Differential Projections of Thermoreceptive and Nociceptive Lamina I Trigeminothalamic and Spinothalamic Neurons in the Cat

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Craig, A. D. and J. O. Dostrovsky. Differential projections of thermoreceptive and nociceptive lamina I trigeminothalamic and spinothalamic neurons in the cat. J Neurophysiol 86: 856–870, 2001. The projections of 40 trigeminothalamic or spinothalamic (TSTT) lamina I neurons were mapped using antidromic activation from a mobile electrode array in barbiturate anesthetized cats. Single units were identified as projection cells from the initial array position and characterized with natural cutaneous stimuli as nociceptive-specific (NS, n = 9), polymodal nociceptive (HPC, n = 8), or thermoreceptive-specific (COOL, n = 22; WARM, n = 1) cells. Thresholds for nociceptive activation were measured from each electrode in the mediolateral array at vertical steps of 250 μm over a 7-mm dorsoventral extent in two to eight (median = 6.0) anteroposterior planes. Histological reconstructions showed that the maps encompassed all of the lamina I projection targets observed in prior anatomical work, i.e., the ventral aspect of the ventroposterior complex (vVP), the dorsomedial aspect of the ventroposterior medial nucleus (dmVPM), and the submedial nucleus (Sm). The antidromic activation foci were localized to these sites (and occasional projections to other sites were also observed, such as the parafascicular nucleus and zona incerta). The projections of thermoreceptive and nociceptive cells differed. The projections of the thermoreceptive-specific cells were 20/23 to dmVPM, 21/23 to vVP, and 17/23 to Sm, whereas the projections of the NS cells were 1/9 to dmVPM, 9/9 to vVP, and 9/9 to Sm and the projections of the HPC cells were 0/8 to dmVPM, 7/8 to vVP, and 6/8 to Sm. Thus nearly all thermoreceptive cells projected to dmVPM, but almost no nociceptive cells did. Further, thermoreceptive cells projected medially within vVP (including the basal ventral medial nucleus), while nociceptive cells projected both medially and more laterally, and the ascending axons of thermoreceptive cells were concentrated in the medial mesencephalon, while the axons of nociceptive cells ascended in the lateral mesencephalon. These findings provide evidence for anatomical differences between these physiological classes of lamina I cells, and they corroborate prior anatomical localization of the lamina I TSTT projection targets in the cat. These results suggest that differences in the ventral aspect of the basal ventral medial nucleus is important for thermosensory behavior in cats, consistent with the view that this region is a primordial homologue of the posterior ventral medial nucleus in primates.

INTRODUCTION

Lamina I of the trigeminal and spinal dorsal horn is an integral component of the central neural representation of pain and temperature sensations (for review: Craig and Dostrovsky 1999). Lamina I receives input from Aδ and C primary afferent fibers, including specific nociceptors and thermoreceptors, and it is the major source of ascending output from the superficial dorsal horn. Lamina I cells contribute about half of the direct projections to thalamus from the dorsal horn in cats and monkeys, and their projections in the trigeminothalamic and spinothalamic (TSTT) tracts are behaviorally and clinically critical for pain and temperature sensations.

The original physiological description of spinal lamina I neurons by Christensen and Perl (1970) provided clear evidence that different response classes can be recognized. They described nociceptive-specific (NS) cells responsive to noxious mechanical stimulation or noxious heat or both and, in addition, thermoreceptive cells responsive to cooling the skin with ethyl chloride spray, some of which were specific, but some of which responded also to noxious stimuli. Mosso and Kruger (1973) reported similar findings in the trigeminal dorsal horn. Lamina I TSTT projection cells in the trigeminal dorsal horn were identified in the cat by Dostrovsky and Hellon (1978), who reported corresponding response characteristics and who documented thermoreceptive lamina I units sensitive only to warming as well. Kumazawa et al. (1975) described similar cells in the spinal cord of the monkey. Thermoreceptive and nociceptive lamina I TSTT projection neurons have since been documented in the spinal cords of both cat (Craig and Bushnell 1994; Craig and Dostrovsky 1991; Craig and Hunsley 1991; Craig and Kniffki 1985a) and monkey (Dostrovsky and Craig 1996). Consistent with the earlier descriptions, these studies recognized three categories of lamina I TSTT cells: NS neurons, thermoreceptive-specific neurons (WARM or COOL, formerly “COLD”), and polymodal nociceptive neurons sensitive to noxious heat, noxious pinch, and noxious cold (HPC). These categories not only have qualitatively different response properties but also different ascending conduction velocities and different susceptibilities to descending or pharmacological modulation (Craig and Serrano 1994; Dawson et al. 1981; Dostrovsky et al. 1983). Evidence has recently been obtained that they can be distinguished morphologically as well (Han et al. 1998).

A natural inference is that these distinct classes of lamina I TSTT neurons might have distinct projection targets in the thalamus. The present study was motivated by the contrary observation (Craig and Dostrovsky 1991; Dostrovsky and Broton 1985) that both COOL and NS lamina I TSTT cells can be
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Antidromically activated from the submedial nucleus (Sm) in the medial thalamus of the cat. Anatomical evidence indicates that Sm is one of three main sites in cat thalamus in which ascending lamina I TSTT fibers terminate (Craig 1987, 1991), in addition to the ventral aspect of the ventroposterior complex (vVP) and the dorsomedial aspect of the ventroposterior medial nucleus (dmVPM). The suggestion that the different classes of lamina I TSTT cells might not have distinct projections contrasted with the distinctive features of these different classes and with the repeated observation that only thermoreceptive-specific neurons can be recorded in the region of dmVPM (Auen et al. 1980; Landgren 1960). Therefore we directly examined whether the thalamic projections of lamina I TSTT neurons are related to their physiological response characteristics by antidromically mapping the projections of single identified units using a mobile array of electrodes that could encompass all of the anatomical projection sites in the cat thalamus. Our results confirm that lamina I TSTT neurons project mainly to these three sites, and they provide evidence that thermoreceptive and nociceptive lamina I TSTT cells do have different thalamic projection patterns. Preliminary reports were made (Craig and Dostrovsky 1991; Dostrovsky and Craig 1993).

