 34° and 31°C, respectively, and both thresholds corresponded to a

temperature change of $\pm 1^\circ\text{C}$. As expected, changes in AT produced orderly shifts in the perceived intensities and perceptual thresholds of both cold and warmth. However, separate repeated-measures ANOVAs of the cooling and heating data also revealed significant interactions between the effects of AT and ΔT [cooling: $F_{(12,324)}=2.50$, $p<0.005$; heating: $F_{(16,496)}=4.12$, $p<0.0001$]. These interactions reflected a tendency for perceptual thresholds to be reached at smaller ΔT 's as the AT was raised for heating or lowered for cooling. For example, when the AT was 35°C , a ΔT of -1.0° was required for cold to be at least 'barely detectable', compared to a ΔT of only -0.5° when the AT was 31°C (Table 1). Similarly, to perceive warmth at the same level required a ΔT of $+1.0^\circ$ from an AT of 31°C compared to a ΔT of $+0.5^\circ$ from an AT of 35°C . The effect of AT diminished as ΔT increased. There were no significant differences in perceived intensity among the 3 AT's for cooling to 28.5°C and below, or for warming to 37°C and above (Tukey HSD, $p<0.05$).

ANOVAs also revealed significant interactions between the effects of AT and ΔT for nociceptive ratings [cooling: $F_{(10,270)}=6.23$, $p<0.0001$; heating: $F_{(10,310)}=4.73$, $p<0.0001$]. However, the effects were manifest in different ways and to different degrees for cooling and heating, and the perception of nociceptive sensations required larger ΔT 's than the perception of cold and warmth. To perceive at least 'barely detectable' nociceptive sensations during cooling (Fig. 1c) required a ΔT of at least -2.5° when the AT was 35°C , and the required ΔT decreased to only -1.5° when the AT was lowered to 31°C . Even so, the perceptual thresholds for nociceptive sensations were only 1° – 2°C higher than the thresholds for cold sensations (Table 1). From an AT of 35°C , nociceptive sensations began to be perceived after cooling to only 32.5°C . The latter finding together with the ability of the two lower adaptation temperatures to shift the perceptual threshold for nociception to lower temperatures implies the afferent fibers that mediate cold LTN have thresholds above 32°C , and possibly as high as 34°C .

Consistent with the relatively higher nociceptive thresholds compared to cold thresholds, the slopes of the functions for nociceptive sensations were lower than for cold sensations, and the effect of AT did not disappear until cooling fell below 29°C (Tukey HSD, $p<0.05$). However, the latter point of convergence was only 0.5°C lower than for sensations of cold.

The ΔT 's required to evoke nociceptive sensations during heating (Fig. 1d and Table 1) were also larger than those required to perceive warmth, and the relationship with AT was less systematic than for cooling. Raising the AT from 32° to 34°C produced a $+1.5$ to $+2.0^\circ$ shift in the nociceptive threshold, whereas the difference in perceptual threshold between AT's of 30° and 32°C was at most only $+0.5^\circ\text{C}$. Indeed, for these two AT's, log-mean intensity ratings at 34.5° and 35°C were not significantly different from one another (Tukey HSD test; $p>0.05$). Thus, adaptation to 30°C had no statistically significant effect on the threshold for perception of nociceptive sensations during heating. In contrast, adaptation to 34° clearly shifted the threshold to a higher temperature. Together these two findings imply that the most sensitive afferent fibers responsible for heat LTN probably have thresholds between 32° and 34°C .

Experiment 2: Effect of rate of temperature change on LTN vs. thermal sensation

As in experiment 1, analyses were conducted only on the data from subjects who reported nociceptive as well as thermal sensations. The criteria for inclusion were ratings of at least 'barely detectable' stinging/pricking or burning for cooling to 28°C at $-4.0^\circ/\text{sec}$ or for heating to 38°C at $+4.0^\circ/\text{sec}$. The resulting subsets included 21 subjects (56.7%) for cooling and 29 subjects (78.4%) for heating. The data from these subjects are shown in Fig. 2a–d.

