Response of C fibre nociceptors in the anaesthetized monkey to heat stimuli: estimates of receptor depth and threshold

Donna-Bea Tillman, Rolf-Detlef Treede, Richard A. Meyer* and James N. Campbell

*Departments of Neurosurgery and Biomedical Engineering, and *The Applied Physics Laboratory, Johns Hopkins University, Baltimore, MD 21205, USA*

1. Responses to ramped or stepped temperature stimuli were obtained from fifty-three cutaneous C fibre mechano-heat nociceptors (CMHs) in the hairy skin of the pentobarbitone–morphine anaesthetized monkey. A three-layer heat transfer model was developed to describe the temperature distribution within the skin and to estimate receptor depth and heat threshold.

2. Surface heat threshold, defined as the surface temperature when the first action potential occurs, increased as: (a) the rate of temperature rise for the ramped stimuli increased from 0·095 to 5·8 °C s^{-1}; (b) the duration of stepped heat stimuli decreased from 30 to 1 s; and (c) the base temperature of stepped heat stimuli decreased from 38 to 35 °C. These results suggest that the heat threshold for CMHs is determined by the temperature at the depth of the receptor.

3. Receptor depth estimates from responses to ramped stimuli ranged from 20 to 570 μm with a mean of 201 μm. The estimated mean receptor heat threshold was 40·4 $\pm$ 2·2 °C ($\pm$ S.D.). No correlation was observed between depth and thermal or mechanical threshold. The average receptor depth and threshold, estimated from the responses to stepped heat stimuli, were 150 μm and 40·2 °C, respectively.

4. We conclude that: (a) the receptor endings of CMHs occur in the epidermis and dermis; (b) temperature at the level of the receptor determines threshold; (c) temperature at the receptor ending is much lower than skin surface temperature at threshold; and (d) the tight distribution of receptor heat thresholds suggests a uniform transducer mechanism for heat in CMHs.

The location of the endings of cutaneous afferents responsible for the sensation of pain has been disputed for many years. Many anatomists have reported intraepidermal C fibres in both the glabrous skin (Weddell, 1947; Cauna, 1980; Munger & Halata, 1983; Novotny & Gommert-Novotny, 1988) and the hairy skin (Woollard, 1936; Miller, Ralston & Kasahara, 1958; Arthur & Shelley, 1959; Cauna, 1973; Kruger, Perl & Sedivec, 1981; Munger & Halata, 1983). However, the observation that much of the epidermis can be painlessly shaved off suggests that these intra-epidermal fibres may not play an important role in pain sensation (Woollard, 1936).

Several investigators have used psychophysical measures of heat pain to estimate the depth of heat-sensitive nociceptors to be between 100 and 200 μm (Buettner, 1951; Stolwijk & Hardy, 1955; Stoll & Greene, 1959). The basis for these estimates was that pain threshold for the different stimulus conditions correlated with the temperature at the depth of the receptor reaching a fixed level. To estimate receptor depth, a one-dimensional heat transfer model was used to calculate that depth at which the temperature at the end of a liminally painful stimulus was the same for all stimuli used in the study.

A principal finding in those studies was that the threshold temperature for heat pain increased with the intensity of a constant power heat source, i.e. with the rate of temperature change. This apparent increase in heat pain threshold can be due to two independent factors: (1) heat transfer – as the rate of temperature change increases, the difference between the temperature at the skin surface and the temperature at the receptor increases (Hensel, 1950); and (2) reaction time – skin temperature continues to

* To whom correspondence should be addressed at the Applied Physics Laboratory, Johns Hopkins University, Johns Hopkins Road, Laurel, MD 20723, USA.

change during the time it takes the subject to react to a noxious heat stimulus (Yarnitsky & Ochoa, 1990). The latter factor confounds all previous estimates of the intracutaneous depth where heat-sensitive nociceptors terminate.

The aim of the present paper was to estimate the intracutaneous depth of nociceptive terminals by using ramped and stepped heat stimuli in conjunction with a multi-layer thermal model of the skin. In contrast to previous studies, we evaluated neural responses from C fibre mechano-heat-sensitive nociceptors (CMHs) instead of psychophysical measures. Whereas many authors have measured nociceptor heat thresholds, this is the first study in which stimulus parameters were systematically varied to estimate receptor depth.

METHODS

In fourty-five experiments standard single fibre recording techniques (Campbell & Meyer, 1983) were used to record from the medial antebrachial cutaneous ($n = 19$), superficial radial ($n = 5$), upper ulnar ($n = 4$), saphenous ($n = 15$), sural ($n = 1$), and superficial peroneal ($n = 1$) nerves of anaesthetized male monkeys (*Macaca fascicularis*). The monkeys were initially sedated with an intramuscular injection of ketamine (10 mg kg^{-1}), and anaesthesia was maintained by a continuous intravenous infusion of sodium pentobarbitone (3 mg kg^{-1} h^{-1}) and morphine sulphate (0·5 mg kg^{-1} h^{-1}). Adequate depth of anaesthesia was ensured by monitoring heart rate with an electrocardiogram. An increase in heart rate associated with a noxious stimulus was interpreted as a sign of inadequate anaesthesia. Dextrose (5%) in normal saline was infused intravenously throughout the experiment to maintain hydration (4–6 ml kg^{-1} h^{-1}). The monkeys were artificially ventilated, and end-tidal CO_2 was maintained at 35–45 mmHg. When it became apparent that an animal was breathing spontaneously, a bolus dose of pancuronium bromide (0·1 mg kg^{-1}) was given. At the same time, absence of motor responses to noxious stimuli could be checked (every 2–3 h). Core temperature was measured with a rectal thermometer and kept within the range of 36–37·5 °C with heated water pads. At the beginning of each experiment, penicillin G (450 000 units) was administered for prophylaxis against infection. These procedures have been approved by the Johns Hopkins Committee on Animal Care and Use.

Small nerve filaments, cut proximally from the nerve trunk, were wrapped around an electrode for recording. The amplified and filtered signal was relayed to a differential amplitude and time discriminator, as well as to an oscilloscope for monitoring. A microcomputer recorded the time of occurrence of discriminated action potentials, and off-line programs were later used to analyse the data.

Neurophysiological protocol

The receptive field of each nociceptive afferent was first identified by firmly squeezing the skin. The hair in the region of the receptive field was cut with a fine pair of scissors while viewing the area with a microscope. The extent of the receptive field and the mechanical threshold were then determined using von Frey monofilaments. Receptive fields were mapped with a 10 bar (200 mN) von Frey filament, and mechanical threshold was defined as the minimum pressure that reliably evoked a response. CMHs whose mechanical threshold was so low that they responded to the pressure of the thermal stimulator were excluded from the study.

Conduction velocity was obtained by dividing the latency of the response to suprathreshold electrical stimulation at the receptive field by the conduction distance. Units with conduction velocities less than 1·5 m s^{-1} were designated C fibres. Only C fibres that responded to both mechanical and heat stimuli, and whose receptive field was located on the hairy skin, were included in this study. In order to obtain a precise temporal alignment of the stimulus and the response, the conduction time from the receptive field to the recording electrode was subtracted from the latency of all recorded action potentials.

Thermal stimulators

Heat testing was conducted using either a contact stimulator that operated under the Peltier principle (Wilcox & Giesler, 1984) or a feedback-controlled laser thermal stimulator (Meyer, Walker & Mountcastle, 1976). Both devices were under computer control.

Peltier stimulator. The effects of varying stimulus ramp rate were examined using a feedback-controlled Peltier device (model LTS 3, Thermal Devices Incorporated, Golden Valley, MN, USA). The active surface (8 × 8 mm) of the device consists of two copper plates lying on either side of a glass-encapsulated thermistor. The signal from the thermistor is used by the feedback electronics to control the delivered thermal stimulus. To prevent electrolytic deposits from forming on the skin, the active surface of the stimulator was tightly covered with a 10 μm thick layer of plastic wrap (polyvinylidene chloride). The thermal diffusivity of the plastic wrap (0·0003–0·0010 cm^2 s^{-1}) is similar to that of skin (0·0004–0·0012 cm^2 s^{-1}). A small amount of thermal conductive gel (Type 25 Silicone Compound, GC Electronics) was placed between the plastic wrap and the skin to ensure uniform heat transfer.

