Trigeminohypothalamic and Reticulohypothalamic Tract Neurons in the Upper Cervical Spinal Cord and Caudal Medulla of the Rat

AMY MALICK, ANDREW M. STRASSMAN, AND RAMI BURSTEIN

Department of Anesthesia and Critical Care, Beth Israel Deaconess Medical Center; and Department of Neurobiology and the Program in Neuroscience, Harvard Medical School, Boston, Massachusetts 02115

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INTRODUCTION

Trigeminal sensory information that arises in orofacial organs facilitates exploratory, ingestive, and defensive behaviors that are essential to overall fitness and survival (Dubner et al. 1978; Feindel 1956; Geppetti et al. 1988; Guyton 1971; Lund and Dellow 1971). In the rat, tactile signals that arise in orofacial organs such as the vibrissae, nose, lips, and tongue contribute to the perception of subtle external cues that assist in the execution of feeding-related activities such as chewing, swallowing, licking, and suckling, while painful sensation that originates in these organs can alert the animal to potential dangers and allow for preservation of structures that subserve additional sensory modalities including vision, olfaction, and audition.

Because organs such as the mouth, nose, and eyes serve functions that must be performed continuously for survival, pain that originates in these structures is repeatedly aggravated and is therefore one of the most commonly cited sources of discomfort in human patients. Injuries such as facial lacerations (Bakay and Glasauer 1980) and diseases such as trigeminal neuralgia (Kugelberg and Lindblom 1959), headache (Olesen et al. 1993), sinusitis (Saunte and Soyka 1994), toothache (Sharav 1994), and temporomandibular joint pain syndrome (Sessle and Hu 1991) are believed to activate trigeminal nociceptors and consequently second-order trigeminal brain stem nuclear complex (TBNC) neurons in nucleus caudalis and
microstimulation and single-unit recording techniques. Our
1983). To fill this gap, we sought to identify and physiologi-
and Burstein 1998; Newman et al. 1996; Ring and Ganchrow
Carstens et al. 1990; Iwata et al. 1992; Li et al. 1997; Malick
gans innervated by the trigeminal nerve (Burstein et al. 1990;
thalamus sensory information that originates in orofacial or-
zyk et al. 1997; Zhang et al. 1995) and cervical dermatomes
(Zhang et al. 1999b), upper limbs (Dado et al. 1994a; Kostarc-
limbs (Burstein et al. 1991), abdominal region and bile duct
convey to the hypothalamus sensory signals originating in the
monosynaptic pathways that originate in spinal cord and med-
polysynaptic pathways.

Somatosensory signals reach the hypothalamus.

Complex hypothalamic-mediated functions are commonly
influenced by somatosensory and visceral signals from the
body and cortical signals arising from cortical and sub-
cortical brain regions. The integration of sensory, physiological,
cal, and cognitive signals by hypothalamic neurons that regu-
late both hormonal secretion and the activity of brain stem and
spinal cord neurons that mediate autonomic responses could
provide a partial answer to the question of how sensory signals
produce endocrine, autonomic, and affective responses. To be
in a position to integrate somatosensory and visceral informa-
tion with endocrine and autonomic responses, hypothalamic
neurons must receive somatosensory and visceral inputs. The
afferent inputs that the hypothalamus receives from brain stem
nuclei, such as the parabrachial nuclei (Cechetto et al. 1985;
Saper and Loewy 1980; Slugg and Light 1994), nucleus of the
solitary tract (Menetrety and Basbaum 1987; Ricardo and Koh
1978), periaqueductal gray (Beitz 1982; Eberhart et al. 1985;
Lima and Coimbra 1989; Liu 1983), and caudal ventrolateral
medulla (Lima et al. 1991; Sawchenko and Swanson 1981),
and the identification of neurons in these nuclei that respond to
noxious and innocuous somatosensory and visceral stimulation
(Bernard and Besson 1990; Kannan et al. 1986; Pan et al. 1999;
Person 1989; Zhang et al. 1992) contributed to the notion that
somatosensory signals reach the hypothalamus through several
polysynaptic pathways.

Recent anatomical studies showed that somatosensory and
visceral information can also reach the hypothalamus through
monosynaptic pathways that originate in spinal cord and med-
ullary dorsal horn neurons. The electrophysiological studies
that followed described the course of sacral, lumbar, thoracic,
and lower cervical spinal somatosensory tract (SHT) axons that
convey to the hypothalamus sensory signals originating in the
perineum and colorectal canal (Katter et al. 1996a), lower
limbs (Burstein et al. 1991), abdominal region and bicep
(Zhang et al. 1999b), upper limbs (Dado et al. 1994a; Kostarc-
zyk et al. 1997; Zhang et al. 1995) and cervical dermatomes
(Dado et al. 1994b). Currently, however, no information is
available on axons of TBNC neurons that carry to the hypo-
thalamus sensory information that originates in orofacial or-
gans innervated by the trigeminal nerve (Burstein et al. 1990;
Carstens et al. 1990; Ivata et al. 1992; Li et al. 1997; Malick
and Burstein 1998; Newman et al. 1996; Ring and Ganchrow
1983). To fill this gap, we sought to identify and physiologi-
cally characterize hypothalamic projection neurons in the cau-
dal medulla and upper cervical spinal cord using antidromic
microstimulation and single-unit recording techniques. Our
hypothesis was that the trigeminohypothalamic tract (THT) is
capable of transferring to the hypothalamus nociceptive and
nonnociceptive information that arises in all orofacial organs
innervated by the trigeminal nerve.