Increasing the rate of cooling or heating from 0.5° to $4.0^\circ/\text{sec}$ increased the log-mean intensity ratings of both thermal and nociceptive sensations. At the most extreme temperatures tested (28° and 38°C), the $\pm 4^\circ/\text{sec}$ rate produced cold-thermal and heat-thermal sensations that were

twice as intense as those produced by the $\pm 0.5^{\circ}\text{C}$ rate (log-mean differences of +0.30 and +0.31, respectively). The increase in intensity between the two rates was slightly greater for cold-nociceptive sensations, which intensified by a factor of 2.5 to 1 (log-mean difference of +0.40), and was much greater for heat-nociceptive sensations, which intensified by a factor of 5.4 to 1 (log-mean difference of +0.73). Separate repeated measures ANOVAs conducted on the thermal and nociceptive ratings revealed main effects of rate of temperature change for both categories of sensation [thermal sensations: $F(1,20)=33.9$, $p<0.0001$ for cooling, $F(1,28)=30.3$, $p<0.0001$ for heating; nociceptive sensations: $F(1,20)=5.8$, $p<0.05$ for heating, $F(1,28)=43.7$, $p<0.0001$ for cooling]. The same ANOVAs revealed significant rate $\times$ temperature interactions for nociceptive sensations [cooling: $F(2,40)=3.40$, $p<0.05$; heating: $F(2,56)=13.3$, $p<0.0001$] but not for thermal sensations. It is apparent from Figs. 2c and 2d that the source of the interaction for nociceptive sensations was the absence of an effect of rate of cooling and heating at the two mildest temperatures (32° and 34°C), where nociceptive sensations averaged less than 'barely detectable' for the $\pm 0.5^{\circ}\text{C}/\text{sec}$ rate and did not increase at $\pm 4.0^{\circ}\text{C}/\text{sec}$ (Tukey HSD test, $p>0.05$).

Two other ANOVAs compared the effects of rate of cooling and heating between the two categories of sensation. For cooling there was a significant main effect of rate of temperature change [$F(1,20)=15.6$, $p<0.001$] but no interaction between rate of temperature change and sensation quality [$F(1,20)=.90$, $p=0.35$]. The 3-way interaction among the effects of temperature, rate and sensation quality fell just short of significance [$F(2,40)=2.6$, $p=0.085$]. The ANOVA conducted on the heating data showed a stronger effect of rate of temperature change. In addition to a significant main effect of rate [$F(1,28)=47.3$, $p<0.0001$], there were significant interactions between rate and sensation quality [$F(1,28)=16.5$, $p<0.0005$] and among temperature, rate, and sensation quality [$F(2,56)=11.1$, $p<0.0001$]. In summary, rate of temperature change had similar effects on (1) cold- and heat-thermal sensations, and (2) cold-thermal and cold-nociceptive sensations, but (3) had its greatest effect on heat-nociceptive sensations.

Discussion

The present results support the hypothesis that low-threshold thermoreceptors with thresholds in the range of warm fibers and cold fibers relay stimulation to the nociceptive system. The thresholds for nociceptive sensations differed from the thresholds for thermal sensations by at most 3.5° and by as little as 1.0° , with some subjects reporting LTN at 32.5°C during cooling and 34.5°C during warming, temperatures that are far below the thresholds of identified cold-sensitive (Campero et al., 1996; Simone and Kajander, 1996) and heat-sensitive (Van Hees and Gybels, 1981; Bessou and Perl, 1969; Yarnitsky et al., 1992) nociceptors. The results are therefore consistent with the evidence that some STT neurons that encode pain (e.g., WDRs) receive input from primary afferent fibers that have thermal thresholds in the innocuous range (Christensen and Perl, 1970; Price and Mayer, 1975; Zhang et al., 2006; Zanutto et al., 2007). A few A δ - and/or C-fibers that respond strongly to painfully cold temperatures have been reported to have thresholds above 20°C (Georgopoulos, 1976; LaMotte and Thalhammer, 1982; Campero et al., 2001), but none have been reported that have thresholds above 30°C . Moreover, the likelihood that nociceptors would have thermal thresholds low enough to yield perceptual thresholds just 1.0 – 1.5°C higher or lower than those produced by cold fibers and warm fibers stretches the definition of nociceptors to the extreme. A more parsimonious explanation is that activity in some fibers that express TRP channels that have temperature sensitivity in the innocuous range (e.g., TRPM8, TRPV3 or TRPV4) relay stimulation to the nociceptive pathway, either exclusively or in addition to the thermal pathways (Green, 2002; 2004; Green et al., 2008). Belmonte and Viana (2008) recently reviewed in detail the mounting cellular and molecular evidence that multimodal signal processing of this kind takes place in somatosensory neurons, and summarized this evidence in what they aptly described as the

“Blurry Picture” model. In this model all afferent fibers express multiple receptor channels that render each fiber sensitive to multiple stimulus types over potentially wide ranges of stimulus energy.