Calibration. The thermistor on the surface of the Peltier device was calibrated by immersing the device in a temperature-controlled water bath. (The temperature in the water bath was maintained for 10 min prior to each measurement.) However, this calibration procedure did not adequately predict the temperature at the Peltier–skin interface during rapid temperature changes. The skin surface temperature measured with a thermocouple wire was significantly lower than the temperature reported by the thermistor within the Peltier device.

To quantify the difference between the Peltier temperature and the skin surface temperature, the temperature at the stimulator–skin interface was measured in four experiments on the volar forearm of the anaesthetized monkey using a calibrated copper–constantan thermocouple (diameter, 50 μm). For the three nominal ramp rates used in this study (10, 1 and 0·1 °C s^{-1}), the skin surface temperature ramp rates were approximately 5·8, 0·85 and 0·095 °C s^{-1}. (The skin surface ramp rates were not perfectly linear; the values used are based on a 14 °C temperature step.) Note that the difference between the nominal and the measured temperatures decreased as the stimulus ramp rate decreased. This is consistent with the hypothesis that there is a thermal insulator (perhaps the glass bead that covers the thermistor) located between the Peltier–thermistor combination and the skin surface. All temperatures in the analyses that follow refer to the temperatures measured at the skin–stimulator interface.

Heat stimulus protocol. Heat stimuli were delivered from a base temperature at ramp rates of 5·8, 0·85 and 0·095 °C s^{-1}. The base temperature was 32, 35 or 38 °C. The base temperature was applied for 1 min at the beginning of each run. During the interstimulus interval, temperature was maintained at 32 °C. The temperature at the end of the ramp varied from 36 to 48 °C and, unless specified, was maintained for 1 s. Temperature was brought back to the base temperature by cooling at a rate of 2·6 °C s^{-1}. The interstimulus interval was 10 min to minimize stimulus interaction effects (LaMotte & Campbell, 1978; Tillman, 1992). This stimulus protocol does not produce thermal sensitization in CMHs (Tillman, 1992).

Laser thermal stimulator. In a second set of experiments with a different population of CMHs, a CO_2 laser under radiometer feedback control provided stepped increases in skin temperature without contact with the skin. The rise time to the desired temperature was about 100 ms. A circular area of 7·5 mm diameter was heated uniformly (for detail see Meyer *et al.* 1976).

The threshold for heat stimuli was tested with runs of ascending stimulus intensities in 1 °C steps. A run was terminated when at least one action potential was elicited or a temperature of 49 °C was reached. Stimuli of 1 s duration were presented every 30 s. The base temperature (38 °C) was maintained for 1 min at the beginning of the run and between stimuli during the run. If the CMH responded to the base temperature ($n = 9$), the base temperature was lowered to 35 °C. No fibre responded to the 35 °C base. In a second set of runs, thresholds were determined for stimuli of 30 s duration given every 60 s. Runs were repeated at least twice with a 2 min inter-run interval. The high reliability of this threshold testing protocol has been previously documented (Treede, Meyer & Campbell, 1990).

RESULTS

A total of fifty-three CMHs were studied in forty-five experiments. The mean receptive field size was 20 ± 14 mm^2 (mean ± S.D.), as determined by mapping with a 10 bar (200 mN) von Frey filament. Mechanical thresholds, also determined by von Frey testing, ranged from 0·69 to 10 bar, with a mean (± S.D.) of 3·4 ± 1·8 bar (range, 0·6–200 mN; mean, 27 ± 33 mN). The mean (± S.D.) conduction velocity was 0·85 ± 0·17 m s^{-1}.

Effects of stimulus ramp rate on nociceptor discharge

The response of a typical CMH to heat stimuli presented at three different stimulus ramp rates is shown in Fig. 1. The surface temperature when the first action potential occurred increased from 39·6 °C at the slowest ramp rate (Fig. 1*C*) to 41·7 °C at the fastest ramp rate (Fig. 1*A*).

The effect of stimulus ramp rate on surface heat threshold is shown in Fig. 2 for thirteen CMHs that responded to all three ramp rates (base temperature was 32 °C ($n = 1$), 35 °C ($n = 5$) or 38 °C ($n = 7$)). Surface heat threshold, defined as the skin surface temperature when the first action potential occurred, increased with stimulus ramp rate. The differences in threshold between ramp rates are significant ($P < 0·001$, Student's paired t tests). For eleven of twelve other CMHs for which a response was obtained for only two of the ramped stimuli, the surface heat threshold at the faster ramp rate was also greater than the threshold at the slower ramp rate ($P < 0·01$, Wilcoxon signed-rank test). These results indicate that, as stimulus ramp rate increases, a higher surface temperature is required to evoke the first action potential.

Heat transfer model

Heat stimuli applied to the surface of the skin are attenuated as they are conducted through the epidermis and dermis to the receptor. To estimate the temperature at the depth of the receptor, we must be able to infer intracutaneous temperature profiles from surface temperature measurements. Intracutaneous temperature gradients are difficult to measure directly, because even the smallest thermocouples (50 μm) are large with respect to epidermal (40 μm) and dermal (1100 μm) thicknesses in the hairy skin. Therefore, we developed a heat transfer model to describe mathematically how a surface temperature waveform is transmitted through the skin.

As the stimulated area is large (e.g. 8 × 8 mm for the Peltier device and 7·5 mm diameter for the laser) with respect to the presumed receptor depths (< 1 mm), a one-dimensional model is sufficient. The simplest one-dimensional, homogeneous model can be represented by a single parameter, thermal diffusivity. However, Hensel (1950) has shown that thermal diffusivity varies with depth. Therefore, we modelled skin as a three-layer composite medium in which the three layers correspond to the epidermis, dermis and subcutaneous tissue.

The one-dimensional diffusion equation is given by:

$$\frac{\partial T}{\partial t} = \frac{k\,\partial^2 T}{\rho C \partial x^2},$$

where T is temperature, t is time, x is depth, k is thermal conductivity, C is heat capacity, and ρ is density. This equation was evaluated for each layer of the model subject to the appropriate boundary conditions. As shown in detail elsewhere (Tillman, 1992), the equations were solved numerically using a modified Crank–Nicholson finite difference scheme (Smith, 1975; Press, Flannery, Teukolsky & Vetterling, 1988). The model parameters used in this analysis are indicated in Table 1. As shown in the Appendix, temperatures predicted by the three-layer model were relatively insensitive to changes in model parameters within the range reported in the literature (Tillman, 1992).

Effects of stimulus ramp rate on predicted intracutaneous temperature profiles

The heat transfer model was used to predict the temperature waveform below the skin surface for each stimulus

waveform. The predicted temperature waveforms at three different depths following a 5·8 °C s^{-1} heat stimulus applied to the surface of the skin are shown in Fig. 3*A*. After 1 s, the surface temperature had risen to 44·6 °C, but the temperature at 100 μm was only 41·7 °C. The temperatures at deeper locations were even lower. Thus, the temperature at the receptor is not the same as the stimulus temperature measured at the skin surface.

Figure 3*B* illustrates the effects of changing stimulus ramp rate on the predicted intracutaneous temperatures at a single depth (100 μm). In this figure intracutaneous temperature is plotted *versus* surface temperature, instead of time, so that the three different ramp rates can be compared. The more the curves diverge from the diagonal, the greater the difference between the applied surface temperature and the temperature at 100 μm. As indicated by the dotted lines, the surface temperature required for the intracutaneous temperature to reach 43 °C ranged from 43·3 °C at the slow ramp to 46·4 °C at the fast ramp. If the temperature at the receptor determines threshold for activation, this model predicts that the surface temperature when the first action potential occurs (the surface heat threshold) will increase as stimulus ramp rate increases. This result is consistent with the neurophysiological results (Fig. 2).

