Quantitative Analysis of Orofacial Thermoreceptive Neurons in the Superficial Medullary Dorsal Horn of the Rat

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Hutchison, W. D., J. Tsoukatos, and J. O. Dostrovsky. Quantitative analysis of orofacial thermoreceptive neurons in the superficial medullary dorsal horn of the rat. J. Neurophysiol. 77: 3252–3266, 1997. Surprisingly little is known concerning the central processing of innocuous thermal somatosensory information. The aim of the present study was to obtain quantitative data on the characteristics of neurons in the rat superficial medullary dorsal horn (sMDH) that responded to innocuous thermal stimulation of the rat’s face and tongue. Single-unit extracellular recordings were obtained in chloralose-urethane anesthetized rats. A total of 153 thermoreceptive neurons was studied. Of these, 146 were excited by cooling and inhibited by warming and were classified as COLD cells. The remaining seven cells were excited by innocuous warming of the skin or tongue. Of 123 COLD cells tested, 33% were excited by touch and 22% by pinch stimuli delivered to the thermoreceptive field. Of the 50 COLD cells tested, 46% were excited also by noxious heating (≥50°C for 5 s). Most (82/121) of the receptive fields were located on the upper lip, 25 on the tongue, and most of the remaining on the lower lip. Receptive fields were generally small (1–5 mm²). In some experiments, electrical stimulation in the thalamus was performed, and nine COLD cells could be activated antidromically. The responses of 38 COLD cells to incremental 5°C cooling steps were examined quantitatively. Thermal stimuli were applied to facial or lingual receptive fields of sMDH neurons with a computer-controlled Peltier thermode starting from 33°C, decreasing to 8 or 3°C, and returning to 33°C. Most COLD cells (26/38) had both static and dynamic responses; 7 had mainly dynamic and 5 mainly static responses to step decreases in temperature. Rat sMDH COLD cells could be classified into three groups depending on their stimulus-response functions. The first group (Type 1, n = 19) had a bell-shaped static stimulus response function. The second group (Type 2) had a high maintained or increasing static firing rate as the temperature decreased <18°C (n = 10). Type 3 COLD cells had mainly dynamic properties (n = 7). Many of the cells in all groups were excited by noxious mechanical stimulation. Type 2 cells differed from the other two groups in that most did not respond to noxious thermal stimuli (hot) and many responded to innocuous tactile stimuli. Neurons from each of the three groups of COLD cells could be activated antidromically from contralateral thalamus. These data suggest that there is little central processing of thermal information at the first central synapse for Type 1 neurons, however, the responses of the other two types may be due to central processing and convergence. The demonstration of rat sMDH COLD cells with distinctive stimulus-response functions to thermal shifts suggests separate functional roles of these neurons in the ascending thermal sensory pathway.

INTRODUCTION

In the nearly 60 years since Zotterman first recorded from specific thermoreceptors in the lingual nerve of the cat (Zotterman 1936), there have been many investigations of the firing characteristics of thermoreceptors in the orofacial region in many species (mouse, rat, guinea pig, rabbit, bat, cat, monkey, and human) (Boman 1958; Davies et al. 1985; Dubner et al. 1975; Duclaux et al. 1980; Heinz et al. 1990; Hellon 1983; Hensel and Kenshalo 1969; Molinari and Kenshalo 1977; Perl 1990; Poulos and Lende 1970a, b; Sumino and Dubner 1981). Most of these studies reported the existence of cooling specific (COLD) primary afferents and failed to find afferents responding to innocuous warming of the orofacial region, probably because WARM fibers are less numerous and more difficult to record from because they are unmyelinated. COLD primary afferent fibers are excited by cooling stimuli and decrease their firing rate in response to warming of the receptive field. They have pronounced dynamic responses to cooling steps and adapt to a static firing rate that is dependent on skin temperature and that is maximal at ~18–25°C. Most reports indicate that static firing of COLD primary afferents drops markedly or ceases at temperatures <18°C, although a few reports have revealed the existence of some afferents that maintain or even increase their firing at lower temperatures (Hensel and Kenshalo 1969; LaMotte and Thalhammer 1982). COLD fibers have small punctate receptive fields, and slowly conducting axons, which are primarily in the Aδ range although some COLD C fibers have been reported in the limb (LaMotte and Thalhammer 1982). Most studies have reported that thermoreceptors are not excited by innocuous mechanical stimuli (Darian-Smith 1984; Hellon 1983), although two studies in the rat reported mechanically sensitive Aδ COLD fibers (Davies 1984; Pierau et al. 1975). It is also well known that some low-threshold mechanoreceptors respond phasically to rapid and large drops in skin temperature (Burton et al. 1972; Hensel and Zotterman 1951; Iggo and Muir 1969). It appears that at least some COLD afferents may respond to noxious mechanical stimuli, but this has not been studied systematically or quantitatively (Darian-Smith 1984; Hellon 1983). However, many of the COLD afferents are activated by noxious heat stimulation, and this discharge is frequently termed a “paradoxical” response (Darian-Smith 1984; Dubner et al. 1975; Long 1977). These studies largely confirm the predictions of the psychophysicists of the last century such as von Frey and Blix, who observed discrete COLD and WARM spots on the skin surface and proposed that they give rise to the specific sensations of cold or warmth, respectively, when stimulated (see Hensel 1973a).