METHODS

General procedures

All procedures were conducted in accordance with the guiding principles for the care and use of animals approved by the American Physiological Society, and the protocols were approved by the appropriate institutional animal care and use committees. Experiments were performed on 46 domestic cats (2.0–5.9 kg, mean 3.3), approximately half male and half female. The animals were anesthetized with pentobarbital sodium (40 mg/kg ip), and a catheter in the right cephalic vein was used to administer intravenous supplements to maintain areflexia. The head, neck, back, face, and forelimb or hindlimb were shaved. Eye salve was administered, 0.5% marcaine was injected subcutaneously at all incision sites, cetacaine was sprayed in the ear canals, and a single intravenous injection of dexamethasone (10 mg) was administered. The left common carotid artery was catheterized to monitor blood pressure, and the trachea was cannulated. Paralysis was induced with pancuronium bromide (0.04 mg/h), and a positive pressure respirator was used to maintain end-tidal CO₂ at 3.5–4.5%. Anesthetic depth (areflexia) was verified every hour with 0.35-mm diameter tubing to minimize deviation. Monopolar search stimuli were delivered to each of the electrodes in the thalamic array with the following parameters: 500 μA, 2-msec duration, three or four pulses at 200–333 Hz, inner (or outer) contact negative. The indirect stimulus electrode was attached to the incised cranial skin.

Implantation of thalamic electrode array

Tungsten-in-glass microelectrodes (tip diameter ~25–40 μm) were used to make single- and multi-unit recordings of somatosensory responses in the ventroposterior (VP) thalamus to determine the initial placement of the antidromic stimulating electrodes. Electrode penetrations commenced anteriorly and laterally (AP9.5, ML6.5) to identify the junction of the cutaneous and deep representations of the forepaw and then moved posteriorly and medially to locate the small (approximate diameter, ~0.25 mm) site at the dorsomedial aspect of the face representation where units responding to cooling the ipsilateral tongue can be found (Auen et al. 1980; Landgren 1960) and to identify as well the ventral border of VP at the external medullary lamina. Based on these results, the array of antidromic electrodes was implanted so that it was situated 1 mm dorsal to the intended initial location (dmVPM, vVP, or Sm); it was moved into the intended position after a lamina I unit was isolated. The array consisted of seven (6 experiments) or eight (40 experiments) concentric bipolar electrodes evenly spaced at 1-mm intervals in a mediolateral row. Custom-made Rhodes (Kopf Instruments, Tujunga, CA) MCE-100X electrodes were used that were 45 mm in length with an inner contact diameter of 25 μm and an outer contact 100 μm higher that had a diameter of 150 μm; these were reinforced to within 5 mm of the tip with 0.35-mm diameter tubing to minimize deviation. Monopolar search stimuli were delivered to each of the electrodes in the thalamic array with the following parameters: 500 μA, 2-msec duration, three or four pulses at 200–333 Hz, inner (or outer) contact negative. The indifferent stimulus electrode was attached to the incised cranial skin.

Characterization of lamina I TSTT units

Recordings were obtained from cells in the superficial dorsal horn near the dorsal root entry zone with platinum-plated tungsten-in-glass microelectrodes having tip sizes of ~15 μm. Recordings were made in the trigeminal dorsal horn at the University of Toronto, and recordings were made in the spinal cord at the Barrow Neurological Institute. In general, whether in the trigeminal, cervical, or lumbosacral dorsal horn, lamina I was recognized at depths of 200–600 μm by the presence of units with slow, irregular ongoing activity; such activity was often inhibited by radiant heat and excited by application of an innocuous cool stimulus to the face, forepaw, or hindpaw, indicative of COOL cells (see following text). In the spinal cord, lamina I is just below the Group I–II afferents that have regular ongoing discharge. (Below this, we find a 200-μm-thick zone, in the outer substantia gelatinosa, where our electrodes rarely pick up unitary discharges, followed by field potentials and multi-unit responses to low-threshold mechanical stimulation in inner lamina II and laminae III–V.) The identification of TSTT projection neurons at these depths provides assurance that the units were lamina I neurons because TSTT neurons are rarely located in laminae II–III in the cat (Zhang et al. 1996).

The microelectrode was moved so that a single unit was isolated on the basis of spike amplitude, and then activation from the thalamic electrode array was tested. The antidromic nature of each unit’s response was determined on the basis of a constant latency, a discrete all-or-none threshold to stimulation, the ability to follow a 200- to 333-Hz train of at least three stimuli with constant latencies, and collision by a closely preceding orthodromic spike (Fig. 1). All units that fulfilled these criteria demonstrated a definitive critical interval for collision with orthodromic spikes when tested (Ranck 1975). The orthodromic and antidromic spike waveform of every unit studied was constantly monitored to ensure that recordings from the same single unit were maintained throughout the characterization and antidromic mapping. Lesions were made at these recording sites (5–20 μA cathodal current for 20–40 s), and every one found (40 in 46 cats) was located in lamina I of the trigeminal or spinal dorsal horn (Fig. 2).
Antidromically activated single units were tested with natural cutaneous stimulation, including innocuous and noxious mechanical (brush, cotton wisp, and flat forceps), innocuous cooling (beaker of wet ice), innocuous warming (radiant heat lamp), noxious heat (heat lamp), and noxious cold (ice-cold beaker) stimuli (Craig and Dostrovsky 1991; Craig and Hunsley 1991; Craig and Serrano 1994). The cells were classified as: thermoreceptive-specific cells sensitive to cooling (COOL) or warming (WARM); NS cells sensitive only to heat, pinch, or both; and HPC cells sensitive to noxious heat, pinch, and noxious cold. Wide dynamic range cells also occur in lamina I, but these rarely project to thalamus in the cat (Craig and Kniffki 1985a; Craig and Serrano 1994; Hylden et al. 1986).