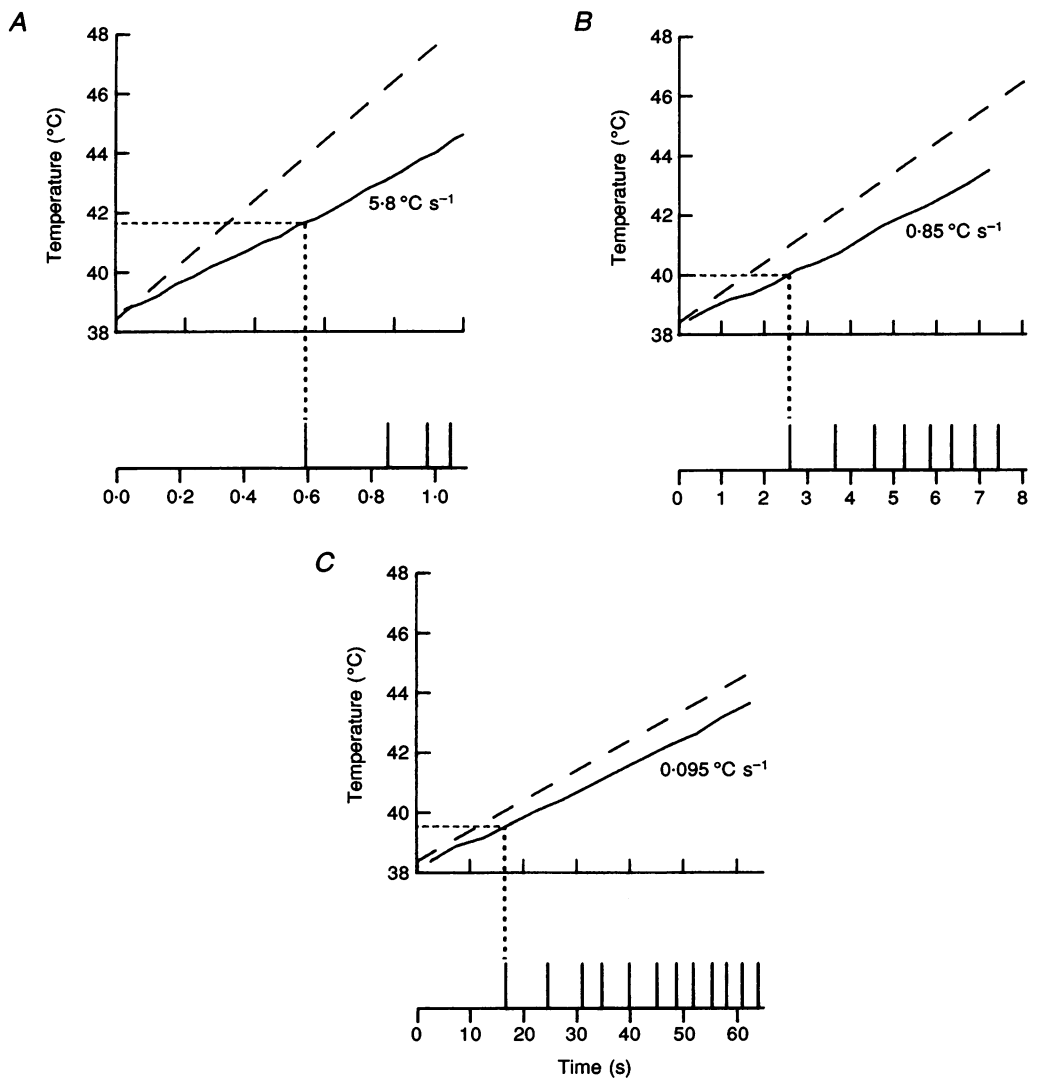

Figure 1. Response of a typical CMH to heat stimuli at three different ramp rates

Under each stimulus waveform is the response of the same CMH to the three different stimuli. *A*, stimulus ramp rate was 5·8 °C s^{-1}. *B*, stimulus ramp rate was 0·85 °C s^{-1}. *C*, stimulus ramp rate was 0·095 °C s^{-1}. Note that time scale is different for each figure. The dashed line indicates the temperature measured by the internal thermistor of the Peltier divice, and the continuous line indicates the temperature measured by a thermocouple at the skin–Peltier interface.

Table 1. Parameters used in the heat transfer model for skin

Skin layer	Thickness (mm)	Thermal conductivity (mcal cm^{-1} s^{-1} $°C^{-1}$)	Heat capacity (cal g^{-1} $°C^{-1}$)	Density (g cm^{-3})
Epidermis	0·040 (0·02–0·10)	0·5 (0·3–0·6)	0·77 (0·70–0·85)	1·2
Dermis	1·10 (0·7–1·6)	1·2 (0·8–1·4)	0·86 (0·75–0·95)	1·2
Deep tissue	10 (9–11)	1·2 (0·8–1·4)	0·91 (0·81–1·0)	1·2

Thermal conductivity from Tillman (1992) as derived from Hensel (1950). Heat capacity from Henriques & Moritz (1947). Density from Lipkin & Hardy (1954). Tissue thickness from Southwood (1955) and Whitton & Everall (1973). Numbers in parentheses indicate ranges considered in sensitivity analysis discussed in the Appendix.

Estimates of receptor depth and threshold

The heat transfer model can be used in conjunction with the neurophysiological data to estimate receptor depth and threshold temperature at that depth (see Stoll & Greene, 1959, for a similar approach). Several assumptions are inherent in this estimation technique: (1) the receptor region over which the action potential can be generated does not extend over a large vertical distance, but rather can be associated with a single depth; (2) the receptor utilization time is negligible; (3) the skin can be adequately modelled by the three-layer heat transfer model; and (4) the response at threshold of a CMH depends only on the temperature at the receptor, and not on the rate of temperature change or any other such variable.

Given these assumptions, receptor depth and threshold can be estimated as follows. For each of the three ramped stimuli, the time of the first action potential is determined. (The number of runs at each ramp rate used to determine this time varied among CMHs, ranging from 1 to 10.) Using the heat transfer model, the temperature *versus* depth profile for that instant in time is then computed. The temperature *versus* depth profiles for each of the three different ramped stimuli are superimposed. If the assumptions are correct, the profiles should intersect at a single point. The co-ordinates of this point are the receptor depth and the threshold temperature at the receptor.

Temperature *versus* depth profiles were obtained for twenty-three CMHs. For twelve CMHs, responses were obtained

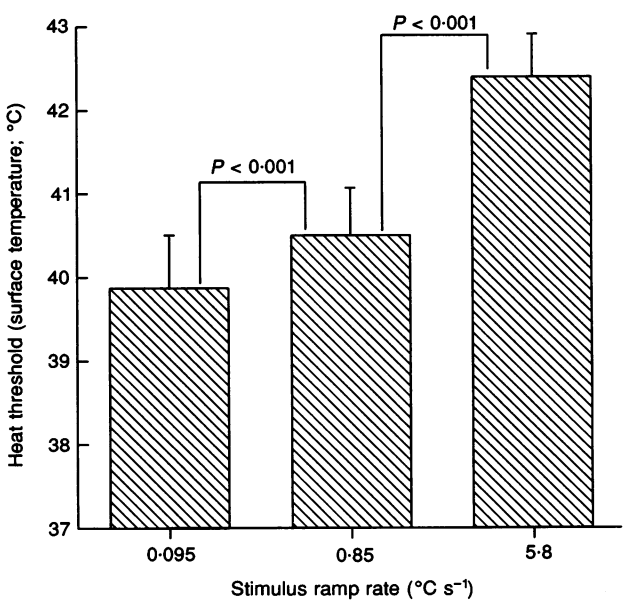

Figure 2. Effects of stimulus ramp rate on surface heat thresholds of CMHs

Mean (± S.E.M.) surface heat thresholds of 13 CMHs obtained for stimuli at three different ramp rates. Surface heat threshold increased significantly with ramp rate (paired *t* tests).

for all three stimulus ramp rates. Temperature *versus* depth profiles for four of these CMHs are shown in Fig. 4*A*. For the eleven other CMHs, responses were obtained from only two stimulus ramps. Temperature *versus* depth profiles for four of these CMHs are shown in Fig. 4*B*. Six of the latter received all three ramps, but failed to respond to the fastest ramp (e.g. Y1901 and Y8602 in Fig. 4*B*). For these six CMHs, the temperature profile that existed at the end of the fastest ramp stimulus was calculated (dashed lines). This profile, which represents the lower limit for possible receptor threshold and depth points, was below the intersection from the other two profiles in all cases.