Thermoreceptors are believed to terminate exclusively in the superficial laminae (laminae I and outer II) of the spinal and medullary dorsal horn (Willis and Coggeshall 1991). This assumption is strengthened by anatomic reconstructions of two intracellularly labeled COLD thermoreceptors in the
RAT MEDULLARY COLD CELLS

Animal preparation

Male Wistar rats weighing between 200 and 440 g (mean 323, n = 63) were anesthetized with a chloralose-urethane mixture (50:900 mg/kg, ip). Facial fur, vibrissae, scalp, and throat were closely clipped and bupivacaine HCl 0.5% (Marcaine, 0.2 ml) was infused into the surgical sites to reduce nociceptive input. Dexamethasone 2% (Dexamone, 0.2 mg) was given intraperitoneally to reduce edema. Tracheal and venous cannulae were inserted to aid breathing and administer drugs, respectively. End-tidal percentage CO₂ was monitored throughout the experiment, and the level of anesthesia was maintained with supplemental doses of anesthetic given as required by assessing a limb withdrawal reflex. A feedback-controlled heating pad maintained core temperature at 37 ± 1°C. Rats were mounted in a Stoeling stereotactic head holder without the mouth piece plate. A rod extending to the rostral cranium was screwed into the frontal bone to stabilize the skull. This allowed greater access to the facial region. The dorsal aspect of the medulla was exposed through the cisterna magnus by removing the atlanto-occipital ligament and the dura was reflected for insertion of electrodes. In some experiments, a craniotomy was made to allow introduction of a stimulating electrode (Rhodes MCE-100) into thalamus to activate antidromically neurons in the superficial MDH (sMDH). A stereomicroscope (Nikon SMZ-2B) was used to visualize contact of the electrode tip with the surface of the medulla and ease of electrode penetration.

Recording procedures

Extracellular recordings were made with silver-plated, carbon-fiber electrodes (Millar and Williams 1988) with resistances of 0.5–2 MΩ or parylene-C-insulated tungsten electrodes (Microprobe, initial resistances ~1.3 MΩ) from locations measured rostral and lateral to the bifurcation of the dorsal column nuclei (termed obex in this study even though the true obex is slightly more rostral). An electrode was advanced into the superficial laminae of the trigeminal nucleus caudalis with a microdrive (Burleigh InChivorm). Depths of the recorded cells below surface were noted. Usually the first penetration was made 1 mm caudal and 2 mm lateral to the obex. Single-unit activity was amplified (DAM 80 WPI Instruments) with a gain of 1,000 and initially filtered at 0.1–10 kHz. The signal was further filtered at ~200 Hz to 5 kHz (Krohn-Hite model 3700 filter), and the signal fed to a dual window discriminator (Bak Electronics, model DD1-1), audio monitor (Grass AM 8), and oscilloscopes. The waveform of the unit was monitored on a digital oscilloscope (Iwatsu DS-6411) with a stored “reference spike” to ensure recordings were made from the same unit throughout the experiment. The ratemeter histogram of the single unit and the temperature of the Peltier thermode and thermistor on the rat skin were led to a digital interface (CED 1401) and displayed on the computer monitor using Spike2 software and thermal stimuli and have been termed heat, pinch, cold (HPC) cells. The response of HPC cells to cooling is also different in that they are activated only at cooler temperatures and have maximal static firing rates at colder temperatures. Our preliminary studies in the rat MDH suggested that there may be more than one group of cooling-sensitive cells, and the main purpose of this study was to examine this in greater detail. Some of these data have been reported briefly (Hutchison and Drostovsky 1992; Hutchison et al. 1995).

METHODS

Physical characterization

Units in the sMDH with spontaneous ongoing discharge were tested for inhibition by radiant warmth applied to the face and tongue with a 50-W projector lamp. This search stimulus enabled rapid identification of COLD and WARM units. Receptive fields of some units initially were localized by moving a spot of radiant heat over the surface of the facial skin. This was performed with a heat shield made of reflective metal foil with various sized holes to allow the passage of the warming rays. In some experiments, a thermistor (Physitemp BAT-12 with IT-2 probe, response time constant of 0.15 s) was fixed to the center of the receptive field with cyano-acrylate glue. Four metal probes of different diameters were used to cool different sized areas of facial skin (or tongue) that were smaller, co-extensive, or larger than the receptive field to examine the existence of spatial summation or presence of inhibitory fields. The probes were numbered 1–4, had diameters of 1,
Thermal ramps