The COOL cells were identifiable by irregular spontaneous activity that could be silenced by innocuous radiant warming of the skin, by vigorous excitation on contact or near contact with a cold object, and by their insensitivity to mechanical stimulation with a probe warmed to neutral skin temperature. They displayed graded responses to innocuous cooling but did not show increased responses to noxious cold (see Fig. 1). Some COOL units were weakly excited by noxious heat (reflecting the “paradoxical” discharge of some cooling-specific primary afferents) (see Hensel 1981) or phasically excited by a strong pinch. The rare WARM cells (only 2 were encountered) were identified by their response to innocuous warming, inhibition by cooling, and their insensitivity to noxious or mechanical stimuli. In contrast, polymodal nociceptive HPC lamina I TSTT neurons were identifiable by their lower ongoing discharge (see Andrew and Craig 2001), their insensitivity to warming, their phasic excitation by innocuous cooling, and their sustained, graded discharge to noxious cold, noxious heat, and pinch. The thresholds of HPC cells to noxious cold vary (see Craig and Serrano 1994). They are insensitive to innocuous mechanical stimulation with thermally neutral probes. Finally, we characterized NS lamina I TSTT cells by their selective, graded sensitivity to pinch or noxious heat (see Fig. 1) or both pinch and heat. In the present experiments, we included as NS cells two units that were briskly responsive to noxious heat and pinch but appeared to have been sensitized by prior stimulation (one responded intermittently to hair movement and another was sensitive to a cotton wisp slowly drawn across the glabrous skin at the tip of the nose but to no other innocuous stimuli).

Receptive fields (RFs) were determined by manual mapping with threshold stimuli and indicated on figurines. Receptive fields were located on glabrous or hairy skin of the face, the forepaw, or the hindpaw according to the segmental location of the recording sites and were consistent with prior descriptions (Craig and Dostrovsky 1991; Craig and Hunsley 1991; Craig and Kniffki 1985a; Craig and Serrano 1994; Dostrovsky and Hellon 1978). In the spinal experiments, a series of computer-controlled cooling and warming steps was applied with a Peltier thermode (20 × 20 mm) placed on the RF (see Fig. 1) (Craig and Hunsley 1991; Craig and Serrano 1994).
of at least one electrode track in each plane, but a different pattern was used in each plane to facilitate histological reconstruction; lesions were also made at or just above critical low-threshold foci.

In general, monopolar stimuli were used as search stimuli for threshold mapping because this produced antidromic activation from a broad area and thereby provided assurance that projection areas between the electrodes or between mapping planes were not overlooked. In some experiments, however, monopolar stimulation was effective at very low currents, resulting in activation by the search stimulus from very wide areas and in less steep threshold gradients in the neighborhood of low-threshold foci; in these cases, bipolar stimulation between the inner and outer conductors of individual electrodes was used as an adjunctive measure to confirm localization of the low-threshold foci of activation. Bipolar stimulation resulted in higher thresholds that increased more sharply with increasing distance from the focus, presumably due to the more focused current spread, and thereby provided greater spatial definition of low-threshold foci. The adjunctive comparison of monopolar and bipolar stimulation thresholds often helped distinguish and cleanly separate neighboring antidromic projection foci.

Anatomical reconstructions

To reconstruct anatomical maps of the sites of antidromic activation, each mapping plane was first identified histologically on the basis of the pattern of the lesions made. The shrinkage within each brain was estimated based on the distance between lesions made at the top and the bottom of one electrode track (usually 10–15%). The bottoms of the tracks of the individual electrodes were identified within each plane, and vertical distances were measured from these points, taking into account the shrinkage and the locations of other lesions made at strategic sites. The antidromic activation sites and the measured thresholds were then plotted on cytoarchitectonic drawings made from the individual thalamic sections according to Craig and Burton (1985), as in the example shown in Fig. 3. In most cases, the sections were fairly well aligned with the electrode tracks so that at least half of the vertical extent could be seen in one or two adjacent sections, but in some cases, the sections were misaligned due to the plane of sectioning or due to progressive edema during the mapping procedure. In these cases, portions of the electrode tracks were visible on successive sections, and vertical depths from the marking lesions were interpolated to the appropriate histological level.

The antidromic mapping data were compiled for systematic analysis by transposing the histological reconstruction of each individual unit onto a standard series of coronal drawings of the cat thalamus made at ~0.5-mm intervals (the same series used by Norrsell and Craig 1999). In these standardized summaries, three different dot sizes were used to represent the number of consecutive mapping points at which thresholds ≤60 μA were measured: a small dot was placed if only one point had a threshold ≤60 μA, a medium-sized dot was placed if two consecutive points had such thresholds, and a large dot was used to represent three such consecutive mapping points. The sizes of the medium and large dots were such that the dot covered both or all three of the low-threshold points. Comparison of the original reconstruction for unit c13-11.1 (Fig. 3) with the standardized summary in Fig. 4 shows that the localization and the distinctness of the low-threshold projection foci were preserved during this transfer.

The final compilation of the antidromic mapping data was made by superimposing the standardized summary maps of all 40 thoroughly mapped cells, using a constant gray level for the threshold dots (in the program Adobe Photoshop), sorted according to cell type and RF location (Fig. 6). With this method, anatomical areas of overlap between the antidromic projection foci of these cells were signified with progressively darker gray levels. Statistical comparisons of the characteristics of different populations of neurons were made with the program CSS Statistica (Statsoft; Tulsa, OK).
RESULTS

Characteristics of the total sample of antidromically activated lamina I TSTT units