The temperature *versus* depth profiles have a number of characteristic features. (1) The temperature at '0' depth corresponds to the surface temperature at the instant that the action potential was generated. For each fibre, this surface temperature threshold was highest for the fastest ramp rate and lowest for the slowest ramp rate. (2) The steepest profiles correspond to the fastest ramp rate, and

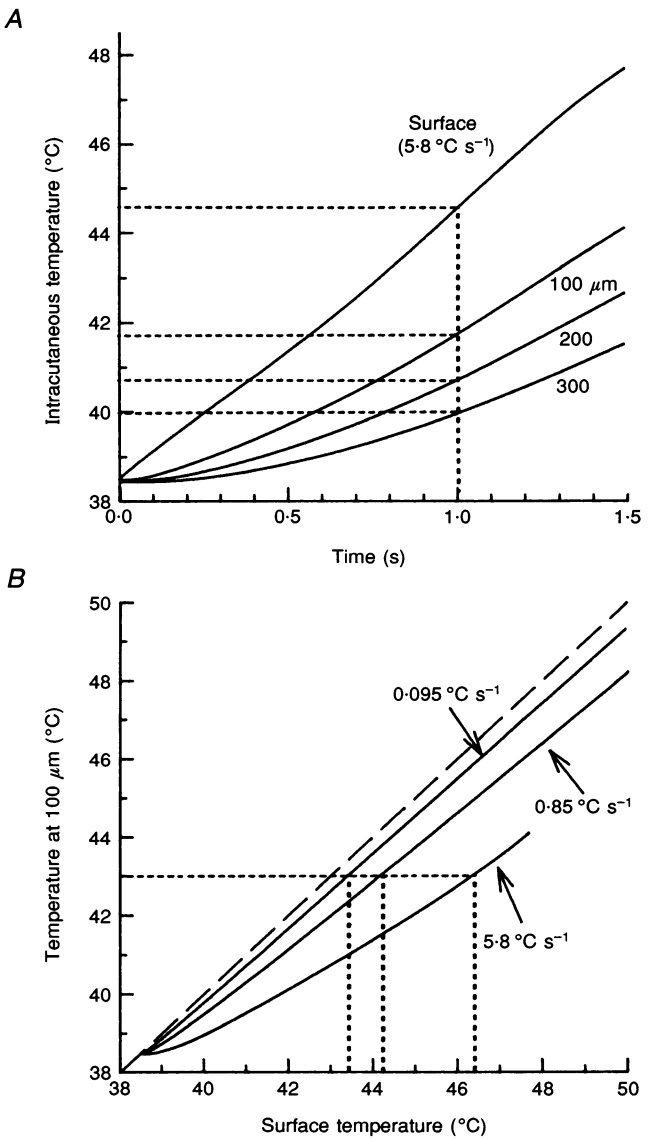

Figure 3. Intracutaneous temperature profiles predicted by the three-layer model of skin

A, intracutaneous temperature profiles at three different depths. The surface temperature waveform was a 5·8 °C s^{-1} ramp. At any instant (e.g. at 1 s as indicated by the dotted lines) the temperature decreased with depth. *B*, predicted intracutaneous temperature profiles at three different stimulus ramp rates. Temperature at a depth of 100 μm is plotted *versus* surface temperature. Note that the surface temperature required to attain an intracutaneous temperature of 43 °C (dotted lines) increases as stimulus ramp rate increases. For extremely slow ramp rates, the surface temperature and the temperature at 100 μm would be equal (dashed line).

the shallowest profiles correspond to the slowest ramp rate. (3) Each profile exhibits a distinct change in slope at a depth of 40 μm due to the change in thermal properties of the model at the epidermal–dermal junction. (4) The point at which the profiles intersect corresponds to the depth of the receptor and the threshold temperature at the receptor.

For six of twelve CMHs, all three profiles intersected at a single point (e.g. Y4005 and Y6102 in Fig. 4*A*). For the remaining six CMHs, the intersections span a small triangle for which a weighted median was calculated. The purpose was to weight more heavily those intersection points that were based on a larger number of neurophysiological runs. Each temperature *versus* depth profile was assigned a weight corresponding to the number of stimulus presentations used to determine that particular threshold. Each intersection point (corresponding to a depth estimate, based on two temperature profiles) was then assigned a weighing factor corresponding to the smaller of the two weights of its intersecting curves. These weighted intersection points were then used to determine a median depth and threshold.

Estimated receptor depths ranged from 20 to 570 μm, with a mean of 201 $\pm$ 173 μm ($\pm$ S.D.) (Fig. 5*B*). The mean ($\pm$ S.D.) receptor threshold was 40·4 $\pm$ 2·2 °C (Fig. 5*A*). The majority of CMHs (14 of 23) had receptor heat thresholds within a small temperature range (39–41 °C). These temperatures are substantially lower than the surface heat thresholds measured with the 5·8 and 0·85 °C s^{-1} ramps, but are nearly equal to the surface heat threshold measured with the 0·095 °C s^{-1} ramps. Thus surface threshold is only a good indicator of receptor threshold when the stimulus ramp rate is very slow, or the stimulus duration is very long.

The distribution of the receptor heat thresholds (Fig. 5*A*) and the depths (Fig. 5*B*) are not statistically different for the two-ramp and the three-ramp data (Mann–Whitney U test, $P > 0{\cdot}1$). The scatter plot of receptor threshold *versus* depth shown in Fig. 5*C* indicates that the receptor threshold did not correlate with depth (Spearman's rank test, $r = 0{\cdot}07$). The apparent negative correlation between receptor depth and threshold for the three-ramp data (filled