Quantitative assessment of static and dynamic sensitivity of COLD cells was carried out with a computer-controlled Peltier thermoelectric thermode (surface dimensions 2 x 2.5 cm; LTS 3, Thermal Devices). The thermode head was held in contact with the receptive field using an adjustable arm clamped to a magnetic stand so there was no movement during thermal stimulation. Considerable time and care was taken to ensure that the thermode covered and made good contact with the receptive field by using the following techniques: 1) positioning—the head of the thermode was adjusted to be parallel with the receptive field. For receptive fields on the upper lip, a cotton pledget was placed under the lip to extend it outward and onto the surface of the probe. 2) Pretest—a test thermal shift was used to determine whether there was a dynamic response. If no dynamic response was seen, the probe was repositioned. 3) Contact—a silicone-based, heat-sink compound was used to aid thermal conduction to the skin. The computer was programmed to drive the thermal stimulator to produce a series of 50-s duration 5°C descending steps. This 50-s time period was considered to be sufficient adaptation time for neuronal responses to the cooling steps on the basis of previous findings (Broman 1958; Craig and Dostrovsky 1991; Dostrovsky and Hellon 1978). The “staircase”-shaped ramp started from an adapting temperature of 33°C, descended to either 8 or 3°C, and ascended back to 33°C. The rate of cooling during each shift of temperature produced by the Peltier device ranged from 3.3 °C/s at 13°C to 4.2 °C/s at 33°C, and the rate of warming was approximately constant over the range of temperatures employed and was 6.6 °C/s. The existence of a “paradoxical” response was tested by delivering a 5-s step from 43 to 50 or 52°C.

Data analysis

Neuronal responses to static and dynamic thermal stimuli were calculated from single pass poststimulus time histograms with 1-s binwidths. The static rate of discharge was calculated from an average of the counts in each successive 1-s bin in the last 10 s of each step. The peak discharge frequency was defined as the maximum frequency attained in one 1-s bin within the first 10 s after the thermal shift. The dynamic difference was calculated by subtracting the static rate of discharge before the thermal shift from the peak discharge frequency to the thermal shift. A similar series of calculations were made over the rewarming phase of the staircase ramp, i.e., the static discharge frequency before the warm pulse minus the minimum trough discharge frequency. An index of relative dynamic-to-static properties (D:S ratio) was calculated for each cell by dividing the maximal dynamic difference response by the maximum static response (minus background activity at baseline temperature). Ratios ≥3 were considered “mainly dynamic” from 3 to 1 were considered both “dynamic and static”, and <1 were considered “mainly static” response properties. Statistical analyses of data were carried out using parametric (Student’s t-test) and nonparametric (Mann-Whitney rank sum) tests where appropriate using a statistical computer program (SigmaStat, Jandel Scientific Software) and a significance level of P < 0.05. Linear or higher order regression analysis of dynamic and static responses to obtain the population stimulus-response function was carried out with Sigmaplot (Jandel), and 95% confidence limits were plotted.

RESULTS

Location of thermoreceptive cells, receptive fields, somatotopy

A total of 153 neurons responding to innocuous thermal stimulation was studied, of which 146 were excited by cooling and were classified as COLD cells and the remaining 7 were excited by warming and were termed WARM cells. The recording positions relative to the obex were measured for 141 of the 153 units investigated and were found to range from 0.8 mm rostral to 1.5 mm caudal to obex and 1.1–3.2 mm lateral to the midline. The histological identification of 22 recording sites confirmed that the units were in the rostral superficial layers of the MDH (see Fig. 1) and corresponded...
well with the micromanipulator readings. COLD cells with receptive fields in the mandibular division of the trigeminal nerve tended to be located dorsomedial to those with maxillary receptive fields. On average, the locations (distance from the midline) of neurons with tongue receptive fields were more medial (1.8 ± 0.3 mm; mean ± SD) compared with the upper lip (2.2 ± 0.3 mm; t = 5.01, df = 53; P < 0.01), even though the variation in width of the brain stem in the rostrocaudal direction was not considered.

Most of the receptive fields (121 COLD; 6 WARM) were on the face, and the remaining 25 COLD and 1 WARM cell had receptive fields on the tongue (see Fig. 2). Two WARM cells and most (82/121) of the COLD cells had receptive fields on the upper lip below the most ventral row of vibrissae, and lower lip receptive fields were encountered less frequently (32 COLD; 2 WARM). One COLD cell had a receptive field beside the nares (nostril), and one had a receptive field on the lower gingivum. The receptive fields of the remaining seven COLD cells and two WARM cells were not localized precisely. Only one neuron was found that had a receptive field around the eye, but this was likely due to sampling bias because the ventrolateral portion of sMDH, where one would expect to find such receptive fields (Dickenson et al. 1979; Dostrovsky and Hellon 1978), was not routinely studied. Receptive fields were small (generally 1–2 mm long and 1 mm wide on the upper lip) and located exclusively ipsilateral to the recording site. All facial receptive fields were confined to one division of the trigeminal nerve, except for six COLD cells whose receptive fields extended around the corner of the mouth on both lips. Receptive fields on the tongue were also ipsilateral and sometimes larger (~4 mm²) but did not cross the midline. No clear evidence of the presence of inhibitory surrounds was obtained for 14 COLD cells tested using semiquantitative methods. Stimulation of the receptive fields with large probes that extended beyond the boundaries of the receptive field produced responses similar to (n = 7) or slightly larger (n = 5) than those elicited by the small probes applied within the receptive field (see Fig. 3). In two other cases, although the dynamic response was smaller with the larger probes, the total number of spikes during the stimulation was comparable.