Consistent with this view, Campero and Bostock (2009) recently reported cold-sensitive C-fibers in human hairy skin that have the response characteristics of A δ -cold fibers, including spontaneously discharge at temperatures as high as 35°C. Although the kinds of sensations these fibers mediate is uncertain, the existence of low-threshold C-fibers have been hypothesized (Fruhstorfer, 1984) to explain why pressure or ischemic A-fiber block causes innocuous cooling to be perceived as burning or stinging (Wahren et al., 1989; Yarnitsky and Ochoa, 1990), and to account for the perception of synthetic heat (the Heat Grill Illusion) at mild temperatures (Fruhstorfer et al., 2003). In addition, because menthol evokes nociceptive as well as thermal sensations (Green, 1992; Green and Schoen, 2007; Albin et al., 2008), TRPM8 must be expressed on low-threshold cold fibers that converge on STT nociceptive neurons. It remains to be learned whether these fibers are A δ - or C-fibers, as TRPM8 has been reported to be expressed in both types of fibers (Kobayashi et al., 2005). It is important to note, however, that as yet there is no published evidence of STT nociceptive neurons that respond to warming at temperatures as low as 35°C or to cooling at temperatures as mild as 32°C. It is possible that responses in STT neurons at mild temperatures may be difficult to detect because of relatively sparse inputs from low-threshold neurons, and/or because synaptic contacts from such neurons may be relatively weak. Weaker contacts with STT neurons might also explain the greater lability of ‘LTN spots’ compared to ‘warm spots’ and ‘cold spots’ (Green et al., 2008) as well as the greater susceptibility of LTN to tactile inhibition produced by dynamic mechanical contact (Green and Pope, 2003; Green and Schoen, 2005; Green et al., 2008).

Effect of adaptation temperature on thermal and nociceptive thresholds

The parallel psychophysical functions in Fig. 1a & b show that the 3 warm and 3 cold ATs produced adaptation of warmth and cold in similar amounts, which indicates that all ATs were above the thresholds of the thermoreceptors that mediate those sensations (Sumino and Dubner, 1981). The similarity in slopes of the initial portions of the thermal functions is also consistent with data showing that the dynamic response of primate warm fibers is unchanged between ATs of 30° and 35°C (Dubner et al., 1975).

The psychophysical functions for cold-LTN (Fig. 1c) were also parallel and shifted in amounts proportional to the changes in AT. This implies the subset of thermoreceptors that mediate cold-LTN lie within the same sensitivity range as those that mediate cold sensations, since decreasing the AT from the mildest temperature produced a comparable shift in ratings of cold and nociception. On the other hand, the higher perceptual thresholds for cold-LTN (Fig. 1c) suggest that nociceptive sensations may not be mediated by the most sensitive thermoreceptors. As noted above, the cold-sensitive channel TRPM8 is assumed to mediate both the cold and nociceptive sensations produced by menthol, and thus might also mediate both normal cold sensations and cold-LTN. Evidence has recently been found that the thermal thresholds of cultured, cold-sensitive trigeminal ganglion neurons that express TRPM8 depend on the relative expression of TRPM8 and the potassium channel Kv1, which is expressed on the same neurons (Madrid et al., 2009; Belmonte et al., 2009). Higher levels of expression of TRPM8 together with lower levels of expression of Kv1 were correlated with high cold sensitivity, with the most sensitive neurons having cold thresholds as high as 34°C. This finding raises the interesting possibility that neurons that have lower levels of TRPM8 expression, and thus are relatively insensitive, may more often project to nociceptive STT neurons, although it is unclear why such an association might occur. One possibility is that the sensitivity of all neurons that express TRPM8 with other temperature-sensitive channels may depend on the relative expression level of the two (or more) channels (i.e., the channels may interact). Alternatively,

expression of TRPM8 may simply be lower in neurons that express at least one other TRP-channel. Thus, neurons that express TRPM8 together with the nociceptive channel TRPV1 (McKemy et al., 2002; Abe et al., 2005; Xing et al., 2006; Okazawa et al., 2004; Hjerling-Leffler et al., 2007; Takashima et al., 2007) might have higher thermal thresholds than those that express TRPM8 alone, and expression of TRPV1 may be associated with projection to nociceptive STT neurons.