During the search for THT neurons, we occasionally en-
countered neurons that projected to the hypothalamus and
responded to noxious stimulation of both orofacial and extrac-
ephalic receptive fields. Later anatomical analysis revealed
that these neurons were located along the poorly defined ven-
tromedial border of lamina V, in an area previously identified
as the lateral reticular formation (Nord and Kyler 1968). The
input that neurons in the lateral reticular formation (LRF) re-
ceive from nociceptive neurons in the spinal cord (Westlund
and Craig 1996) and their well-documented projections to the
hypothalamus (Cunningham and Sawchenko 1991; Loewy
et al. 1981; McKellar and Loewy 1981; Sawchenko and Swanson
1981) make them additional candidates to transmit nociceptive
information to the hypothalamus. Because our aim is to under-
stand as completely as possible how sensory trigeminal informa-
mation reaches the hypothalamus, these reticulohypothalamic
tract (RHT) neurons were also studied.

METHODS

Surgical preparations, neuronal recording, and identification of
hypothalamic projecting neurons

Male Sprague-Dawley rats weighing 400–600 g were anesthetized
with urethan (1.2 g/kg). A metal tube was inserted into the trachea for
artificial ventilation, and the rat was mounted in a stereotactic appa-
ratus. Core temperature was maintained at 37°C by a feedback-
controlled heating pad, and end-tidal CO2 was monitored and kept at
4.0–4.5%. A laminectomy was carried out to expose the first cervical
segment of the spinal cord (C1), and portions of the occipital bone
were removed to allow complete access to nucleus caudalis (VC) in
the caudal medulla. The dura was retracted, the pia removed, and a
pool of warm mineral oil formed over the exposed area. Large
portions of the frontal and parietal bones were removed on both sides
to allow introduction of stimulating electrodes into the hypothalamus,
basal ganglia and midbrain. Rats were then paralyzed with gallamine
triethiodide (1 g/kg) and artificially ventilated.

Using stainless steel (8–12 MΩ) or tungsten (4–6 MΩ) microelec-
trodes (FHC), single units were recorded within the dorsal horn of C1,
the medullary dorsal horn of Vc, and the lateral reticular formation
(LRF). To search for neurons that project to the hypothalamus, one or
two monopolar stimulating electrodes were lowered into the contralat-
eral hypothalamus and cathodal current pulses were delivered (500
µA, 200 µs, 10 Hz). When one stimulating electrode was used, it was
placed in the anterior-lateral hypothalamus. When two stimulating
electrodes were used, the second was placed in the dorsal-medial area
of the posterior hypothalamus. After isolating the spikes of an anti-
dromically activated neuron, the stimulating electrode from which the
unit was antidromically activated was moved systematically through
the hypothalamus (as described in Burstein et al. 1991; Dado et al.
1994a) until a point was found from which a current of ≤50 µA was
capable of inducing consistent antidromic spikes in the neuron. Cri-
teria for antidromic activation included constant latency (total varia-
tion ≤0.2 ms), ability to follow trains of high-frequency stimuli
(>333 impulses/s), and collision of antidromically induced spikes
with those induced orthodromically (Lipski 1981). All neurons de-
scribed in this study were antidromically activated from at least one
point in the hypothalamus by a current of ≤50 µA. Locations from
which neurons were activated antidromically by currents of ≤50 µA
were defined as low-threshold points. Action potentials were ampli-
ified, sent to a window discriminator, collected by computer, analyzed
quantitatively by Neuro-spike software (Pearson Technical Software), and presented as peristimulus time histograms (500-ms binwidth).

Receptive-field mapping

Following the identification of neurons that project to the hypothalamus their cutaneous and intraoral receptive fields were mapped by applying brief innocuous (vibrissae and hair deflection, air puff, and brush) and noxious (pressure, pinprick, and pinch) mechanical stimuli to the nose, vibrissal pad, upper and lower lips, tongue, skin areas above the eye (ophthalmic) and below the eye (maxillary), on the ventral surface of the face (mandibular), and on the entire body. An area was considered outside the neuron’s cutaneous receptive field if no stimulus was capable of producing a response in ≧50% of the trials. Neurons exhibiting restricted orofacial receptive fields were classified as “trigeminal” neurons (i.e., trigeminohypothalamic tract units) and neurons exhibiting both orofacial and extracephalic receptive fields (e.g., abdomen, limbs, tail) were classified as “non-trigeminal” neurons (i.e., reticulohypothalamic tract units). Noncutaneous receptive fields such as the cornea and intracranial dura were mapped by sliding a brush over these organs and by indenting them with calibrated von Frey hairs.