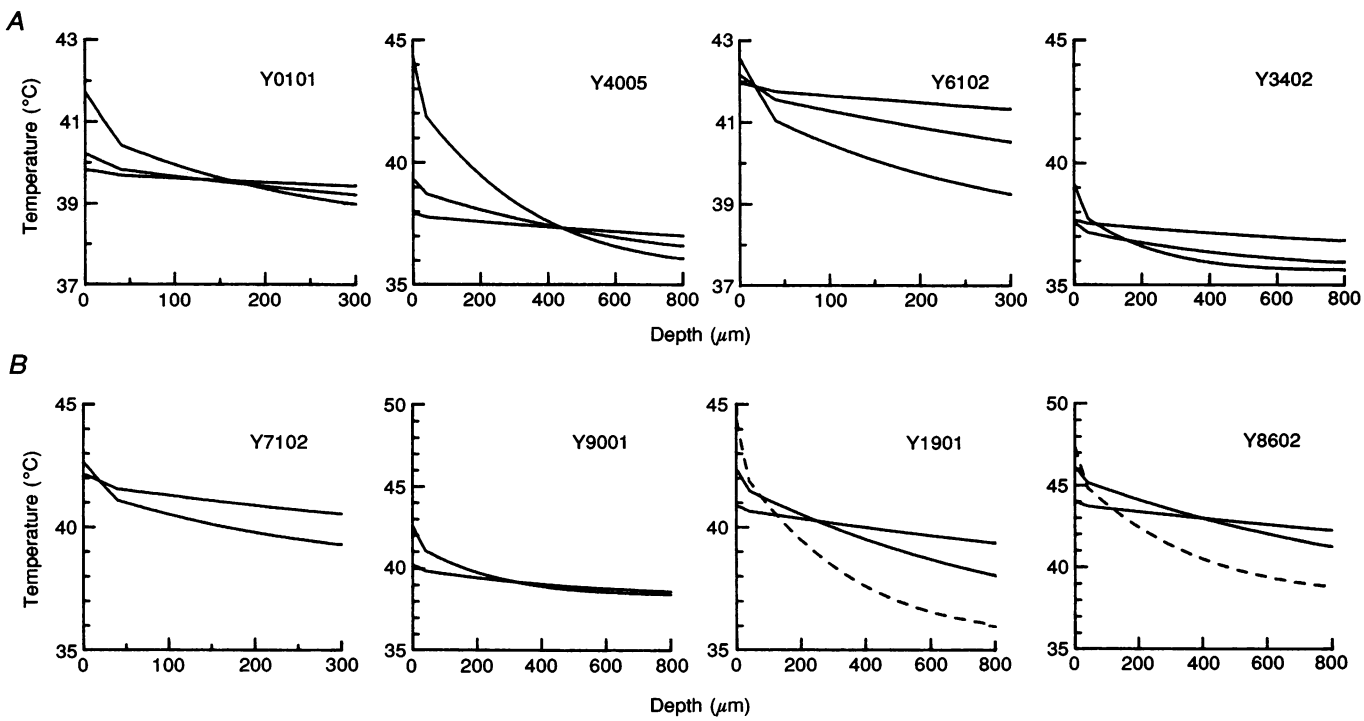

Figure 4. Temperature–depth profiles

A, temperature–depth profiles for 4 CMHs that responded to the three different stimulus ramp rates. Intracutaneous temperature predicted from a three-layer heat transfer model is plotted *versus* depth for the instant at which the first action potential occurred. Each curve corresponds to the profile for a different ramp rate. The locus of intersections of the 3 curves corresponds to the predicted depth of the receptor and threshold temperature for activating the receptor. *B*, temperature–depth profiles for 4 CMHs that either received only two of the ramp rates (Y7102 and Y9001) or did not respond to the 5·8 °C s^{-1} ramp (Y1901 and Y8602). The lack of a response to the 5·8 °C s^{-1} ramp establishes a lower boundary (dashed line for fibres Y1901 and Y8602) for the temperature–depth profile. The actual threshold/depth point must lie above this line.

squares in Fig. 5*C*) is in fact only a sampling artifact due to limitations in the heat stimuli that could be delivered by the stimulator. The highest surface temperature that could be achieved with the fastest ramp rate (5·8 °C s^{-1}) was 45 °C (from a 35 °C base). As mentioned previously, six of the eleven CMHs that are included in the two-ramp group actually received all three ramps, but did not respond to the 5·8 °C s^{-1} ramp (open triangles in Fig. 5*C*). These CMHs were either deep or had high heat thresholds, and thus the intracutaneous temperatures produced by the fast ramp were insufficient to activate them.

There was no correlation between mechanical threshold and receptor depth (Spearman's rank test, $r = 0{\cdot}05$), although the deepest CMH had the highest mechanical threshold, and the most superficial CMH had the lowest (Fig. 6*A*). There was also no correlation between receptive field size (Fig. 6*B*) or conduction velocity and receptor depth.

Effects of base temperature and stimulus duration on thresholds of CMHs to stepped heat stimuli

A second group of CMHs with receptive fields in the hairy skin were studied with stepped heat stimuli applied by the laser thermal stimulator. In one set of experiments, nine CMHs were studied with 1 s duration heat steps at 1 °C intervals from two different base temperatures (35·4 or 38 °C). For all nine CMHs the size of the temperature step was higher from the 35·4 °C base temperature. As shown in

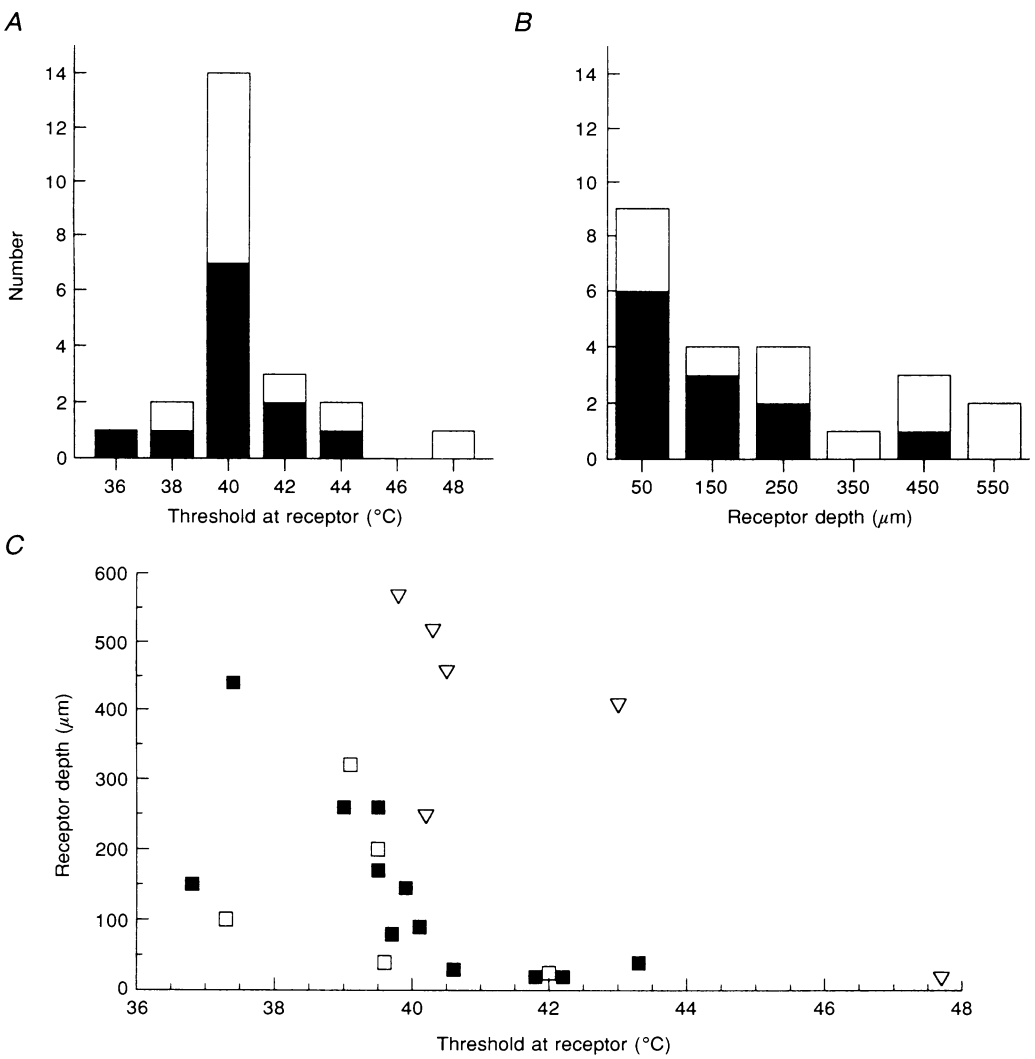

Figure 5. Relationship between receptor depth and receptor heat threshold

A, distribution of heat thresholds at the receptor (bin width, 2 °C; stacked bar). Data obtained from CMHs that responded to all three stimulus ramp rates are shown by the filled bars ($n = 12$), while data based on only two ramp rates are shown by the open bars ($n = 11$). *B*, distribution of receptor depths (bin width, 100 μm; stacked bar). *C*, relationship between receptor depth and threshold at the receptor. ▽, those CMHs that received but did not respond to the 5·8 °C s^{-1} ramp; □, those CMHs that only received two ramp rates; ■, those CMHs that responded to all three stimulus ramps.

Fig. 7*A*, the surface temperature step at threshold from the lower base was twice as large as the step from the higher base. These data indicate that CMHs are not sensitive to the size of the surface temperature step, but rather to the final temperature attained. In fact, the final surface temperature at threshold from the lower base (42·4 ± 0·6 °C; mean ± S.E.M.) was slightly but significantly higher than that from the higher base (41·4 ± 0·4 °C; $P < 0·05$, paired t test).

The three-layer heat transfer model was used to predict the temperature waveforms for stepped heat stimuli at the two threshold temperatures for a range of intracutaneous depths. The depth at which the intracutaneous temperatures at the ends of the two stimuli are the most similar gives an estimate for the average depth of the nociceptor terminals. Figure 7*B* shows the temperature waveform at this depth that was graphically determined to be 150 μm. The temperature at the end of the two stimuli was 40·4 °C, which is the same as the mean receptor heat threshold found with the ramped stimuli.