However, there are at least two factors that together could produce higher *perceptual* thresholds for nociceptive sensations even if the thermoreceptors that mediate nociception and cold have similar temperature thresholds. First, if only a small fraction of cold-sensitive thermoreceptors relay stimulation to the nociceptive pathway, colder temperatures might be required to recruit enough of these fibers to produce a sensation (i.e., spatial summation may be required). This possibility is supported by a recent study that demonstrated the existence of low-threshold 'nociceptive spots' in the skin (Green et al., 2008) and found that such spots were rare when compared to warm spots and cold spots. Given that thermoreceptive fibers terminate in one (or at most two) spots in the skin (Darian-Smith et al., 1979; Hensel and Iggo, 1971; Kenshalo and Gallegos, 1967), the scarcity of nociceptive spots likely reflects a sparse innervation by low-threshold thermoreceptors that relay stimulation to the nociceptive pathway. Second, innocuous cold has an analgesic effect (Nahra and Plaghki, 2005) that has been attributed to inhibition of STT nociceptive neurons by STT cold-specific neurons (Craig and Bushnell, 1994), which are assumed to receive input from A δ -cold fibers (Fruhstorfer, 1984; Kanui, 1987; Wahren et al., 1989; Yarnitsky and Ochoa, 1990; Craig and Bushnell, 1994; Wasner et al., 2004). This means that cold-LTN always occurs in a background of cold-induced inhibition. Such inhibition could easily account for the only 1°C difference between thermal and nociceptive thresholds from ATs of 31° and 33°C.

On the other hand, the effect of AT on thresholds during heating indicates unambiguously that heat-LTN (Fig. 1d) is not mediated by the most sensitive thermoreceptors that respond to warming. From an AT of 30°C, log-mean ratings of warm sensations exceeded 'barely detectable' when stimulus temperature increased just 1° to 31°C, whereas nociceptive sensations did not rise to the same level until temperature rose 3.5° to 34.5°C (Table 1; Fig. 1d). In addition, the psychophysical functions for heat-LTN shifted only slightly between ATs of 30° and 32°C, which implies the thresholds of most thermoreceptors that serve heat-LTN lie above 32°C, and that many probably lie at or above 34°C. Indeed, from ATs of 30° and 32° C stimulus temperatures above 34°C generated nearly identical psychophysical functions, whereas adapting the skin to 34°C shifted the psychophysical function by approximately +1.5°.

Too few putative warm fibers have been studied in microneurography experiments to determine whether humans possess two fiber types with different sensitivities that could account for the present findings (Hallin et al., 1982; Konietzny and Hensel, 1977; Konietzny and Hensel, 1975). Nor is there evidence of such a dichotomy from studies in other species. Hensel and Iggo (1971) reported finding primate warm fibers that differed in their response to temperatures above 45°C but the two types had similar thresholds. Although data exist which suggest that the two TRP-channels known to be sensitive to mild heating, TRPV3 and TRPV4, may have different activation thresholds, the evidence so far is inconclusive. Lower thresholds have been reported for TRPV4 (e.g., 25°–27°C) (Guler et al., 2002; Watanabe et al., 2002; Chung et al., 2003) than for TRPV3 (31°–39°C) (Peier et al., 2002; Xu et al., 2002; Smith et al., 2002), but these differences must be interpreted with caution because the sensitivity of the channels can vary depending on the conditions under which they are expressed and studied (Guler et al., 2002). Nevertheless, it is notable that TRPV3, but not TRPV4, has been reported to be co-expressed in DRG neurons (Smith et al., 2002) with TRPV1, which is considered a marker for nociceptive neurons (Caterina et al., 1999; Szolcsanyi, 2004). In addition, TRPV3 continues to respond to painfully hot temperatures (Peier et al., 2002) whereas TRPV4 does not, and

knockout mice that lack TRPV3 show deficits in response to both noxious and innocuous heat (Moqrich et al., 2005). Together with the present threshold data, this evidence points to TRPV3 as the most likely candidate receptor for heat-LTN.