Only seven WARM cells were found in the present study. They were located usually near other COLD cells and did not appear to be located in clusters. They were inhibited totally by cooling stimuli to 10°C and excited by warming stimuli ≤43°C. Quantitative thermal stimulation was performed for only three of these cells. Without thermal stimulation their spontaneous firing ranged from 5 to 20 Hz. They had low levels of firing (<2 Hz) below 20°C and in the noxious range. The cells were excited on rewarming from cold temperatures. The responses were primarily static in nature both in warming and cooling directions.

Mechanosensitivity of MDH COLD cells

Many COLD cells in the sMDH of the rat responded additionally to innocuous brushing and noxious mechanical stimulation of the receptive field. Of 123 units with orofacial receptive fields, excluding tongue, 41 (33%) responded to low-threshold mechanical stimulation (brush, touch) and additional 27 (22%) responded only to noxious pinch. The remaining neurons (45%) did not respond to brush or pinch. Of 18 COLD cells tested with a receptive field located on the tongue, only 1 responded to low-threshold mechanical stimuli, and 7 responded to noxious pinch.

An example of the responses of a mechanosensitive COLD cell is shown in Fig. 3. The spontaneous activity of ~7 Hz was suppressed totally by radiant warming of the receptive field; this heated the skin from a temperature of 28 to 30°C. Each of the cold metal probes of increasing diameter (1–4: small to large) cooled larger areas of skin but all produced similar responses. Brushing the skin of the upper lip produced a weak response. This unit was excited more vigorously by noxious pinch of the receptive field. The slight degree of inhibition preceding the pinch-evoked response was due to the initial contact of the prewarmed tips of the forceps. The location of the unit in the sMDH is shown on the histological section labeled “upper lip” in Fig. 1. The response of a COLD cell to irrigating the tongue with water at different temperatures is shown in Fig. 4. The spontaneous ongoing activity of this unit at 34°C was inhibited by water at 39°C and excited by water at 13°C. The unit was excited by squeeze, pinch, and noxious radiant heat (51°C).

Classification according to dynamic and static properties

Quantitative analysis of 38 COLD cells (10 tongue, 23 upper lip, 3 lower lip, 1 nose, 1 vibrissal pad) in rat sMDH

FIG. 2. Locations of all of receptive fields of neurons where it was possible to delineate their boundaries.
revealed that 26 (68%) had both dynamic and static properties, i.e., a D:S ratio between 1 and 3. An example of this type of COLD cell with a D:S ratio of 2.1 is shown in Fig. 5. This unit had a receptive field on the ipsilateral dorsum of the tongue adjacent to but not crossing the midline. The unit did not respond to brush but was excited by noxious pinch (not shown) and noxious heat applied to the receptive field (shown in Fig. 5).

Five of the neurons (13%) had mainly static responses (ratios <1). The COLD cell shown in Fig. 6 is representative of this group and had a D:S ratio of 0.35. It had a receptive field on the upper lip and responded also to brushing, noxious pinch, and noxious heat (not shown). The cooling steps produced immediate responses (indicating that the thermode was close to the axon terminal), but lacked an immediate high-frequency, rapidly adapting dynamic component although there was some adaptation of the initial response. Also the warming steps failed to produce a large inhibition of activity. The static responses increased in magnitude down to the lowest temperature delivered (3°C).

Seven neurons were found to have mainly dynamic responses and had D:S ratios >3. An example of one these COLD cells that had a D:S ratio of 8.3 is shown in Fig. 7. It responded to the staircase cooling of its receptive field with rapidly adapting responses with the peak sensitivity at the first step from 33 to 28°C. The dynamic response was greatest for the first thermal shift and decreased progressively for cooling steps at lower holding temperatures. In contrast to cooling, rewarming the receptive field did not produce comparable inhibitory responses, because the cell was not silenced for >1 s. This cell also responded to brushing of the receptive field on the upper lip. The COLD cell illustrated in Fig. 8 showed a dynamic response to cooling steps that decreased in magnitude with colder steps to 23 and 18°C and an additional increase in static and dynamic activity at temperature steps to 8 and 3°C, suggestive of an input from high-threshold or ‘cold pain’ fibers. It did not respond to brush or pinch but did respond to noxious heat. Other evidence for input from high-threshold COLD fibers is seen in the Type 2 cells, where firing rates were maintained or increased (3 cells) at the lowest temperatures of 8 and 3°C (see Fig. 10A).