Physiological characterization

Neurons were then physiologically characterized according to their responsiveness to a series of brief (10 s) innocuous and noxious mechanical stimuli applied to the most sensitive portion of their cutaneous receptive field. Innocuous stimuli consisted of slowly passing a soft bristled brush across the cutaneous receptive field and pressure applied with a loose arterial clip. Noxious stimuli consisted of pinch with a strong arterial clip and crush with nonserrated forceps. To avoid inducing prolonged changes in spontaneous neuronal discharge or response properties, more intense or prolonged stimuli were not used. Neurons classified as low threshold (LT) responded maximally or exclusively to innocuous mechanical stimulation. Neurons classified as wide dynamic range (WDR) responded to brush and also to noxious mechanical stimulation in a graded fashion. Neurons designated as high threshold (HT) did not respond to brush but responded to more intense mechanical stimuli (pressure, pinch, and crush) of their cutaneous receptive fields (Dado et al. 1994b; Palecek et al. 1992). To further characterize the neurons, their responses to thermal stimulation were determined following the application of thermally conductive paste to the skin. In most cases, thermal responses were determined by rapidly (10°C/s) heating (to 39, 41, 46, 50, and 55°C) or cooling (in most cases to 20, 10, and 0°C, in several cases to 30, 25, 20, 15, 10, 5, 0, and −10°C) the skin with a 9 × 9 mm contact thermal stimulator (Yale University) for 30 s. The data obtained from the rapid-ramp thermal stimuli were used for the quantitative analyses of the response magnitude. Thermal responses were also determined by slowly heating (35–55°C at a rate of 2.4°C/s) or cooling (35–0°C at a rate of 2.0°C/s) the skin (Burstein et al. 1998) for 10–30 s. The data obtained from the slow-ramp thermal stimuli were used to determine response thresholds (Burstein et al. 1998). The skin surface was maintained at 35°C during the periods between stimuli. This period is defined as the interstimulus interval, which was 180 s. Because we examined only a small number of lamina I neurons and because not all were examined for their responses to heat, cold, and mechanical stimuli, we opted not to use the classification of thermoreceptive-specific (cold), and polymodal nociceptive (HPC) neurons (Craig and Dostrovsky 1991; Craig and Serrano 1994; Dostrovsky and Craig 1996; Han et al. 1998).

Physiologically characterized units were further classified according to whether they process sensory information that arises in the vibrissae, tongue, cornea, intracranial dura, or the entire body. To identify neurons that respond to vibrissal stimulation, we deflected individual vibrissae within the neuron’s receptive field. The vibrissa that induced the largest response was then manually deflected in four orthogonal directions (in 90° increments relative to the horizontal alignment of the whisker row) at 10-s intervals. Each deflection lasted 5 s. To identify neurons that respond to intraoral stimulation, the tongue was gently pulled out and exposed to the same mechanical and thermal stimuli that were applied to the skin. Cornea-sensitive neurons were identified by gently sliding a brush over the corneal surface; activating LT Aβ rapidly adapting mechanosensitive receptors (Giraldez et al. 1979; Maclver and Tanelian 1993a,b). To activate other corneal receptors and nociceptors (i.e., C-fiber cold receptors, Aβ high-threshold mechanosensitive nociceptors, and C-fiber chemosensitive receptors) a small portion of gel foam dipped in 0.1 M of nicotine (temp = 35°C, pH = 7.4) was laid on top of the corneal surface for 30 s (Maclver and Tanelian 1993a,b; Tanelian and Bisla 1992), then rinsed with physiological saline. Dura-sensitive neurons were identified by applying single shocks (0.8 ms, 0.5–4.0 mA, 1 Hz) through a bipolar stimulating electrode placed on the dura overlying the ipsilateral transverse sinus (Burstein et al. 1998). These stimulus parameters were capable of activating both Aβ and C fibers that innervate the dura (Strassman et al. 1996). The sinus area from which the lowest current was capable of activating the neuron was then explored by mechanical stimuli such as dural indentation with calibrated von Frey hairs (Stoelting, flat and round tip shape, diameter range = 0.15–0.38 mm) and gentle rubbing with a brush.

When neurons were found to have both orofacial and extracephalic receptive fields, identical series of mechanical and thermal stimuli (described above) were applied to each area (on the face, limbs, etc.) to determine whether the information they process from different skin regions is qualitatively and/or quantitatively similar. Each series of stimuli was separated by ≧3 min. These neurons were classified separately for their responses to orofacial versus extracephalic stimulation.