Effect of rate of temperature change on thermal and nociceptive thresholds

The very similar effects of rate of temperature change on thermal sensations indicates that more rapid cooling and heating leads to stronger afferent input in both the warm and cold thermal pathways. This finding differs from the results of studies of the effect of rate of temperature change on the thresholds for detection of warmth and cold (Kenshalo, Sr. et al., 1968; Pertovaara and Kojo, 1985; Harding and Loescher, 2005; Reulen et al., 2003), and from the results of a single previous study of the effect of rate of change on suprathreshold warmth and cold (Molinari et al., 1977). Threshold measurements have generally shown that faster rates ($> \pm 0.5^\circ\text{C}/\text{sec}$) lead to higher rather than lower warm thresholds, whereas cold thresholds are either slightly higher or unchanged. The higher thresholds for warmth have been attributed to reaction time artifacts associated with use of the psychophysical method of limits (Yarnitsky and Ochoa, 1991), although other factors [e.g., temporal summation (Palmer et al., 2000)] have also been suggested to play a role. In the earlier study of suprathreshold temperature perception, mean magnitude estimates indicated that increasing the rate of temperature change from $\pm 0.5^\circ/\text{sec}$ to $\pm 1.5^\circ/\text{sec}$ did not increase the intensity of either warmth or cold. However, Molinari et al. (1977) tested a much smaller range of rates of temperature change (a 3-fold range compared to an 8-fold range) and collected data from many fewer subjects.

Whatever the reasons may be for the difference in psychophysical findings, the present data are consistent with the dynamic discharge of primate (Sumino and Dubner, 1981; Duclaux and Kenshalo, Sr., 1980) and human (Konietzny and Hensel, 1977) warm fibers and of primate cold fibers (Kenshalo and Duclaux, 1977), which predicts that sensation should increase with the rate of heating or cooling. Both the present data and the electrophysiological data imply that the putative cold and warm receptors TRPM8, TRPV4, and possibly TRPV3, must have similar sensitivities to rate of temperature change. Although there is evidence that transfected TRPV3 channels are highly rate-sensitive (Xu et al., 2002), we could find no published data comparing the rate-sensitivity of the various thermal TRP channels.

Rate of temperature change affected nociceptive sensations in somewhat different and more complicated ways. Much of the difference appears to be attributable to the weakness of nociceptive sensations at the mildest temperatures (cf. Fig. 1c and Fig. 2c). Ratings of nociceptive sensations averaged less than 'barely detectable' at both rates of change when the temperature change was only $\pm 1.0^\circ$ from 33°C . This finding is consistent with the recent psychophysical evidence (Green et al., 2008) that only a small subset of thermoreceptors may relay stimulation to the nociceptive system. It would not be surprising if stimulating a small number of fibers more strongly by increasing the rate of temperature change is simply insufficient to induce a reliable sensation. When skin temperature was 28°C , the magnitude of the rate effect was similar for nociceptive sensations and for cold, which is consistent with the hypothesis that both cold and cold-LTN are mediated by afferent fibers that express TRPM8. The much more pronounced effect of rate found for heat-LTN at skin temperatures above 34° raises the possibility of differences in the response characteristics of the receptors that serve warmth and heat-LTN. If, as we speculate, TRPV3 is the receptor primarily responsible for heat-LTN and TRPV4 is the receptor primarily responsible for warmth, our results predict that TRPV3 has a higher rate-sensitivity than does TRPV4.

It is interesting to note that the type of afferent fiber stimulated by heating the skin of rats is rate-dependent, with slower rates (e.g., $< 1.0^\circ\text{C}/\text{sec}$) tending to stimulate c-fibers and faster rates tending to stimulate A δ fibers (Yeomans and Proudfoot, 1996). This raises the possibility that the large increase in nociceptive sensations obtained with the faster rate of heating may

have been due to recruitment of heat-sensitive A δ -fibers, and implies that if TRPV3 is in fact the primary receptor for heat-LTN, it should be differentially expressed on such fibers. To our knowledge there are no published data on the expression of TRPV3 in different fiber types.

Individual differences

Discussion of the receptors and afferent fibers that may underlie LTN must take into account the fact that not all subjects experience the phenomenon. In the present study about 60% of subjects reported cold-LTN and about 75% reported heat-LTN. It is difficult to speculate about the source of these individual differences when so little is known about the neural basis of LTN. However, because subjects who report LTN appear to have otherwise normal thermal perception, it is unlikely the variation is due to the lack of expression of a specific thermoreceptive channel in some individuals. A more likely source of the variation is the extent to which low-threshold temperature-sensitive channels are expressed on afferent fibers that make synaptic contact with STT nociceptive neurons. Alternatively, the strength of the synaptic contact between low-threshold thermoreceptors and STT nociceptive neurons, and/or the degree of inhibition of nociceptive stimulation by the warm and cold pathways, may vary across individuals.