**Paradoxical discharge**

Heating of the receptive field of COLD cells produced a discharge when the temperature was in the noxious range in 29 of 70 (42%) cells tested. It is likely that a higher percent-
age of COLD cells would have had paradoxical discharges if a higher temperature stimulus had been used. For example, the unit shown in Fig. 6 was not excited by the standard noxious heating step of 50°C for 5 s but did respond to a higher skin temperature produced by radiant heat (not shown). Figure 5 shows the excitatory response of a COLD cell to heating the facial skin to 50°C for 5 s. The response of this cell to cooling immediately after the heat-induced discharge was reduced greatly, i.e., there was only a small discharge during the cooling shift from 38 to 33°C. However, this phenomenon was observed in only 2/20 COLD cells tested with noxious heat. Another example of paradoxical discharge during the cooling shift from 38 to 33°C is illustrated in Fig. 7. This neuron, which had a large dynamic response, was not inhibited after the paradoxical discharge and responded well to the thermal shift from 48 to 33°C. Also noteworthy is the appearance of some static discharge to the large cooling step after the noxious heat. The five COLD cells classified as having mainly static responses did not show any paradoxical discharge and all of the five cells showing high dynamic properties did show paradoxical discharges. Figure 9 shows that neurons with no paradoxical discharge had lower dynamic-to-static ratios than those that did have a paradoxical response (median value of 0.88 vs. 2.45, Mann Whitney rank sum test, T = 80, P < 0.002).

Separation of COLD cells into three types based on static firing properties

Three major types of static stimulus-response functions were identified, as illustrated in Fig. 10A. One type of COLD cell (Type 1) had the classic bell-shaped curve usually observed in primary afferents with maxima at temperatures in the 18–28°C range. The population mean curve for this type of neuron had a maximum static activity at 23°C and a pronounced decrease in static rates of neuronal firing at temperatures less than 18°C. Type 1 neurons (n = 19) included five cells with static maxima at 28°C, five cells with maxima at 23°C, four cells with 18°C static maxima, and five with a maximal static response at a temperature of 13°C. These neurons were usually insensitive to brush (only 5/17 responded) but most responded to pinch (10/16 tested) and noxious heat (10/18). Three were shown to project to the contralateral thalamus (see Table 1). The second type (Type 2, n = 10) comprised neurons having static responses that did not decrease more than 10 imp/s at lower temperatures. The population mean response curve had a more gradual shift from 48 to 33°C. Also noteworthy is the appearance of some static discharge to the large cooling step after the noxious heat. The five COLD cells classed as having mainly static responses did not show any paradoxical discharge and all of the five cells showing high dynamic properties did show paradoxical discharges. Figure 9 shows that neurons with no paradoxical discharge had lower dynamic-to-static ratios than those that did have a paradoxical response (median value of 0.88 vs. 2.45, Mann Whitney rank sum test, T = 80, P < 0.002).

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FIG. 5. Quantitative testing of rat superficial MDH neuron to decremental 5°C thermal shifts and 50-s holding temperatures. A range of cooling steps were delivered sequentially from an adapting temperature of 33 to 3 or 8°C and rewarming followed. Example of most common thermoreceptive neuron encountered exhibiting both dynamic and static properties over studied range from 33 to 8°C. This unit also showed a paradoxical discharge to criterion test temperature of 52°C for 5 s. It had a D:S ratio of 2.1.

(4/6), but only one of five tested responded to brush; all three types were shown to project to thalamus (Table 1). The static responses of these groups of COLD cells are shown over the rewarming phase in Fig. 10B. The shapes of the mean static response curves for rewarming are quite similar to those for cooling, although the variability in response function is higher. The sites from which these nine units could be antidromically activated were in the medial ventroposteromedial nucleus and/or ventral ventroposteromedial and ventroposterolateral thalamus.

Dynamic responses of COLD cells

For each COLD cell, the “dynamic difference” between the peak dynamic response to a thermal shift and the previous static rate was calculated (see METHODS). An analysis of the population response for the dynamic difference shows that the most sensitive region of thermal-shift coding for COLD cells is from 33 to 23°C where the change is ~3.4 imp s⁻¹ °C⁻¹; <23°C, the slope of the stimulus-response function is much lower at only 0.7 imp s⁻¹ °C⁻¹ (Fig. 11, ●). The dynamic responses (reductions in firing rate) for warming steps during the rewarming phase are plotted in Fig. 11 (○) and reveal a maximal sensitivity at ~25°C. The dynamic reduction of the static neuronal activity during rewarming appears to be a mirror image of the dynamic excitatory response to cooling over the lower range (i.e., the curves overlap). However, above 23°C there is a decrease again in the mean dynamic difference values, and this is likely due to the loss of static COLD cell activity on which the reduction due to warming can be imposed. Figure 12 plots the relationship of peak (or trough) firing frequency for cooling and warming steps as a function of temperature. Although a higher order function describes the effects of cooling on the dynamic differential response, a simple linear function fits the data quite well for the peak frequency relationship. The slope of the line fitted through the points is 0.9 imp s⁻¹ °C⁻¹ with a y intercept of 22 imp/s. The rewarming curve shows a flattened bimodal shape similar to the dynamic difference curve. The rewarming function for the trough frequency was in the same direction as the cooling function only in the region from 8 to 18°C. This most likely resulted from the presence of a sufficient background level of static activity in many COLD cells at these temperatures for a dynamic inhibition to be coded. Over the 18–38°C range, there was a lower mean trough value likely due to lower static levels of firing and larger numbers of neurons being totally inhibited during the warm pulse.
FIG. 6. Example of a COLD cell showing only static properties and having a D:S ratio of 0.35.