The units selected for antidromic mapping were taken from a total sample of 130 lamina I neurons that were identified from the initial array position as TSTT cells by the demonstration of a high-frequency, constant latency antidromic train response (Fig. 1). These lamina I units were recorded in the trigeminal dorsal horn (nucleus caudalis) in 29 cats, in the cervical C8 segment in 2 cats, and in the lumbar L7 segment in 15 cats. Of this total sample, 89 lamina I TSTT units were recorded in the trigeminal dorsal horn, and these had an average conduction velocity of 5.71 m/s based on a mean latency of 5.25 ms (range 1.9–13.0) and a mean distance of 30 mm. A total of 15 units recorded in the C8 segment had an average conduction velocity of 3.41 m/s based on a mean latency of 30.8 ms (range 12–90 ms) and a mean distance of 105 mm, and 26 units recorded in the L7 segment had an average conduction velocity of 2.56 m/s based on a mean latency of 133 ms (range 38–360) and a mean distance of 340 mm. The latencies recorded were the shortest obtained from the site of the initial array placement in thalamus (targeted in different cases at vVMb, Sm, or dmVPm; see following text). These latencies and mean conduction velocities are consistent with prior observations of lamina I trigeminothalamic and spinothalamic cells in the cat (Craig and Kniffki 1985a; Dostrovsky and Hellon 1978). The present data at three spinal levels demonstrate for the first time that there is a progressive decrease in lamina I TSTT conduction velocities with increasing conduction distance.

The conduction velocities of the characterized lamina I TSTT cells differed according to their physiological class. For the entire sample of 20 characterized lamina I TSTT cells recorded in the L7 segment, where the differences were greatest, the mean conduction velocities were: for NS cells, 1.83 ± 0.76 (SD) m/s, n = 5; for HPC cells, 3.24 ± 1.67 m/s, n = 6; and for COOL cells, 5.78 ± 1.77 m/s, n = 9. Despite the small numbers, these conduction velocities are pairwise significantly different (NS vs. HPC, P = 0.011; NS vs. COOL, P < 0.001; HPC vs. COOL, P < 0.02; t-tests), consistent with the significant differences observed in prior samples (Andrew and Craig 2001; Craig and Serrano 1994).

Characteristics of the antidromically mapped lamina I TSTT cells

We selected 49 of the total sample of 130 identified lamina I TSTT cells for mapping with the mobile electrode array, and we mapped their thalamic projections in one to eight anteroposterior planes. The antidromic identification of each mapped unit as a TSTT cell was verified by demonstration of its critical collision interval (see Fig. 1). In each experiment, the array was implanted initially to stimulate one of the main lamina I projection sites. That is, each cell studied was selected because it was initially antidromically activated from vVP, dmVPm, or Sm. Accordingly, the initial location of the electrode array was varied in order that this selection would not produce a bias. The number of cells selected for antidromic mapping that were initially identified from each histologically verified location was: 16 from vVP, 13 from dmVPm, and 20 from Sm.

The final data set comprised 40 of the 49 antidromically mapped cells. Two of the 49 units were discarded because of inadequate histology, and 7 were discarded because the histological reconstruction showed that the region mapped had not encompassed all three major lamina I termination sites; these units included primarily cells for which a map had been obtained in only one or two planes. We initially focused on COOL cells in this study (Craig and Dostrovsky 1991), and of the 40 thoroughly mapped cells, 23 were thermoreceptive-specific (COOL or WARM) cells, while 8 were HPC cells, and 9 were NS cells. The final sample of 40 well-mapped lamina I TSTT units included 28 trigeminothalamic cells and 12 spinothalamic cells recorded in the L7 segment. The antidromic maps of these 40 cells comprised an average of 5.6 planes (median 6.0).

The histological reconstruction of the complete mapping results from one representative experiment is shown in Fig. 3. In this experiment, the antidromic electrode array was initially positioned at AP 6.5, where electrodes 3 and 4 were meant to be situated at or near dmVPm, and from this site, we antidromically identified five lamina I trigeminothalamic COOL cells with RFs on the nose or the eyelids. The COOL cell (c13-11.1) that we selected for antidromic mapping was initially activated at a monopolar threshold of 270 μA on electrode 3 with an antidromic latency of 1.9 ms, and it had a large, well-isolated and stable action potential and a RF on the dorsal and ventral eyelids. Antidromic thresholds were mapped first at AP 6.5, then successively at AP 7.25, 8.0, 6.0, 5.25, and 4.25, using both monopolar and concentric bipolar stimulation.

This representative example shows the broad coverage and the clear localization of projection sites provided by antidromic mapping with the mediolateral array of electrodes. The unit was consistently activated by the 500–μA monopolar search stimulus from points rostrocaudally, mediolaterally, or dorsoventrally adjacent to the projection foci, indicating that the search coverage exceeded the 1-mm mediolateral spacing between electrodes, the 0.75- or 1.0-mm spacing between antero posterior planes, and the 0.25-mm spacing between vertical test points. Nevertheless, clearly delimited foci with thresholds <20 μA were obtained. For example, at AP 5.25, the unit illustrated in Fig. 3 was antidromically activated with stimulus thresholds <200 μA over an extent of 2.5 mm from electrode 3, yet two clearly distinguishable low-threshold foci were defined, one at a depth of 4.5 mm (in dmVPm) and the other at 5.5 mm (in the ventral aspect of VMb, which is part of the
WARM
c4-3.1
nose

COOL
c5L-3.1
nose

COOL
c13-11.1
eye

COOL
c25-4
nose

COOL
c20-1
v hp
vVP region). These two low-threshold foci had focal monopolar thresholds of 20 μA and were separated by a sequence of monopolar thresholds of 55, 125, and 60 μA at the intervening 0.25-mm steps. The localization of these foci was confirmed with the use of bipolar stimulation, with which these sites had focal thresholds of 35 and 45 μA and were even more clearly separated by a sequence of thresholds of 150, 230, and 90 μA at the intervening steps.

Low-threshold activation points with thresholds ≤60 μA with monopolar stimulation were found for all of the 40 thoroughly mapped cells. The average threshold at these foci was 20.5 μA ± 15.0 SD (range 2–60 μA), with a 25th percentile of 9.0 μA, a median of 17.0 μA, and a 75th percentile of 28.0 μA.