Axonal mapping in the midbrain, hypothalamus, and basal ganglia

Once physiological characterization of TH and RHT neurons was completed, we mapped the course of their axons in the midbrain, hypothalamus, and basal ganglia by using the antidromic microstimulation mapping technique (for detailed description, see Burstein et al. 1991; Dado et al. 1994a,c; Fields et al. 1995; Zhang et al. 1995). To determine the course of the axon, the hypothalamic-stimulating electrode had to be repositioned. Before moving this electrode, a second stimulating electrode was inserted into the contralateral midbrain and placed −1 mm lateral to the periaqueductal gray at the level of the superior colliculus. The position of the midbrain stimulating electrode was adjusted until the same neuron was activated using a current of ≧50 μA. To avoid damage to the parent axon, no attempt was made to lower the current by placing the electrode closer to the axon. The midbrain stimulating electrode was used to ensure that the neuron was not lost during the mapping of its axon when we were unable to activate it from other hypothalamic areas, to recognize the changes that occurred in the amplitude of the spike during the search, and to confirm that the spike elicited from the two stimulating electrodes propagated in the same axon. This last task was achieved by demonstrating that stimulation at the midbrain and hypothalamus (or any other more rostral point) induced spikes that were similar in shape, duration and amplitude, and antidromic spikes that collided with each other when the interspike interval was shorter than the time required for the spike to travel between the two stimulating electrodes.

To determine whether the axon of the examined neuron terminated in the contralateral hypothalamus, the hypothalamic stimulating electrode was moved as far rostral as the craniotomy allowed and reinserted into the brain in a systematic way that enabled us to determine thresholds for antidromicity at points separated by 200 μm dorsoventrally and 300–500 μm mediolaterally. If the neuron was not activated from any point within the most anterior level, the stimulating elec-
trode was moved 500–1,000 μm posteriorly, and a similar search was made. At each anteroposterior level, the presence of the axon was indicated by a shift in latency of the antidromic spike to a value longer than that recorded from the contralateral midbrain. If a low-threshold point in the contralateral hypothalamus could be surrounded anteri-orly, medially, laterally, dorsally, and ventrally by points from which higher currents were required to activate the neuron and if the spikes elicited from that point collided with spikes elicited from the mid-brain, the axon was considered to terminate in the contralateral hypo-thalamus.

To determine whether the axon of the examined neuron crossed the midline, the stimulating electrode was moved to the ipsilateral side (1 mm from the midline) and repeatedly inserted along the midline (intervals of 500 μm) from the anterior diencephalon to the midbrain. In cases in which the neuron was antidromically activated from the ipsilateral side, the systematic mapping of the axon continued on both sides of the brain. In cases in which the neuron was not activated from the ipsilateral side, attempts were made to determine whether the parent axon issued collateral branches in the hypothalamus. Detailed description of collateral branches mapping with antidromic stimula-tion technique and their limitations are given in our recent paper (Fields et al. 1995). Briefly; the presence of a branch was indicated by a shift in latency of the antidromic spike to a value longer than that of the parent axon at the same anteroposterior level. The criteria used to confirm that the longer-latency spike was elicited from a branch of the parent axon were that the position of the low-threshold point for the putative branch be in one of the hypothalamic nuclei and at a clear distance from the parent axon in the supraoptic decussation (where most parent axons are found), and that the minimum current sufficient to activate the branch be too low to activate the parent axon by current spread.

Anatomical analysis

At the conclusion of each experiment, the recording site and the low-threshold points for antidromic activation were marked with electrolytic lesions (anodal DC of 25 μA for 20 s). Only one neuron was studied in each animal. In cases in which multiple low-threshold points were found, lesions were made at those points from which a clear shift in latency could be demonstrated. Conduction distances were measured between the recording site and midbrain by placing the midbrain stimulating electrode over the recording site and then cal-culating the differences from the anteroposterior, dorsoventral, and mediolateral stereotaxic coordinates as the shortest distance between the two points. Similar measurements were made between the mid-brain low-threshold point and each of the hypothalamic low-threshold points. Rats were perfused with 1% potassium ferrocyanide in 10% formalin. The brain, brain stem, and upper cervical spinal cord were removed and postfixed for 5 days, during which time they were also reacted for Prussian blue stain of ferric ions. The tissue was cut transversely on a freezing microtome (50 μm) and examined under dark field illumination, which allowed clear identification of laminar borders in C1 and Vc. The tissue was then stained for Nissl substance, and the sections were reexamined under bright field illumination that reacted for Prussian blue stain of ferric ions. The tissue was cut transversely on a freezing microtome (50 μm) from the anterior diencephalon to the midbrain. The database consisted of measurements of the number of spikes/second (response) recorded in C1-THT, Vc-THT, and LRF-RHT neurons. Data organization and analysis were done on the Prophet System (release 4.1), a national computing resource for life science research sponsored by the National Institutes of Health, Division of Research Resources. Response magnitude to each stimulus was calculated by subtracting the mean ongoing activity occurring before the first stimulus (10 s for mechanical, 30 s for thermal and chemical) from the mean firing frequency that occurred throughout the duration of each stimulus. The means of the measurements were plotted against the mechanical (brush < pressure < pinch < crush, expressed as scale data 1, 2, 3, 4), heat, and cold stimuli. The resulting distributions were tested for normality using the D’Agostino test (D’Agostino 1986), and their central measures were computed. Comparison of responses among the respective levels of mechanical, heat, and cold stimuli were performed using appropriate multiple sample comparison procedures [Newman-Keuls if the data were normally distributed (parametric), Kruskal-Wallis if nonparametric].