**DISCUSSION**

Several previous studies have examined various aspects of thermoreceptive processing in the rat MDH (Cahusac et al. 1990; Davies et al. 1985; Dickenson et al. 1979; Mokha 1993; Young and Dawson 1989). The results of the present study in general have confirmed the findings of these previous studies but also extended them in several important ways. In particular, this study has revealed the existence of several different types of cooling sensitive neurons in the rat sMDH. On the basis of static stimulus-response functions, three types of COLD cells could be distinguished. Type 1 had maximal static responses to temperatures in the 18–28°C range; Type 2 had maintained static responses down to the coldest temperatures; and Type 3 showed little static changes with temperature but good dynamic responses. Further evidence suggesting that these are distinct groups is the marked difference in incidence of neurons responding to innocuous and noxious mechanical stimuli and noxious thermal stimuli (see Table 1). Importantly, neurons of all three types were shown to project to contralateral thalamus and therefore are likely to be involved in perception and discrimination of temperature stimuli.

Previous studies of lamina I COLD cells have tended to emphasize their similarities and have implied that they are a relatively homogeneous group of cells. The “typical” COLD cell has been reported to have dynamic and static responses to cooling steps, increasing static firing rate with decreasing temperatures from normal skin temperature down to 25–20°C, and then decreasing rates below this point (Craig and Hunsley 1991; Davies et al. 1985; Dawson et al. 1982; Dickenson et al. 1979; Dostrovsky and Hellon 1978; Poulos and Molt 1976; Young and Dawson 1989). The COLD cells in our study that were classified as Type 1 are similar to these typical COLD cells. The static stimulus-response functions of this group of neurons are similar to those of the primary afferents (Davies et al. 1985; Dubner et al. 1975; Heinz et al. 1990; Poulos and Lende 1970a; Poulos and Molt 1976) except that they tend to fire at higher rates, especially at lower temperatures. These neurons also have similar characteristics to those described in the MDH of the rabbit and cat (Davies et al. 1983; Dickenson et al. 1979; Dostrovsky and Hellon 1978; Poulos et al. 1979) and the superficial spinal dorsal horn in the cat and monkey (Craig and Hunsley 1991; Dostrovsky and Craig 1996).

Our study, however, also has revealed the existence of a sizeable population of cooling-sensitive neurons whose stimulus response functions differ from those of the typical thermo-
receptive neuron (Type 1). However, the mean static activation curve for all COLD cells obtained by summing all three types of curves (not shown) is similar to that of Type 1 alone and to that reported by other authors (Craig and Hunsley 1991; Davies et al. 1983, 1985; Dickenson et al. 1979; Dostrovsky and Hellon 1978; Poulos and Molt 1976). Therefore, it is possible that neurons of Type 2, and maybe also 3, were in deeper layers of the skin, and these respond at longer latency and decreased frequency to stimuli delivered to the skin surface because of the temperature gradient (Ivanov 1990). Such input would be expected to give rise to increased responses at lower skin temperature because the stimulus response function of these receptors is shifted to lower temperatures. However, if such inputs exist in the rat, they apparently do not selectively converge on neurons in the MDH as we did not observe any neurons with stimulus-response functions attributable only to such inputs. Another possibility is that these neurons receive input from polymodal nociceptors that respond at low temperatures. This, however, also is considered unlikely because many of these neurons did not respond to noxious thermal stimuli.

It is not clear what gives rise to the response of Type 3 neurons. Cold primary afferents with purely dynamic responses have not been reported. The large, phasic, and short
latency responses indicate that the thermode was close to and in good thermal contact with the primary afferents that were activated by the stimuli. They are unlikely to be due to the known dynamic thermal sensitivity of some low-threshold mechanoreceptors (Burton et al. 1972; Hensel and Zotterman 1951) because only one responded to low-threshold mechanical stimuli, their sensitivity to thermal steps was much higher, and such neurons and primary afferents are not located in the marginal layer. Thus these findings suggest that either there exists a previously unreported group of COLD primary afferents with primarily phasic responses to cooling or that the responses are due to central processing that essentially filters out the slowly adapting component of the incoming signals.