Different antidromic latencies were generally observed at different projection foci, indicative of the different conduction times in different collaterals. Slightly longer latencies were also recorded in the periphery around each projection focus, consistent with longer onset activation times associated with activation from a distance. Significantly, at the projection focus of 22 cells we observed discrete latency shifts at different stimulus intensities (“latency jumping”) as we have described before (Craig and Dostrovsky 1991). These shifts were as large as several milliseconds for spinal units; the largest was a shift from 52 to 45 ms at higher intensities at the projection focus in Sm for cell c40-1. Such shifts were observed in each of the three main projection targets (dmVPM, vVP, Sm). These discrete latency shifts indicate that different terminals were activated within the terminal arborization field of the cell’s axon (Lipski 1981), which provides direct evidence supporting the conclusion that these antidromically mapped activation foci identify the terminal projection sites of these axons.

Distribution of antidromically mapped projection foci

Reconstructions of the projection maps of several thermoreceptive-specific (COOL and WARM) cells and nociceptive (NS and HPC) lamina I TSST cells are shown at the standardized thalamic levels (see METHODS) in Figs. 4 and 5. These units are representative of the major projection patterns, and they also show a variety of individual ancillary projections. Both trigeminothalamic and spinothalamic cells are illustrated; RF locations for each cell are denoted in the figures. If a particular level of the thalamus was not mapped in an experiment, then the image of that level is not included in the charts. The fidelity of the transposition of individual histological reconstructions onto the standardized series can be appreciated by comparing the complete reconstruction of cell c13-11.1 in Fig. 3 with its standardized representation in Fig. 4. Note that the sizes of the dots in Figs. 4 and 5 represent the spatial extent of low-threshold antidromic activation (≤60 μA), not absolute thresholds (see METHODS). The projections of individual cells varied, yet several patterns were apparent. The overall patterns can be appreciated from the summary charts in Fig. 6, in which the projections of all 40 cells are superimposed.

The predominant projection targets were vVP, Sm, and dmVPM, consistent with anatomical observations based on anterogradely transported PHA-L (Craig 1987, 1991). Nearly all (37/40) of the thoroughly mapped lamina I TSST cells projected to vVP (including the ventral aspect of VMb, the region of VPI, and the ventral aspect of VPL). Most (32/40) projected to Sm. About half (21/40) projected to dmVPM.

The projections of the thermoreceptive-specific (COOL and WARM) lamina I TSST cells clearly differed from the projections of the nociceptive (NS and HPC) cells. Whereas most cells in both populations projected to vVP and to Sm, the thermoreceptive-specific cells had a characteristic projection to dmVPM, and in contrast, almost none of the nociceptive lamina I TSST cells projected to dmVPM. Thus of the 23 thoroughly mapped thermoreceptive-specific cells, 21 projected to vVP, 17 projected to Sm, and 20 projected to dmVPM. Of the nine thoroughly mapped NS cells, all nine projected both to vVP and to Sm, but only one projected to dmVPM. Similarly, of the eight thoroughly mapped HPC cells, seven projected to vVP and six to Sm, but none projected to dmVPM. The distinction between the projections of thermoreceptive-specific cells and NS or HPC nociceptive lamina I TSST cells is statistically significant (P < 10⁻³, General Linear Model, CSS Statistica).

The characteristic projection of thermoreceptive-specific lamina I TSST cells to dmVPM was focused at levels two and three in the standard series, as shown in Figs. 4 and 6. Corroborative evidence for this characteristic projection is provided by the observation that we consistently identified many additional COOL lamina I TSST cells when searching for a TSST unit to map with the array initially positioned at dmVPM, as noted in the detailed description of experiment c13 in the preceding text.

The thermoreceptive-specific and nociceptive lamina I TSST cells also differed with respect to the distribution of their projections within vVP (Figs. 4 and 6). The projections of thermoreceptive-specific (COOL and WARM) cells were particularly concentrated within the medial portion of vVP. Nearly all of these cells projected to the ventrolateral aspect of VMb and/or the adjacent medial portion of VPI. There was even an indication of somatotopographic order because every trigeminothalamic thermoreceptive-specific cell (except the 2 that did not project to vVP) projected to this region at the two or three most posterior standard levels (Fig. 6), whereas every spinthalamic thermoreceptive-specific lamina I cell projected to this region at more anterior levels (especially levels 3 and 4).

In contrast, the projections of the nociceptive cells within vVP were more widely distributed throughout its caudal, rostral, medial, and lateral extents (Figs. 5 and 6). Lateral projection foci in the ventral aspect of VPL were regularly observed for nociceptive cells, regardless of RF location, but such projections were almost never observed for COOL cells. Some nociceptive cells projected throughout much of the mediolateral extent of vVP, whereas for others, discrete foci occurred in

FIG. 4. Summaries of the antidromically identified projections of representative thermoreceptive-specific lamina I TSST units. The 3 dot sizes indicate the spatial extent at each projection focus over which a unit was antidromically activated at threshold values ≤60 μA (see METHODS for further explanation) and not absolute threshold values. The response type, unit number, and receptive-field location are noted for each unit. The scale bar and cytoarchitectonic reference guide are shown at the far right in Fig. 5, where the shaded areas indicate the regions in which lamina I TSST terminations have been anatomically observed with anterograde tracing (Craig 1987, 1991).
FIG. 5. Summaries of antidromically identified projections of representative nociceptive lamina I TSTT units. Conventions as in Fig. 4.
Of the 17 nociceptive (HPC and NS) cells, only 1 did not project to vVP [an HPC cell that projected only to caudal posterior nucleus (Po)].

More than two-thirds (17/23) of the thermoreceptive-specific cells and nearly all (15/17) of the nociceptive cells also projected to Sm. The single WARM cell that was mapped did not differ from the COOL cells in this regard (Fig. 4). There was evidence of somatotopography in this region as well (Fig. 6): lamina I trigeminothalamic cells (both thermoreceptive and nociceptive) generally projected to the caudal pole of Sm (level 3), whereas spinothalamic lamina I cells (both thermoreceptive and nociceptive) generally projected to the rostral half of Sm (level 4). This is consistent anatomically with the rostrocaudal topographic organization of Sm (Craig 1987, 1991; Craig and Burton 1985).