In a second set of experiments, sixteen CMHs were studied with 1 and 30 s duration heat steps. The base temperature was constant at either 35·4 or 38 °C for each CMH, with the average base temperature being 35·8 °C. For fourteen of the sixteen CMHs, the heat threshold was higher for the 1 s *versus* the 30 s stimulus. As shown in Fig. 7*C*, the threshold for the 1 s stimulus (41·4 ± 0·7 °C; mean ± S.E.M.) was significantly higher than the threshold for the 30 s stimulus (40·1 ± 0·6 °C; $P < 0·001$, paired t test). This difference is again explained by the predicted temperature waveforms at 150 μm (Fig. 7*D*). Although the threshold surface temperature was higher for the 1 s stimulus, the final intracutaneous temperature was nearly the same as that for the 30 s stimulus. All responses to the 30 s stimulus began near the end of the stimulus, when the intracutaneous temperature was increasing slowly, whereas responses to the 1 s stimulus were elicited during a rapid temperature rise. These data indicate that the receptor heat threshold of CMHs is not determined by the rate of temperature change.

DISCUSSION

CMH surface heat threshold increases with stimulus ramp rate

This study shows that the surface heat threshold of CMHs in the hairy skin of the monkey increases with increasing rate of temperature change, decreasing stimulus duration, and decreasing base temperature. These results are consistent with those previously found in the cat (Bessou & Perl, 1969) and rabbit (Lynn, 1979). These observations can all be explained by a simple heat transfer model, if we assume that each CMH starts responding when a liminal temperature is reached at the terminal within the skin.

In contrast, Yarnitsky, Simone, Dotson, Cline & Ochoa (1992) reported no change in surface heat thresholds for CMHs innervating the dorsum of the human hand or foot when stimulus ramp rates ranged from 0·3 to 6·0 °C s^{-1}. This disparity with our results may be due to the presence of a stimulus interaction artifact in the data of Yarnitsky and co-workers. In both man (e.g. Adriaensen *et al.* 1984) and monkey (e.g. LaMotte & Campbell, 1978), stimulus

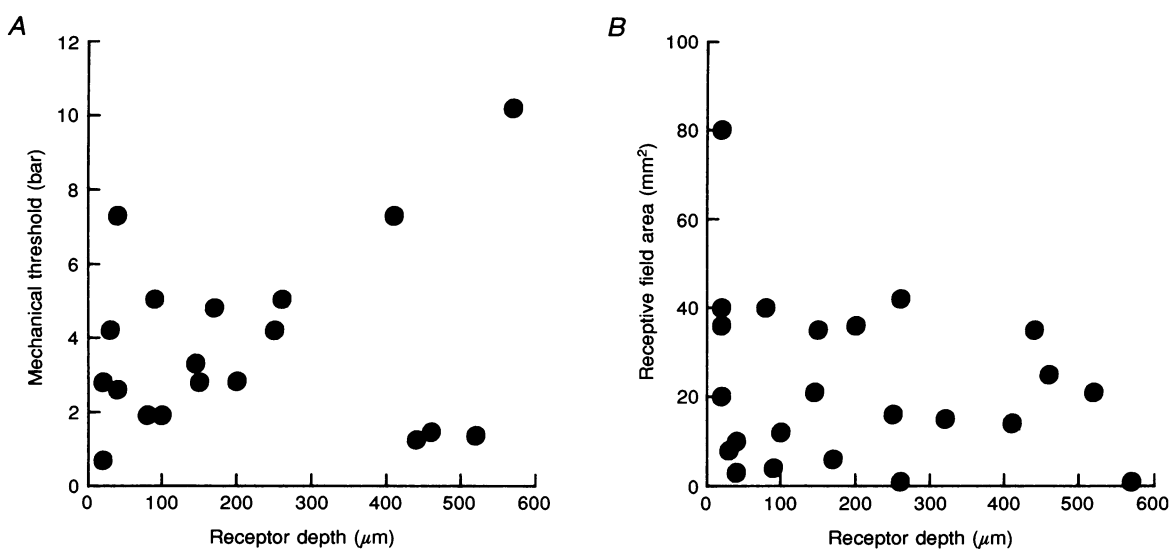

Figure 6. Receptor depth and mechanical response properties

A, relationship between mechanical threshold and receptor depth. There is no statistically significant correlation ($r = 0·05$). *B*, relationship between receptive field area and receptor depth. There is no statistically significant correlation ($r = 0·05$).

interaction effects occur with interstimulus intervals of less than 5 min. Whereas the interstimulus interval in the present study was 10 min, the interstimulus interval in the Yarnitsky *et al.* (1992) study was only 30 s. A second possible explanation is that CMHs in humans are located more superficially or that the technique used to locate CMHs in humans results in a selection bias towards more superficial receptors. The effect of stimulus ramp rate on surface heat threshold increases with receptor depth. If all the CMHs in the Yarnitsky study were intra-epidermal, we would not expect to see any significant dependence of surface heat threshold on stimulus ramp rate.

Croze, Duclaux & Kenshalo (1976) reported that surface heat thresholds for CMHs in monkey skin did not differ significantly for ramp rates of 0·2 and 1·5 °C s^{-1}. However, their stimulus protocol produced sensitization because heat