The studies of Craig and colleagues have identified in the cat and monkey superficial dorsal horn cooling-sensitive neurons that also respond to heat and pinch and that have been termed HPC neurons (Craig and Kniffki 1985; Craig and Serrano 1994; Dostrovsky and Craig 1996). However, the neurons identified in the present study that respond also to noxious mechanical and thermal stimuli do not closely resemble the HPC neurons because the static firing rates of HPC neurons only increase significantly at temperatures >25°C (Craig and Bushnell 1994; Craig and Serrano 1994). It is possible that we may have missed this group of neurons because they are generally not spontaneously active and an antidromic search stimulus was not used as was the case in the above-mentioned studies of Craig.

Mechanosensitivity of MDH COLD cells

Most studies have reported that Aδ COLD primary afferents are not activated by innocuous mechanical stimuli (see reviews by Darian-Smith 1984; Hellon 1983; Willis and Coggshall 1991 and Burton et al. 1972; Darian-Smith et al. 1973; Iggo 1969), and only minimally if at all by noxious mechanical stimuli (Darian-Smith 1984; Iggo 1969). However, two studies, both in the rat, have reported that some Aδ COLD receptors are also mechanosensitive (Davies 1984; Pierau et al. 1975). This difference in properties may be related to species differences as the other studies were conducted primarily in primates and cats. In addition, some Aβ
sMDH (Davies 1984; Dickenson et al. 1979; Dostrovsky and Hellon 1978), there is evidence to suggest the existence of sensitive lamina I cooling responsive neurons that also are activated by innocuous mechanical stimulation. Their much greater sensitivity to cooling than to mechanical stimulation, their good static responses, and their location in lamina I suggest that the responses are not due to direct inputs from temperature-sensitive Aβ mechanoreceptors (spurious thermoreceptors). It is our belief that these neurons are involved in mediating thermoreception. The mechanosensitivity of these neurons may be due to inputs from Aδ mechanosensitive thermoreceptors. Alternatively or additionally it is possible that the responses are due to central convergence from COLD afferents and low-threshold mechanoreceptive afferents. If this is the case, then presumably the tactile inputs are polysynaptic because low-threshold large diameter mechanoreceptors terminate in deeper layers (Willis and Coggeshall 1991) and the dendrites of lamina I COLD cells are unlikely to reach these layers. It is of possible significance that only 1 of the 17 units with receptive fields on the tongue was mechanosensitive to low-threshold stimuli and may indicate that the degree of mechanosensitivity varies depending on the region.

We also found that an additional 40% of COLD neurons were excited by noxious mechanical stimuli. The few studies that have examined the responses of COLD primary afferents to noxious stimuli generally have concluded that the afferents are only very weakly activated if at all by noxious mechanical stimuli (Dubner et al. 1975), thus suggesting that the responses we observed may have resulted from convergence of nociceptive afferents. However, it is also possible that in the rat orofacial region, the COLD primary afferents have a greater sensitivity to noxious mechanical stimuli than in cats and primates, especially as in the rat many apparently respond also to low-threshold mechanical stimuli (Davies 1984). In cat and monkey, lamina I COLD-specific neurons were found to respond only minimally with a few spikes at onset of noxious pressure (Craig and Kniffki 1985; Price et al. 1976). However, as discussed above, Craig and colleagues have reported the existence of a separate group of COLD cells that they termed HPC cells that respond well also to noxious stimuli (Craig and Kniffki 1985; Craig and Serrano 1994; Dostrovsky and Craig 1996) but that do not appear to correspond to the neurons observed in this study.

Receptive field size and location

The finding of COLD cells with receptive fields larger than the reported spot-like receptive fields of COLD fibers, indicates convergence and spatial summation of thermal afferent input onto lamina I COLD cells and is comparable with that observed in other studies in rat, rabbit, and cat (Dickenson et al. 1979; Dostrovsky and Hellon 1978; Kanui 1988). In some cases, it seems likely that many of the responses observed were probably due to inputs from the spurious thermoreceptors (Burton et al. 1970; Rowe and Sessle 1972; Wall and Taub 1962) and therefore may not have had anything to do with thermoreception. Nevertheless, both in the present study where ~30% of the COLD neurons were activated by innocuous mechanical stimuli, and in some other studies on the slowly adapting mechanoreceptors also have been shown to be excited, although to a lesser degree by cooling (Burton et al. 1972; Hensel and Zotterman 1951; Iggo 1969; Poulos and Lende 1970a). There is, however, no direct evidence that these "spurious thermoreceptors" contribute to the sensation of temperature, and there is conflicting speculation on this point (Hensel and Boman 1960; Iggo 1969; Poulos and Lende 1970b).