FIG. 6. Compilation of the standard summary maps of the projections of all 40 thoroughly mapped lamina I TSTT cells, segregated according to response type (WARM or COOL and NS or HPC) and longitudinal location (trigeminal or spinal). The cytoarchitectonic references are provided in the legend at the right of Fig. 5. The most rostral level of the standard series, where very few foci were located, has been omitted. Constant gray tone was used for each cell’s projection foci so that areas of overlap in these projection foci between cells are represented by darker gray levels.
In addition, ancillary antidromic activation sites were observed that were also consistent with the prior anatomical observations, and these too differed according to cell category. Projections to parafascicular nucleus (Pf, \( n = 5 \)), to zona incerta (ZI, \( n = 2 \)), and to supergeniculate nucleus (SG, \( n = 1 \)) were observed for only a few of the 23 thermoreceptive cells, whereas projections to Pf (\( n = 7 \)), to ZI (\( n = 7 \)), to caudal Po (\( n = 9 \)), to dorsal Po (\( n = 4 \)), and to other sites (2 in VM, 2 in central lateral nucleus (CL), 1 in SG) were more common among the 17 thoroughly mapped nociceptive cells. Antidromic activation sites in caudal Po were found only for nociceptive units. It is noteworthy that projections to the dorsal portion of Po were generally located \( \sim 1 \) mm dorsal to the border of VP (e.g., unit c13-11.1, Fig. 5). In two cells, we encountered axon collaterals that ascended rostrally to join the optic tract (e.g., unit c40-1, Fig. 5). Finally, the distribution of the ascending axons of thermoreceptive-specific and nociceptive cells also differed. Lamina I TSTT cells were antidromically activated at isolated sites in the mesencephalon, and these sites were marked with lesions in 13 cases. The ascending axons were activated with a median threshold of \( 40 \) \( \mu A \) (range 6–240 \( \mu A \)) and latencies that were 0.2–0.9 ms shorter for trigeminothalamic cells and 2–6 ms shorter for spinothalamic cells than the shortest respective latencies observed from sites in the thalamus. For seven thermoreceptive cells, five sites were located in the medial half of the rostral mesencephalon (clustered near the border of the periaqueductal gray), and two were found in the lateral half (Fig. 7). For six nociceptive cells (3 HPC, 3 NS), all sites were found in the lateral half of the mesencephalon, clustered near the medial lemniscus in the classical location of the ascending spinothalamic tract. These differences are significant (Fisher exact test, \( P < 0.03 \)).

**DISCUSSION**

**Technical considerations**

The validity of these observations depends on certain considerations. First, antidromic mapping with the mediolateral array of electrodes provided sufficiently broad and complete coverage of the thalamus. The units were generally activated from broad areas by the 500–\( \mu A \) search stimulus, and adjacent activation points were usually observed from neighboring tracks. The array extended across nearly all of the thalamus, and the maps of the final data set of 40 thoroughly mapped cells included all three major anatomically identified lamina I TSTT termination sites, i.e., Sm, dmVPM, and vVP. The electrodes maintained nearly parallel vertical tracks, and the cytoarchitecture of the thalamus was recognizable despite multiple passes of the array. The array did produce compression and distortion of the thalamus in some cases, and progressive edema was also evident where mapping planes made later during an experiment showed curved trajectories. Thus reconstruction measurements were made from the bottoms of the tracks, which were always verified with lesions, and from additional marking lesions made at strategic locations (at or near low-threshold activation sites) during mapping.

Second, this method enabled the localization of projection sites. Low-threshold antidromic activation foci were identified in each experiment with a median threshold of 17 \( \mu A \) using electrodes with 25–\( \mu m \) tips. This implies a spatial resolving power of \( \sim 200 \) \( \mu m \) (Ranck 1975), which justifies the mapping step size used (0.25 mm). Antidromic activation was confirmed at all low-threshold sites by demonstration of a critical collision interval. The low-threshold activation foci were clearly delimited by steeply increasing thresholds at neighboring mapping points. These foci denote terminal projection sites because we observed increases in activation latencies, which indicates conduction slowing in terminal collaterals, and because we observed the phenomenon of “latency jumping,” which indicates activation of different terminals within an arborization field (Lipski 1981). Significantly, the distribution and topography of the projection sites identified in this study are entirely consistent with the anatomically identified terminations of lamina I TSTT neurons observed with anterograde labeling (Craig 1987, 1991).

Third, it should be noted that the simplified compilation method we used did not depend on the representation of the exact numerical values for thresholds at each point, in contrast with the technique for plotting antidromic thresholds that we used for individual histological reconstructions (e.g., Fig. 3) and that is commonly used by others (e.g., Fields et al. 1995; Kitazawa et al. 1995; Zhang et al. 2000). Nonetheless, this compilation method is empirical, and it enables comparison of the histological localization of the projection sites with the spatial resolution of 0.25 mm in these maps. This graphical method also accommodated antidromic activation of axonal projections that terminated between mapping planes. Finally, this method is spatially consistent with the anatomically observed extent of the terminal arbors of lamina I TSTT axons in these regions (Craig 1987, 1991).

**Differentiation of the three major classes of lamina I TSTT neurons**

Consistent with the description of three different physiological response patterns by the earliest studies of lamina I neu-
rons (Christenson and Perl 1970; Mosso and Kruger 1973), we recognize three major and distinct classes of lamina I TSTT projection neurons: NS, COOL or WARM, and HPC. These cell types are distinguished by several features.

First, they have qualitatively different responses to natural cutaneous stimuli in both cats and monkeys (Craig and Serrano 1994; Dostrovsky and Craig 1996). Thermoreceptive-specific (COOL and more rarely WARM) cells respond in a graded manner only to innocuous thermal stimuli, and nociceptive NS and HPC cells respond selectively to noxious thermal and mechanical stimuli. The HPC cells differ from the NS cells in that they respond also to noxious cold, and they differ from COOL cells in that they have lower (colder) thresholds, are not inhibited by warming, and respond in a graded manner to noxious heat and pinch.