The means of the measurements were also subjected to trend analysis using the Spearman rank corre-lation (rₜ) for mechanical stimuli and regression analysis for heat and cold. The responses of LRF-RHT neurons to mechanical stimulation of their trigeminal and non-trigeminal receptive fields were subjected to unpaired two-sample comparison tests (unpaired t-test for paramet-ric data, Mann-Whitney rank-sum test for nonparametric data). Neur-onal responses to thermal stimuli were analyzed in two ways: during the dynamic phase and during the static phase. The dynamic phase (heating or cooling ramp) was defined as the time during which skin temperature is increasing or decreasing and the static phase as the time during which the temperature was maintained at constant temperature.

RESULTS

Physiological characterization

IDENTIFICATION OF HYPOTHALAMIC-PROJECTING NEURONS. Eighty-one neurons (success rate of ~1:3) were antidromically activated from the contralateral hypothalamus with currents of ≥50 μA (mean ± SE was 19 ± 11.8 μA). An example of the localization of a low-threshold point for antidromic activation of a THT neuron from the contralateral hypothalamus is shown in Fig. 1. In the first track from which the neuron was antidromically activated (the most medial track in the hypothalamus), the lowest threshold was 260 μA. After antidromic thresholds were determined every 200 μm throughout the track, the electrode was removed and reinserted 300 μm lateral to the first track. The lowest antidromic threshold in the second track was 70 μA. The electrode was again removed and reinserted 300 μm lateral to the second track. The lowest antidromic threshold in the third track was 8 μA. In the next two tracks (made 300 and 600 μm lateral to the 3rd track),
the lowest antidromic thresholds were 32 and 224 μA, respectively. Since the lowest threshold point in the third track was surrounded medially, laterally, dorsally, and ventrally by points from which higher current was required to activate the neuron, it was considered as the lowest threshold point at this anterior-posterior level. This point was located in the supranaoptic decussation (SOD) within the lateral hypothalamus (Fig. 1A). Antidromic action potentials elicited from this and all other low-threshold points in the hypothalamus for this and all other neurons included in the study fulfilled the standard criteria for antidromic activation: they occurred at constant latency, 6.2 ms in this case (Fig. 1B), collided with orthodromic action potentials elicited by stimulating the cutaneous receptive field (Fig. 1B), and followed a train of high-frequency stimulation (Fig. 1B). The recording site of this HT-THT neuron was found in laminae I-II of Vc (C), and the receptive field was mostly within the territory of the maxillary branch of the trigeminal nerve (D).

RECORDING SITES. Thirty-two neurons were recorded in Vc, 22 in C1, 18 in LRF, and the locations of 9 neurons were not identified. Photomicrographs of lesions made in laminae I–II and IV–V, and in the LRF are shown in Fig. 2. Reconstructions of the locations of electrolytic lesions marking the recording sites of 72 neurons are illustrated in Fig. 3. Because many lesions were found at the border between laminae II and III and between laminae IV and V, it was difficult to assign each lesion to a particular lamina with certainty. Based on the center of the lesions that were made in Vc and C1, however, it appears that 12 of the neurons were recorded in laminae I-II (20%), 7 in laminae III-IV (15%), and 35 in lamina V (65%). As explained in the preceding text, these 54 neurons were considered trigeminal because their receptive fields were restricted to skin areas innervated by the trigeminal nerve. The lesions of 18 additional recording sites were found in the medullary LRF. Although there is no easy way to differentiate between lamina V and the LRF, neurons assigned to the LRF were found deeper than the lamina V THT neurons and exhibited extracephalic, in addition to orofacial, receptive fields. They were therefore considered non-trigeminal neurons. The recording locations of physiologically characterized neurons in C1 and Vc were distributed as follows: laminae I-II contained 4 HT, 4 WDR, and 1 LT neurons; laminae III-IV contained 1 HT, 3 WDR, and 2 LT neurons; lamina V contained 11 HT, 12 WDR, and 8 LT neurons; and the LRF contained 7 HT, 7 WDR, and 2 LT neurons (LRF classification was based on responses to facial stimulation). Of the unclassified neurons, three were in laminae I-II, one in laminae III-IV, four in lamina V, and two in the LRF.