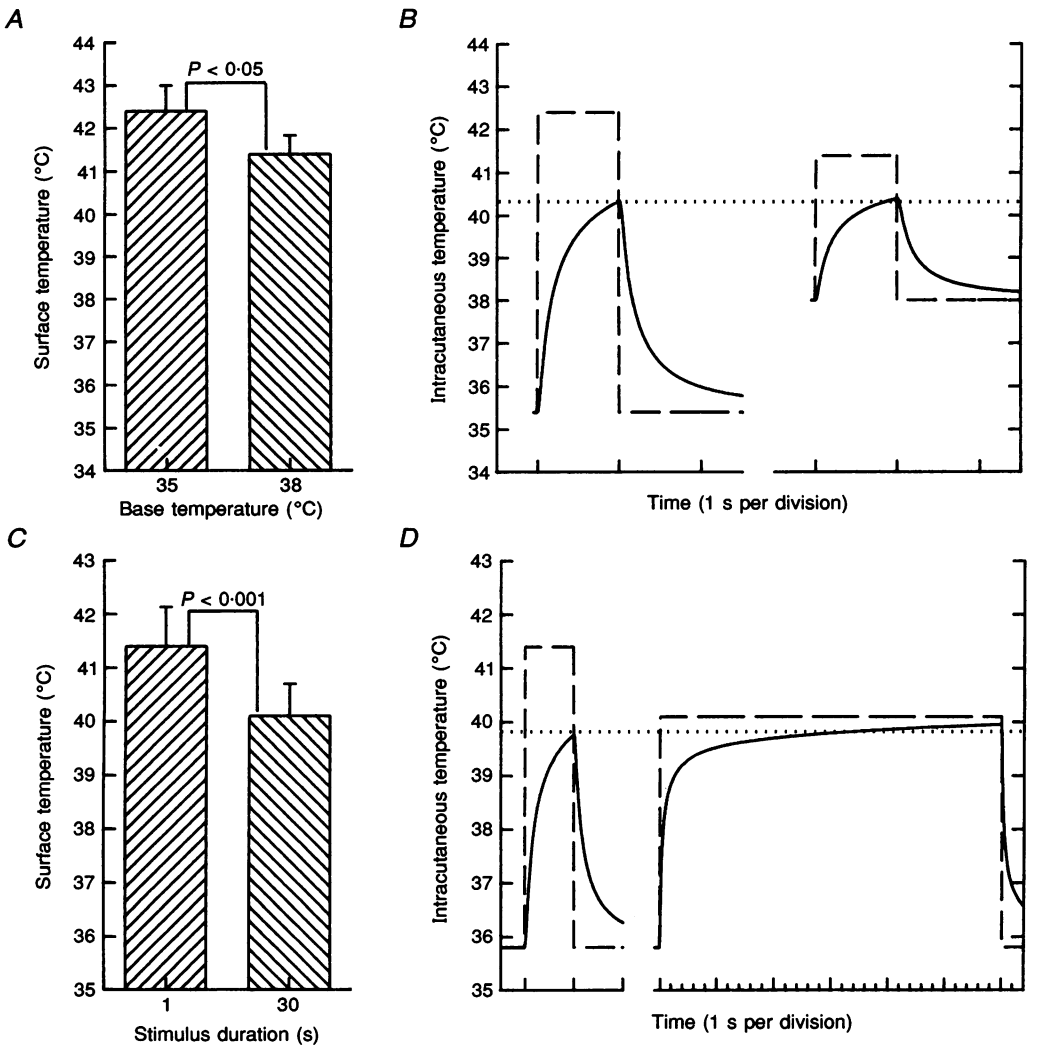

Figure 7. CMH surface heat thresholds for stepped heat stimuli

A, effects of base temperature on surface heat threshold ($n = 9$). Stepped heat stimuli of 1 s duration were delivered by a laser thermal stimulator from an initial temperature of 35·4 or 38 °C. *B*, intracutaneous temperature waveforms at 150 μm (continuous lines) were calculated from the heat transfer model using the mean threshold stimuli from *A* as the surface temperature input waveforms (dashed lines). The surface heat threshold differences in *A* are consistent with an average nociceptor depth of 150 μm and an average receptor heat threshold of 40·4 °C (dotted lines). *C*, effects of stimulus duration on surface heat threshold ($n = 16$). Stepped heat stimuli of either 1 or 30 s duration were delivered by a laser thermal stimulator from an initial temperature of 35·4 or 38 °C (mean, 35·8 °C). CMH surface heat thresholds were significantly higher for the shorter stimulus duration. *D*, intracutaneous temperature waveforms at 150 μm were calculated from the heat transfer model using the mean threshold stimuli from *C* as the surface temperature waveforms (dashed lines). The surface heat threshold differences in *C* are consistent with an average nociceptor depth of 150 μm and an average receptor heat threshold of 39·8 °C (dotted lines).

stimuli were maintained at high levels for 4 min. In addition, stimulus interaction artifacts may have been present due to the 4 min interstimulus interval (LaMotte & Campbell, 1978).

CMH heat threshold is determined by the temperature at the receptor terminal

We have presented three lines of evidence supporting the hypothesis that the receptor heat threshold of a CMH depends primarily on the temperature at the depth of the receptor. (1) For the ramped heat stimuli, the intracutaneous temperature profiles at threshold intersected at or near a single point (Fig. 4). If threshold were dependent on the rate of temperature change, the temperature profiles would not be expected to intersect at a single point. (2) For stepped heat stimuli, the surface temperature step required to evoke a response was smaller at higher base temperatures (Fig. 7*A*; see also Handwerker & Neher, 1976), even though the rate of temperature change was lower at higher base temperatures. (3) For stepped heat stimuli, surface temperature threshold decreased as the stimulus duration increased (Fig. 7*C*), due to the fact that the intracutaneous temperatures more closely approached the surface temperature for long-duration stimuli. The fact that the rate of temperature change with time is much greater for the shorter stimuli (and that threshold is higher for these stimuli) further supports the hypothesis that rate of temperature change does not greatly influence heat threshold.

A spatial temperature gradient (i.e. a change of temperature with depth) also does not appear to be important in determining receptor heat thresholds. If receptor heat threshold were dependent on a spatial temperature gradient, the three experiments described above should have all produced exactly the opposite results. The temperature stimuli that would produce the largest spatial gradients would be those delivered with the fastest ramp rates, from the lowest base temperatures, and for the shortest durations. Cold fibres are also unaffected by spatial gradients (Hensel & Zotterman, 1951); reversing the spatial direction of the cooling stimulus had no impact on the responses of cold fibres.

Stolwijk & Hardy (1955) developed an eight-layer model of the skin for analysis of heat flow during irradiation. Measurements of human pain thresholds were used in conjunction with the heat flow model to examine the roles of thermal gradients, vasomotor reactions and thermochemical changes in the nerve cell membranes (Hardy, Stolwijk, Hammel & Murgatroyd, 1955). They concluded that it was unlikely that intracutaneous temperature gradients were responsible for transient or steady-state pain sensations.

Spatial and temporal temperature gradients are therefore not important for determining the receptor threshold of a CMH. Instead, these results suggest that temperature at the receptor is the adequate stimulus for heat threshold in CMHs.

Receptor depth and threshold determination

The threshold response of a CMH to a heat stimulus is profoundly dependent on heat transfer to the receptor ending. Because the skin is not a perfect conductor, the surface temperature when a CMH first responds will depend on both temporal properties of the stimulus and the depth of the receptor. Although the stimulus is under the investigator's control, cutaneous receptor depth is an unknown factor in almost all neurophysiological experiments. However, from histological investigations we know that free nerve endings can be found within both the dermis and the epidermis.

Stoll & Greene (1959) used heat transfer principles and psychophysical studies to estimate receptor depth for the nerve fibres that mediate the sensation of heat pain. Assuming that the adequate stimulus for heat pain is the temperature at the receptor, they estimated an average receptor depth of 180–240 μm. However, Stoll & Greene neglected to account for reaction time artifacts (i.e. the fact that the difference between the temperature at the time of the nociceptor response and the temperature at the time when pain is first felt increases with stimulus ramp rate), and thus their estimated depths should be greater than the actual depths. Furthermore, as we discuss in the companion paper (Tillman, Treede, Meyer & Campbell, 1995), pain threshold does not directly arise from CMH first response threshold.

Bromm, Jahnke & Treede (1984) determined the intracutaneous temperature profile of painful laser heat stimuli of extremely short duration (50 ms). They reported that only receptors within the first 100 μm of skin would have their temperature elevated above 43 °C, which was assumed to be the threshold temperature for nociceptive endings. Using the threshold estimate from the present study (about 40 °C) extends the range of nociceptive endings possibly activated by this laser pulse to 150 μm. This depth is consistent with the present results.

Using a three-layer heat transfer model of skin and CMH first response thresholds from the monkey, we have estimated that the depths of CMHs can range from 20 to 570 μm. This result provides further evidence for the existence of intra-epidermal as well as intra-dermal C fibre nociceptors in the primate hairy skin. Previous histological studies in human skin have found that free nerve endings terminate within the upper part of the dermis and the epidermis (Cauna, 1973; Breathnach, 1977; Novotny & Gommert-Novotny, 1988). B. L. Munger (personal communication) reports that, in the monkey leg, free nerve ending terminations associated with hair follicles occur down to a depth of 500–600 μm. Our depth estimate range of 20–570 μm is consistent with these results.

Another result from the depth analysis procedure is that there is no correlation between receptor depth and a variety of receptor characteristics, such as mechanical and thermal threshold, receptive field size, and conduction velocity. Thus, differences in thermal and mechanical thresholds of CMHs are not simply a result of some endings being deeper than others.

The estimated receptor thresholds were clustered tightly around 39–41 °C; fourteen of the twenty-three CMHs whose threshold could be estimated had receptor heat thresholds within this range. As noted previously, this uniformity in receptor heat threshold is not simply due to a uniformity in receptor depth. CMHs with receptor heat thresholds in the range 39–41 °C had depths ranging from 20 to 570 μm. This suggests that CMHs may share a common heat transduction mechanism, and that the differential sensitivity to surface heat stimuli may result from different receptor depth within the skin.

In conclusion, variations in apparent surface heat thresholds for CMHs in monkey hairy skin can all be explained by a simple heat transfer model if we assume that each CMH responds when a liminal temperature is reached at its receptive ending. Depth estimates from this model indicate that the receptive endings are distributed throughout the dermis and the epidermis. Surface temperature is only a good indicator of receptor threshold when stimulus ramp rate is very slow, the stimulus duration is very long, or the receptor terminal is very superficial.

APPENDIX

Depth estimate sensitivity analysis

In order for the depth estimate procedure to be useful, it must be relatively insensitive to changes in the model parameters. We considered how variations in the following parameters would affect the estimates of depth and threshold of the receptor: (1) thermal conductivity, heat capacity, and tissue thickness; and (2) core temperature. A detailed description of this sensitivity analysis can be found in Tillman (1992).

Thermal conductivity, heat capacity and tissue thickness

The minimum and maximum values for the thermal conductivity and the thickness of skin were obtained from the literature (Tillman, 1992) (Table 1). A range of values was not available for heat capacity, so this parameter was allowed to vary $\pm$ 10% from its reported values (Henriques & Moritz, 1947).