Second, they have significantly different conduction velocities (Craig and Kniffki 1985a; Craig and Serrano 1994; Dostrovsky and Craig 1996). As confirmed in the present experiments, the NS cells conduct very slowly, many in the range of unmyelinated axons, whereas the HPC and COOL cells conduct progressively faster, consistent with small myelinated fibers. Larger samples of ~200 lumbosacral lamina I TSTT cells support the statistical significance of the pairwise differences observed in the present sample (Andrew and Craig 2001). These data confirm earlier results indicating that the axons of trigeminothalamic lamina I cells conduct on average about twice as fast as the axons of spinothalamic lamina I cells, but the present comparison of trigeminal, cervical, and lumbosacral lamina I TSTT cells indicates that the longer axons are progressively thinner.

Third, they respond differently to descending pharmacological modulation; in particular, COOL cells are not inhibited by brain stem stimulation, whereas NS cells are (Dawson et al. 1981; Dostrovsky et al. 1983) and COOL lamina I TSTT cells are not inhibited by systemic or topical morphine, whereas both NS and HPC lamina I TSTT cells uniformly are (Craig and Hunsley 1991; Craig and Serrano 1994).

The present results add significantly to these distinctions by showing in cats that thermodensitive-specific (COOL and WARM) lamina I TSTT cells have a significantly different projection pattern within the thalamus than NS or HPC cells. Nearly all thermodensitive-specific cells projected to dmVPM, but in contrast almost no NS or HPC cells did. Many NS and HPC cells, but few thermodensitive-specific cells, had ancillary projections to Po, ZI, and other sites. In addition, COOL cell axons ascended within the medial mesencephalon and terminated medially within vVP, whereas NS and HPC cell axons ascended in the lateral mesencephalon and terminated more broadly within vVP.

Thus the present findings clearly indicate that the axons of these lamina I TSTT cells are differentially distributed. This anatomical difference is consistent with the hypothesis that the different physiological classes of lamina I TSTT cells are morphologically distinct. This hypothesis has recently received direct support from observations of the soma of these cells, based on intracellular labeling in cats, that indicate that NS lamina I cells are fusiform neurons, COOL cells are pyramidal neurons, and HPC cells are multipolar neurons, as viewed in horizontal sections (Han et al. 1998; see also Light and Willcockson 1999). Additional evidence supports this anatomical and physiological correspondence in the primate (Craig et al. 1999; Yu et al. 1999). Our physiological observation that NS cells have very slow conduction velocities also supports these findings because Golgi studies reported that fusiform lamina I cells have unmyelinated axons but that pyramidal and multipolar cells have myelinated axons (Gobel 1978; Lima and Coimbra 1986). Thus the present findings add to considerable evidence indicating that the three major lamina I TSTT physiological cell classes that we recognize are robust and biologically relevant.

It is important to note that these classes do not include all lamina I TSTT cells. The lamina I TSTT projection seems to constitute an interoceptive afferent pathway that provides distinct modality-selective sensory channels representing the physiological condition of all tissues of the body (Craig and Dostrovsky 1999; Craig et al. 2000). Some lamina I TSTT cells are selectively responsive to deep (muscle, joint) input (Craig and Kniffki 1985a), and some are selectively responsive to chemical stimulation with histamine or mustard oil (Andrew and Craig 2001). Lamina I neurons that are selectively viscero-or metabo-receptive or responsive to C-fiber mechanoreceptive input probably also exist, although such cells have not been adequately documented yet (Cervero and Tattersall 1987; Light and Wilcockson 1999; Rosas-Arellano et al. 1999; Urban and Gebhart 1999; Vallbo et al. 1999; Wilson and Hand 1997).

**Thalamic projection targets of the different classes of lamina I TSTT cells**

The ascending axons of lamina I spinothalamic cells are concentrated in the middle of the lateral funiculus in cats and primates (Craig 1991, 2000; Ralston and Ralston 1992; Zhang et al. 2000), where lesions in humans disrupt pain and temperature sensation and where lesions in cats interrupt innocuous thermosensory behavior (Norrsell 1979, 1989). Thermoreceptive-specific lamina I TSTT cells form a unique ascending thermosensory pathway and display graded responses that correlate with reports of innocuous thermal sensitivity (Craig and Bushnell 1994; Davies et al. 1983; Hensel 1981). In primates and humans, the thermosensory lamina I pathway is relayed by the thalamic nucleus VMpo (the posterior part of the ventral medial nucleus) and terminates in the dorsal margin of insular cortex (Craig et al. 1994, 2000; Davis et al. 1999; Dostrovsky and Craig 1996). In cats, recent behavioral evidence indicates that the homologous portion of thalamus is the caudointerolateral aspect of the basal part of the ventral medial nucleus (VMb); only a lesion of this part of the thalamus produces a measurable disruption of a cat’s discriminative thermosensory behavior (Norrsell and Craig 1999). Significantly, ventral VMb also projects to the insular cortex (Clasca et al. 1997; Vahle-Hinz and Oertle 1993; see also Yasui et al. 1987). Our present findings provide direct evidence that thermodensitive-specific lamina I TSTT cells terminate in this same part of the thalamus, and thus the present functional anatomic data provide strong corroboration for the behavioral anatomic findings. Thermoreceptive neurons have not yet been recorded in this part of the thalamus in anesthetized cats, but the present findings support the view that this region is a primordial homologue of the much larger VMpo nucleus in primates and especially humans (Blomqvist et al. 2000), where thermodensitive-specific neurons have been identified (Craig et al. 1994, 1999; Davis et al. 1999).
This homology is supported too by the present evidence indicating that nociceptive NS and HPC lamina I TSTT neurons from the trigeminal and the spinal dorsal horn also terminate in ventral VMb. In the cat, nociceptive-specific units have been identified within ventral VMb, and such units include cells with input from the face and the paws (Vahle-Hinz et al. 1987). Similarly, in primates and humans, VMpo also contains many nociceptive-specific neurons (Blomqvist et al. 2000; Craig et al. 1994; Lenz et al. 1993b) that seem to underlie the activation of insular cortex by noxious stimuli in human imaging studies (see Craig and Dostrovsky 1999; Moulton et al. 2000), and stimulation in this region in awake humans evokes discrete, well-localized sensations of pain or cold (Davis et al. 1996, 1999; Lenz et al. 1993a, 1997). The present findings do not differentiate the projections of NS and HPC lamina I TSTT cells and indicate that both project to the ventral VMb region, but other physiological evidence nonetheless indicates that these two classes of nociceptive neurons have differentiable roles in pain sensation (Andrew and Craig 1999; Craig and Andrew 1999; Craig and Bushnell 1994).