RECEPTIVE FIELDS. Twenty of the 22 C1-THT neurons had restricted ipsilateral orofacial receptive fields, and 2 receptive fields were restricted to the ipsilateral neck (Fig. 4A). As shown in the figure, all laminae I and II C1-THT neurons exhibited small to medium receptive fields that extended over facial skin areas innervated by one or two branches of the trigeminal nerve, while many laminae III–V neurons exhibited medium to large receptive fields that extended over facial skin areas innervated by two to three branches of the trigeminal nerve. Similar receptive fields...
were mapped for the 32 Vc-THT neurons (26 of which are shown in Fig. 4B); laminae I–II neurons had primarily small receptive fields, whereas those located in deeper laminae exhibited large receptive fields as well. In general, most HT-THT neurons had small or medium receptive fields, and most WDR-THT neurons had medium or large receptive fields. This tendency, however, was influenced by their location in the different laminae; both HT and WDR neurons had smaller receptive fields if they were recorded in laminae I–II and larger receptive fields if they were recorded in laminae III–V.

Regardless of the neuronal classification, the most sensitive part of the neuronal receptive field correlated somatotopically with the location of the recording site within C1 and Vc. Specifically, neurons exhibiting ophthalmic receptive fields...
neurons. I–V, laminae of the medullary dorsal horn and C1 contained LT and WDR neurons, and lamina V contained all 3 classes of threshold (HT), wide dynamic range (WDR), and low threshold (LT) neurons. Note that lamina I contained HT and WDR neurons, laminae III and IV contained all 3 classes of neurons that receive orofacial input and those that receive orofacial and extracephalic inputs. 

The anatomical segregation between neurons that receive orofacial input and those that receive orofacial and extracephalic receptive fields neurons. Note the anatomical segregation between neurons that receive orofacial input and those that receive orofacial and extracephalic inputs. B: recording sites of functionally identified (i.e., high threshold (HT), wide dynamic range (WDR), and low threshold (LT)) neurons. Note that lamina I contained HT and WDR neurons, laminae III and IV contained LT and WDR neurons, and lamina V contained all 3 classes of neurons. I–V, laminae of the medullary dorsal horn and C1.

correlated with recording sites in the ventrolateral region of the medullary dorsal horn, maxillary receptive fields with recording sites in the mid-dorsal horn, and mandibular receptive fields with recording sites in the dorsomedial region of the medullary dorsal horn.

Seventeen of the 18 neurons that were recorded along the ventral border of the medullary gray matter (LRF-RHT units) had large and complex receptive fields that included not only the ipsilateral orofacial area but also large extracephalic areas such as the limbs, abdomen, back, and tail (Fig. 4C). As shown in the figure, the sensitivity of these complex receptive fields varied from site to site. The most sensitive regions were located primarily within the ipsilateral orofacial field, while less-sensitive areas included the contralateral head and all extracephalic fields.

HIGH THRESHOLD NEURONS. Twenty-four of the 64 (38%) physiologically characterized neurons responded exclusively to noxious mechanical stimuli and were therefore classified as HT. Examples of the responses of two HT-THT neurons are illustrated in Fig. 5. The neuron on the left (Fig. 5A) was recorded in the most dorsomedial portion of lamina V, exhibited a mandibular/maxillary receptive field, and was antidromically activated from the contralateral hypothalamus. It responded to noxious but not innocuous mechanical stimuli (Fig. 5C). The neuron on the right (Fig. 5B) was recorded in the most ventrolateral portion of lamina I, exhibited an ophthalmic receptive field, and was antidromically activated from the lateral hypothalamus. It also responded exclusively to the noxious mechanical stimuli (Fig. 5D). The mechanical response profiles of 23 HT neurons are illustrated in Fig. 5E. Their mean (± SE) firing rates to brush, pressure, pinch and crush were 0.2 ± 0.1, 5.0 ± 1.6, 29.0 ± 5.5, and 36.0 ± 4.8 spikes/s, respectively.

WIDE-DYNAMIC RANGE NEURONS. Twenty-seven of the 64 (42%) physiologically characterized neurons responded to innocuous and noxious stimuli in a graded fashion and were therefore classified as WDR. Examples of the responses of two WDR-THT neurons are illustrated in Fig. 6. The neuron on the left (Fig. 6A) was recorded in the dorsomedial portion of lamina V, exhibited a large mandibular/maxillary/ophthalmic receptive field, and was antidromically activated from the contralateral hypothalamus. It responded most vigorously to innocuous and noxious mechanical stimulation of its mandibular receptive field; stimulation of its ophthalmic receptive field produced smaller responses (Fig. 6C). The neuron on the right (Fig. 6B) was recorded in the most ventrolateral portion of lamina V, exhibited a large ophthalmic/maxillary/mandibular receptive field, and was antidromically activated from the contralateral hypothalamus. It responded most vigorously to innocuous and noxious mechanical stimulation of its ophthalmic receptive field; stimulation of its maxillary and mandibular receptive field produced smaller responses (Fig. 6D). The mechanical response profiles of 27 WDR neurons are illustrated in Fig. 6E. Their mean (± SE) firing rate to brush, pressure, pinch, and crush were 8.3 ± 1.2, 26.6 ± 3.6, 40.2 ± 3.8, and 40.5 ± 2.7 spikes/s, respectively.