The large individual differences must also be considered with respect to the possible function of LTN. The absence of cold-LTN in up to 40% of individuals suggests that it does not serve a critical function in cold thermoreception. On the other hand, both cold- and heat-LTN may impart adaptive advantages in those individuals who experience them. It has been speculated (Green and Schoen, 2005) that LTN may serve the duals functions of providing a nociceptive thermoregulatory signal when skin temperature falls (or rises) to levels associated with dangerous amounts of heat loss or heat gain, and providing an early warning signal that skin temperature is rapidly falling or rising to potentially damaging (freezing or burning) levels. The latter function is supported by the evidence presented here that faster rates of temperature change significantly increase the perceived intensity of nociceptive sensations, particularly during heating.

Peripheral vs. central factors

There is risk in attributing the effects of the present psychophysical measurements solely to the temporal characteristics and sensitivities of receptor channels and primary afferent fibers. As was discussed, inhibition of STT nociceptive neurons by cold stimulation may affect the thresholds for cold-LTN. The possibility must also be considered that the effect of rate of temperature change on thermal and nociceptive sensations is attributable, at least in part, to the response properties of second-order and higher STT neurons. However, the thermoreceptors and afferent fibers do represent rate-limiting steps in the stimulation process. For thermal and nociceptive sensations to increase in intensity with rate of temperature change, thermoreceptors and afferent fibers must respond more vigorously to faster rates of heating and cooling (Sumino and Dubner, 1981; Duclaux and Kenshalo, Sr., 1980). Our results therefore focus attention on the dynamic response characteristics and sensitivity of TRPM8, TRPV3 and TRPV4. Only if the characteristics of these channels and the fibers on which they are expressed fail to account for the psychophysical results will it be necessary to turn to more complex hypotheses involving the properties of central neurons.

On the other hand, the quality of sensation almost certainly depends less on the properties of thermoreceptors than it does on the central neurons on which they converge. Based on early evidence that some STT neurons which project in the nociceptive pathway receive input from afferent fibers that respond to innocuous temperatures (Christensen and Perl, 1970; Price and Mayer, 1975), Price and Dubner (1977) concluded more than three decades ago that "... classification of neurons based on the stimulus energy that produces threshold activation or on

that which produces maximum excitation may be misleading” (p. 332). The present study provides new psychophysical support for this conclusion by demonstrating that depending upon adaptation temperature, nociceptive sensations can be reported at temperatures as little as 1.0° to 1.5°C below the thresholds for cold and 1.5° to 3.5°C for warmth, well within the sensitivity range of classically defined cold fibers and warm fibers.

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Figure 1.

Shown are the log-means of the rated perceived intensities of thermal sensations (a,b) and nociceptive sensations (c,d) as a function of thermode temperature for different adapting temperatures. Data for cooling were obtained using a descending temperature series starting from one of 3 adapting temperature, and those for heating with an ascending temperature series starting from one of 3 different adapting temperatures. The dashed horizontal line in each graph denotes a rating of 'barely detectable' on the intensity ratings scale (gLMS), which was defined as the perceptual threshold. Error bars represent standard errors of the mean.

Figure 2.

Log-mean ratings of perceived intensity of thermal and nociceptive sensations are shown as a function of thermode temperature for two different rates of heating and cooling (filled circles, $\pm 4^\circ/\text{sec}$; empty circles, $\pm 0.5^\circ/\text{sec}$). Error bars represent standard errors of the mean.

Table 1

Estimated thresholds for perception of thermal and nociceptive sensations during cooling and heating from different adapting temperatures. All temperatures are °C.

	Adapting Temperature	Thermal Threshold	Nociceptive Threshold	Threshold Difference
Cooling	35°	34.0°	32.5°	-1.5°
	33°	32.0°	31.0°	-1.0°
	31°	30.5°	29.5°	-1.0°
Heating	30°	31.0°	34.5°	+3.5°
	32°	32.5°	35.0°	+2.5°
	34°	34.5°	36.0°	+1.5°