In the CNS there have been many more reports of neurons that respond both to cooling and to innocuous mechanical stimulation (Auen et al. 1980; Burton 1975; Burton et al. 1970; Dickenson et al. 1979; Dostrovsky and Hellon 1978; Kanui 1988; Poulos and Benjamin 1968; Rowe and Sessle 1972; Wall and Taub 1962), although in many of the studies limited to spinal lamina I neurons none were found to be mechanically sensitive (Cahusac et al. 1990; Craig and Hunsley 1991; Dostrovsky and Craig 1996; Kumazawa and Perl 1978; Poulos et al. 1979; Price et al. 1976). In some cases, it seems likely that many of the responses observed were probably due to inputs from the spurious thermoreceptors (Burton et al. 1970; Rowe and Sessle 1972; Wall and Taub 1962) and therefore may not have had anything to do with thermoreception. Nevertheless, both in the present study where ~30% of the COLD neurons were activated by innocuous mechanical stimuli, and in some other studies on the
not uniformly distributed on the facial skin but rather concentrated on the lips. This is similar to findings in the cat (Dostrovsky and Hellon 1978) and rat (Dickenson et al. 1979) where receptive fields also were found concentrated on the lips and tongue. In the cat, there was also a very prominent concentration of receptive fields on the nose and some on the ear and around the eye. The lack of receptive fields in these locations in our study may represent species differences or possibly is due to failure to sample the appropriate part of lamina I. As mentioned in RESULTS, the paucity of ophthalmic receptive fields is likely due to the fact that the ventrolateral portion of the MDH was not adequately sampled, because COLD cells with receptive fields around the eye have been reported by others (Dickenson et al. 1979).

Paradoxical discharge

Approximately 46% of the neurons were excited by noxious heating of the skin. This proportion of units having a
paradoxical discharge is similar to that reported in COLD primary afferents by noxious heating, a phenomenon described as the paradoxical discharge (Hensel 1973b; Long 1977). However, we cannot rule out that some of these responses may have been due to convergence onto COLD neurons of noxious mechanical stimuli. This paradoxical discharge is unlikely to contribute to the sensation of pain but rather explains the psychophysical observations that noxious heating of “cold” spots on the skin gives rise to a cold sensation.

**WARM cells**

The relatively few WARM cells recorded in rat sMDH in the present study (7 WARM to 146 COLD units) agrees with the relative paucity of this type of thermoreceptive unit reported by others (Dickenson et al. 1979; Dostrovsky and Hellon 1978), as well as the low numbers of WARM fibers in rat split-nerve preparations (Boman 1958). In cat MDH, a ratio of WARM cells to COLD cells was 1:10 (Dostrovsky and Hellon 1978). Although most studies of thermoreceptive primary afferents failed to find any excited by warming, presumably due to their small size (unmyelinated) and relatively low proportion, several studies have reported their existence in primate and cat (Poulos and Lende 1970a), including those with receptive fields on the nose (Sumino and Dubner 1981). If one assumes that low peripheral density corresponds to low incidence of MDH neurons processing these inputs, then these findings are in accord with the findings of early psychophysical studies by Blix, Rein, and Strugold (see Hensel 1973a), showing relatively few warm spots compared with cold spots (from 1:4 to 1:8) on the human face. However, the warm cells we did encounter tended to have small extracellularly recorded action potentials, suggesting that they originated from small cells, and thus for technical reasons, it is likely that the population of WARM cells is larger than that observed. The fact that WARM cells have low or zero spontaneous activity at normal skin temperature also may have contributed to a possible sampling bias. It is interesting, however, that in a study of c-fos labeling in rat sMDH, no cells were found to express the c-fos oncogene after warming stimuli (Strassman et al. 1993).

**Temperature pathways in the CNS**

This is the first study in the rat to demonstrate antidromically activated COLD units from thalamus. Ongoing studies and analysis are examining the termination sites within thalamus and will be the subject of a future report. It is of particular interest that neurons with characteristics of Types 2 and 3 also could be activated antidromically, suggesting that they also play a role in temperature perception and discrimination. There is at the present time very little information regarding the thalamic and cortical processing of thermoreceptive information. Several studies have reported the existence of COLD neurons with receptive fields primarily on the tongue or perioral regions located in the cat dorsomedial ventroposteromedial thalamus (Auen et al. 1980; Bushnell et al. 1993; Emmers 1966; Kawahara et al. 1986; Landgren 1960; Poulos and Benjamin 1968), but it is unclear where neurons with
receptive fields on the rest of the body are located. In the monkey, a newly described region termed VMpo has been shown to receive inputs from COLD neurons in the spinal cord and to contain cooling specific neurons (Craig et al. 1994; Dostrovsky and Craig 1995). A similar region appears to exist in the human as well (Craig et al. 1994; Dostrovsky et al. 1996). This region has recently been shown to project to insular cortex (Craig et al. 1995). It is of interest that in the rat, units responding to cooling the tongue have been described in insular cortex (Kosar and Schwartz 1990), suggesting the possibility that a similar pathway may exist in the rat.

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