LOW THRESHOLD (VIBRISSA-SENSITIVE) NEURONS. Thirteen of the 64 (20%) physiologically characterized neurons responded more vigorously to innocuous than to noxious stimuli and were therefore classified as LT. Most of these LT neurons responded to deflection of a single hair follicle or vibrissa. Examples of the responses of a vibrissa-sensitive LT-THT neuron are illustrated in Fig. 7. This neuron was recorded in the ventrolateral portion of laminae III-IV, exhibited a small receptive field, and was antidromically activated from the contralateral hypothalamus. It responded maximally to the deflection of a single hair follicle or vibrissa in all four directions (Fig. 7B) and to brushing its receptive field (not shown). The mechanical response profiles of 13 LT neurons are illustrated in Fig. 7C. Their mean (± SE) firing rates to
FIG. 4. Orofacial receptive fields of 22 THT neurons in the 1st cervical segment of the spinal cord (A), 26 THT neurons in the trigeminal nucleus caudalis (B), and complex orofacial-extracephalic receptive fields of 16 representative RHT neurons in the lateral reticular formation (C). Black areas indicate most sensitive regions of the receptive fields (defined as the site from which highest response magnitudes were elicited by mechanical stimuli), and gray areas indicate less sensitive regions. Response categories and laminar locations of recording sites are indicated. Note that most LRF-RHT neurons responded to both innocuous and noxious stimulation of orofacial skin areas, but only to noxious stimulation of extracephalic regions.
brush, pressure, pinch, and crush were 34.0 ± 3.0, 21.3 ± 5.5, 22.0 ± 4.7, and 24.5 ± 5.0 spikes/s, respectively.

RESPONSES TO THERMAL STIMULI. Heat. Innocuous and noxious heat stimuli were applied to the receptive fields of 29 neurons. Twenty-seven of these (93%) responded incrementally to graded increases in heat stimuli. Of the 27 heat-sensitive neurons, 5, 3, and 9 were recorded in laminae I-II, III-IV, and V of C1-Vc, respectively, and 10 were recorded in the LRF. Figure 8 illustrates two different response types to heat stimuli: at left; a “static” response, defined as maximal discharge during the steady-state phase of the stimulus, and at right; a “dynamic” response, defined as maximal discharge during the heating ramp.

Eight HT neurons were tested for heat responsiveness; two (25%) did not respond to any heat stimulus, and six (75%) responded only to the noxious heat (Fig. 8E). Their mean heat threshold was 43.6°C and their mean firing rates to 39, 41, 46, 50, and 55°C were 1.4 ± 1.0, 2.0 ± 1.0, 11.0 ± 2.0, 23.0 ± 4.0 and 27.0 ± 6.0 spikes/s, respectively. Seven LT neurons were tested for heat responsiveness, and all but one responded (Fig. 8E). Their mean heat threshold was 40.2°C, and their mean firing rates during the steady-state phase of the heat stimuli (i.e., 39, 41, 46, 50, and 55°C) were 0.8 ± 0.8, 2.5 ± 1.0, 2.8 ± 1.5, 14.5 ± 3.8, and 23.8 ± 3.7 spikes/s, respectively. As shown in Fig. 8E, the static responses of HT, WDR, and LT neurons increased incrementally when the intensities of the noxious heat stimuli increased. Analysis of variances revealed that the static response profiles of HT, WDR, and LT neurons differed only for the 46°C stimulus (P < 0.05, Newman-Keuls multiple range test); only WDR neurons responded to the static phase of this stimulus. The apparent similarity between the static response profiles of HT and LT neurons is somewhat misleading, how-
ever, because none of the HT neurons exhibited a dynamic response. In contrast, 4/6 LT neurons exhibited dynamic responses. Their dynamic responses were characterized by an increase in response magnitude from 39 to 46°C and a sharp decrease from 46 to 55°C (Fig. 8E). During the heating-ramp phase (to 39, 41, 46, 50, and 55°C), the mean firing rates were 10.5 ± 5.6, 20.0 ± 8.0, 59.0 ± 22.4, 47.3 ± 16.3, and 27.1 ± 12.4 spikes/s, respectively.

Cold. Innocuous and noxious cold stimuli were applied to the receptive fields of 25 neurons. Fourteen of these (56%) responded to the cold stimuli. Of the 14 cold-sensitive neurons, 4, 3, and 6 neurons were recorded in laminae I-II, III-IV, and V of C1-Vc, respectively, and 1 neuron was recorded in the LRF. Figure 9 illustrates three different response types to cold: at left, a static response; middle, a dynamic response; and right, a mixed response, defined as a maximal peak during the heating ramp and a slow adaptation during the steady state.