The effects of varying these parameters on the estimated depth and receptor threshold was studied using the neurophysiological data from five typical CMHs. For the worst-case scenario, the depth varied within $\pm$ 50% of its predicted value, while the receptor threshold remained unchanged.

Core temperature

To investigate how changes in core temperature might affect our estimates of receptor depth and threshold, we examined how a decrease in forearm core temperature of 2 °C would affect these estimates for the same five CMHs. In general, decreasing the forearm core temperature had little effect on both the depth and threshold estimates. The effects on receptor threshold were slightly more pronounced for deeper CMHs, but in no case was the threshold changed by more than 0·2 °C.

Adriaensen, H., Gybels, J., Handwerker, H. O. & Van Hees, J. (1984). Suppression of C-fiber discharges upon repeated heat stimulation may explain characteristics of concomitant pain sensations. *Brain Research* **302**, 203–211.

Arthur, R. P. & Shelley, W. B. (1959). The innervation of human epidermis. *Journal of Investigative Dermatology* **32**, 397–410.

Bessou, P. & Perl, E. R. (1969). Responses of cutaneous sensory units with unmyelinated fibers to noxious stimuli. *Journal of Neurophysiology* **32**, 1025–1043.

Breathnach, A. S. (1977). Electron microscopy of cutaneous nerves and receptors. *Journal of Investigative Dermatology* **69**, 8–26.

Bromm, B., Jahnke, M. T. & Treede, R.-D. (1984). Responses of human cutaneous afferents to CO_2 laser stimuli causing pain. *Experimental Brain Research* **55**, 158–166.

Buettner, K. (1951). Effects of extreme heat and cold on human skin. II. Surface temperature, pain, and heat conductivity in experiments with radiant heat. *Journal of Applied Physiology* **3**, 703–713.

Campbell, J. N. & Meyer, R. A. (1983). Sensitization of unmyelinated nociceptive afferents in the monkey varies with skin type. *Journal of Neurophysiology* **49**, 98–110.

Cauna, N. (1973). The free penicillate nerve endings of the human hairy skin. *Journal of Anatomy* **152**, 277–288.

Cauna, N. (1980). Fine morphological characteristics and microtopography of the free nerve endings of the human digital skin. *Anatomical Record* **198**, 643–656.

Croze, S., Duclaux, R. & Kenshalo, D. R. (1976). The thermal sensitivity of the polymodal nociceptors in the monkey. *Journal of Physiology* **263**, 539–562.

Handwerker, H. O. & Neher, K. D. (1976). Characteristics of C-fibre receptors in the cat's foot responding to stepwise increase of skin temperature to noxious levels. *Pflügers Archiv* **365**, 221–229.

Hardy, J. D., Stolwijk, J. A. J., Hammel, H. T. & Murgatroyd, D. (1955). Skin temperature and cutaneous pain during warm water immersion. *Journal of Applied Physiology* **20**, 1014–1021.

Henriques, F. C. & Moritz, A. R. (1947). Studies of thermal injury. I. The conduction of heat to and through skin and the temperatures attained therein. A theoretical and an experimental investigation. *American Journal of Pathology* **23**, 531–549.

Hensel, H. (1950). Die intracutane Temperaturbewegung bei Einwirkung äußerer Temperaturreize. *Pflügers Archiv* **252**, 146–164.

Hensel, H. & Zotterman, Y. (1951). Action potentials of cold fibers and intracutaneous temperature gradient. *Journal of Neurophysiology* **14**, 377–385.

Kruger, L., Perl, E. R. & Sedivec, M. J. (1981). Fine structure of myelinated mechanical nociceptor endings in cat hairy skin. *Journal of Comparative Neurology* **198**, 137–154.

LaMotte, R. H. & Campbell, J. N. (1978). Comparison of responses of warm and nociceptive C-fiber afferents in monkey with human judgements of thermal pain. *Journal of Neurophysiology* **41**, 509–528.

Lipkin, M. & Hardy, J. D. (1954). Measurement of some thermal properties of human tissues. *Journal of Applied Physiology* **7**, 212–217.

Lynn, B. (1979). The heat sensitization of polymodal nociceptors in the rabbit and its independence of the local blood flow. *Journal of Physiology* **287**, 493–507.

Meyer, R. A., Walker, R. E. & Mountcastle, V. B. (1976). A laser thermal stimulator for the study of cutaneous thermal and pain sensations. *IEEE Transactions on Biomedical Engineering* **23**, 54–60.

Miller, M. R., Ralston, H. J. & Kasahara, M. (1958). The pattern of cutaneous innervation of the human hand. *American Journal of Anatomy* **102**, 183–218.

Munger, B. L. & Halata, Z. (1983). The sensory innervation of primate facial skin. I. Hairy skin. *Brain Research Review* **5**, 45–80.

Novotny, G. E. K. & Gommert-Novotny, E. (1988). Intraepidermal nerves in human digital skin. *Cell Tissue Research* **254**, 111–117.

Press, W. H., Flannery, B. P., Teukolsky, S. A. & Vetterling, W. T. (1988). *Numerical Recipes in C: The Art of Scientific Computing*. Cambridge University Press, Cambridge, UK.

Smith, G. D. (1975). *Numerical Solution of Partial Differential Equations*. Oxford University Press, London.

Southwood, W. F. W. (1955). The thickness of the skin. *Plastic Reconstructive Surgery* **15**, 423–429.

Stoll, A. M. & Greene, L. C. (1959). Relationship between pain and tissue damage due to thermal radiation. *Journal of Applied Physiology* **14**, 373–382.

Stolwijck, J. A. J. & Hardy, J. D. (1955). Skin and subcutaneous temperature changes during exposure to intense thermal radiation. *Journal of Applied Physiology* **20**, 1006–1013.

Tillman, D. B. (1992). Heat response properties of unmyelinated nociceptors. Doctoral Dissertation, Johns Hopkins University, Baltimore, MD, USA.

Tillman, D.-B., Treede, R.-D., Meyer, R. A. & Campbell, J. N. (1995). Response of C fibre nociceptors in the anaesthetized monkey to heat stimuli: correlation with pain threshold in humans. *Journal of Physiology* **485**, 767–774.

Treede, R.-D., Meyer, R. A. & Campbell, J. N. (1990). Comparison of heat and mechanical receptive fields of cutaneous C-fiber nociceptors in monkey. *Journal of Neurophysiology* **64**, 1502–1513.

Weddell, G. (1947). The anatomy of cutaneous sensibility. *British Medical Bulletin* **3**, 167–172.

Whitton, J. T. & Everall, J. D. (1973). The thickness of the epidermis. *British Journal of Dermatology* **89**, 467–476.

Wilcox, G. L. & Giesler, G. J. (1984). An instrument using a multiple layer Peltier device to change skin temperature rapidly. *Brain Research Bulletin* **12**, 143–146.

Woollard, H. H. (1936). Intra-epidermal nerve endings. *Journal of Anatomy* **71**, 54–60.

Yarnitsky, D. & Ochoa, J. L. (1990). Studies of heat pain sensation in man: perception thresholds, rate of stimulus rise and reaction time. *Pain* **40**, 85–91.

Yarnitsky, D., Simone, D. A., Dotson, R. R., Cline, M. A. & Ochoa, J. L. (1992). Single C nociceptor responses and psychophysical parameters of evoked pain: effect of rate of rise of heat stimuli in humans. *Journal of Physiology* **450**, 581–592.

Acknowledgements

We thank K. O. Johnson and S. N. Raja for their insight throughout the duration of this research. We greatly appreciate the technical assistance of T. Hartke and J. L. Turnquist. This research was supported by NIH grant NS-14447 and by a research grant from the Bristol-Myers Squibb Corporation. D.-B. Tillman was supported by NIH training grant GM-07057 and R.-D. Treede was supported by the DFG (Tr 236/1-1).

Author's present address

R.-D. Treede: Institute of Physiology and Pathophysiology, Johannes Gutenberg University, Saarstrasse 21, D-55099 Mainz, Germany.

Received 31 January 1994; accepted 6 December 1994.