The present data show that virtually all thermoreceptive lamina I TSTT neurons project to dmVPM in the cat, whereas nociceptive lamina I cells do not. Curiously, the prior behavioral anatomic findings did not reveal a role for this region in thermal sensation; a lesion that damaged this region but not VMb had no measurable effect on a cat’s thermostory behavior (Norrsell and Craig 1999). Thermoreceptive-specific neurons have been recorded in dmVPM (Auen et al. 1980; Landgren 1960; present study), although only orofacial RFs have been reported and most of these were ipsilateral rather than contralateral in the anesthetized cat. The role of dmVPM remains at present undetermined.

The present findings corroborate prior anatomical evidence that the ventral aspect of VPL receives direct lamina I TSTT input from the spinal cord (Craig 1987, 1991; Craig and Burton 1985) and indicate that this is a selectively nociceptive input. Both specific and nonspecific nociceptive neurons have repeatedly been recorded within the ventral periphery of VPL (Bruggemann et al. 1994; Gordon and Manson 1967; Honda et al. 1983; Horn et al. 1999; Hutchison et al. 1992; Kniffki and Mizumura 1983; Vahle-Hinz et al. 1987; Yokota et al. 1988). This region seems to project to the anterior cingulate, area 3a, and SII cortices (Craig and Kniffki 1985b; Musil and Olson 1988), which differentiates it from the ventral aspect of VMb. This differs too from the overall thalamocortical organization of the monkey (Craig and Dostrovsky 1999). Unlike the monkey, where nociceptive neurons have been recorded in VP and in cortical areas 1–2 (reviewed by Kenshalo et al. 2000; Treede et al. 1999), nociceptive neurons have rarely been recorded within the core of the ventrobasal complex in the cat (Davis and Dostrovsky 1988; Golovchinsky et al. 1981; Honda et al. 1983; Kniffki and Mizumura 1983; Rydenhag and Roos 1986). Nonetheless, area 3a is activated by noxious stimulation both in the cat (Iwata et al. 1986) and in the primate (Tommerdahl et al. 1998), where the source of this activation may be a collateral input from VMpo (see Craig and Dostrovsky 1999). Clinical evidence also suggests a role for area 3a in pain sensation (Perl 1984).

Nociceptive neurons have been recorded in Po dorsal to VM and VPL, especially along the border between these nuclei (Bruggemann et al. 1994; Davis and Dostrovsky 1988; Horn et al. 1999; Hutchison et al. 1992; Vahle-Hinz et al. 1987). The present findings provide evidence of lamina I TSTT terminations in Po that are generally quite dorsal to this border. However, other nociceptive inputs to the dorsal and ventral borders of VP could originate from deeper TSTT cells in laminae V–VII (Yen et al. 1991). In contrast, the present findings are certainly consistent with the original description of nociceptive-specific units in caudal Po in the cat (Poggio and Mountcastle 1960), where many of the studies cited in the preceding text reported similar neurons. Recent evidence suggests that this region may project to the most posterior portion of insular cortex in the rat (Shi and Davis 1999), yet the relationship of caudal Po to vVMb remains to be determined.

The present findings corroborate prior work indicating a dense projection of nociceptive lamina I TSTT neurons to Sm (Craig 1987, 1991), and they extend our previous observations (Craig and Dostrovsky 1991) by showing that many thermoreceptive-specific lamina I neurons project to Sm from the spinal cord as well as from the trigeminal dorsal horn. This nucleus in cats and rats contains nociceptive-specific units (Craig 1991; Dostrovsky and Guilbaud 1990; Kawakita et al. 1993; Miletic and Cofferfield 1989), and it projects to ventrolateral orbital cortex, where similar recordings have been obtained (Backonja and Miletic 1991; Snow et al. 1992). In contrast, the analogous lamina I TSTT projection to medial thalamus in the monkey (to the ventral caudal aspect of the medial dorsal nucleus) seems instead to provide the source of thermosensory-modulated nociceptive activation of the anterior cingulate cortex (see Craig and Dostrovsky 1999); this constitutes another profound phylogenetic difference between primates and nonprimates. Nonetheless, the role of convergent thermoreceptive-specific input to medial thalamus may be similar across these species in providing a substrate for the inhibition of nociceptive processing by innocuous thermosensory activity (Craig 1991; Ericson et al. 1996). This is consistent with the observation that lesions of Sm did not affect the thermosensory behavior of cats (Norrsell and Craig 1999).

Conclusion

In summary, the present findings demonstrate with antidromic mapping of single, identified units that the thalamic projections of nociceptive (NS and HPC) and thermoreceptive-specific (COOL and WARM) lamina I TSTT cells differ, which is consistent with the many other features that distinguish these cell classes. These observations corroborate anatomical observations of the projection targets of lamina I TSTT neurons in the cat, they fit with behavioral observations on the role of the ventral aspect of VMb in thermal sensation in cats, and they provide further evidence supporting the view that ventral VMb in cats is a primordial homologue of the VMpo nucleus that is well developed only in primates and especially in humans.

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REFERENCES

ANDREW D AND CRAIG AD. Responses of lamina I spinothalamic tract (STT) cells to tonic noxious mechanical stimuli. IASP World Congress Pain, 1999.
THALAMIC PROJECTIONS OF LAMINA I TSST CELLS