Six HT neurons were tested for cold responsiveness, but only one responded (Fig. 9G). Their mean firing rates to 20, 10, and 0°C were 0.1 ± 0.1, 1.5 ± 0.1, and 2.7 ± 2.0 spikes/s, respectively. Thirteen WDR neurons were tested for cold responsiveness; 54% of them responded incrementally to the increased intensities of the cold stimuli, and 46% did not respond to any cold stimulus (Fig. 9G). The mean firing rates of the responsive neurons to 20, 10, and 0°C were 7.0 ± 3.3, 12.0 ± 5.4, and 21.0 ± 8.3 spikes/s, respectively. Seven LT neurons were tested for cold responsiveness; all responded during either the steady-state or the cooling ramp of the cold stimuli (Fig. 8G). During the steady-state phase, their re-
The response properties of a vibrissa-sensitive LT-THT unit. A: locations of low-threshold points for antidromic activation in the hypothalamus and thalamus and the recording site in lamina III. B: peristimulus time histograms and records of window discriminator output illustrating responses to a 4-way deflection of a single vibrissa. C: stimulus response curves illustrating the response profiles of all LT neurons to mechanical stimulation of their cutaneous receptive fields. Numbers in parentheses depict mean response (spikes/s) to each stimulus. Thick line denotes means of all LT neurons. Note that this THT neuron also projects to the ventroposterior medial thalamic nucleus.

Responses to 20, 10, 0, and −10°C were 4.9 ± 1.4, 9.0 ± 2.2, 13.0 ± 4.1, and 30.8 ± 5.4 spikes/s (Fig. 9G). During the cooling ramp phase (to 20, 10, 0, and −10°C), the mean firing rates were 34.6 ± 11.9, 31.0 ± 11.5, 19.3 ± 7.8, and 10.8 ± 4.2 spikes/s (Fig. 9G). The static responses were characterized by a graded increase in response magnitude as the intensities of the cold stimuli increased. The dynamic responses (calculated by subtracting the mean discharges during the steady state of the cold stimuli from the discharges during the cooling ramp) were characterized by a linear decrease in response magnitude from 20 to −10°C. Analysis of variances revealed no significant differences between the static responses of the three groups (HT, WDR, and LT); a finding we attributed to the small sample size.
FIG. 8. Static and dynamic response patterns of 2 lamina I THT neurons to innocuous and noxious heat stimuli. A and B: locations of low-threshold points for antidromic activation, recording sites, and receptive fields. C and D: peristimulus time histograms illustrating responses to heat stimuli. Stimulus temperature and temperature tracing are shown above histograms. Histograms showing responses to 50 and 55°C in D are truncated to maintain identical y axis. Note that the neuron on the left responded maximally during the steady-state phase of the stimulus (i.e., the static phase) and the neuron on the right during the heating ramp (i.e., the dynamic phase). E: stimulus-response curves illustrating mean responses of individual HT, WDR, and LT neurons during the static phase of the stimulus. Mean responses during the dynamic phase of the heat stimulus are shown only for LT neurons because HT neurons did not respond during this phase and WDR neurons responded rarely. Thick lines show the overall mean responses of all HT, WDR, and LT neurons. Note that during the dynamic phase of the heat stimuli, the discharge rate of LT-THT neurons increased in a near-linear fashion from 39 to 46°C followed by a decrease; suggesting inputs from warm receptors (see text).
FIG. 9. Static, dynamic, and mixed responses of 1 WDR and 2 LT THT neurons to innocuous and noxious cold stimuli. A–C: peristimulus time histograms illustrating responses to mechanical stimuli. D–F: peristimulus time histograms illustrating their responses to cold stimuli. Stimulus temperature and temperature tracing are shown above histograms. Note that the neuron on the left responded maximally during the steady-state phase of the stimulus (i.e., the static phase), the neuron in the middle during the heating ramp (i.e., the dynamic phase), and the neuron on the right exhibited a maximal peak during heating ramp and a slow adaptation during the steady-state (i.e., a mixed response). G: stimulus-response curves illustrating mean responses of individual HT, WDR, and LT neurons during the static phase of the stimulus. Mean responses during the dynamic phase of the cold stimulus are shown only for LT neurons because HT neurons did not respond during this phase and WDR neurons responded rarely. Thick lines show the overall mean responses of all HT, WDR, and LT neurons. Note that during the cooling phase the discharge rate of LT-THT neurons decreased in a near-linear fashion from 20 to −10°C, suggesting inputs from cold receptors (see text).
ORAL-SENSITIVE NEURONS. Twenty-one neurons responded to mechanical stimulation of the oral mucosa, tongue, or lips. They were classified as HT in 9 cases, WDR in 9 cases, and LT in 3 cases. The majority of oral-sensitive THT neurons were recorded in the dorsomedial third of laminae V (11 units) and III-IV (2 units) of C1 (6 units), and Vc (7 units). The other seven oral-sensitive neurons were recorded in the LRF, and the recording site of one neuron was not found. The cutaneous receptive fields of the THT neurons varied; they extended over small mandibular or maxillary areas in three (23%) cases, mandibular and maxillary areas in four (31%) cases, and the entire face in six (46%) cases. Regardless of the cutaneous receptive field size, maximal neuronal responses were most commonly induced by stimulating intraoral structures. An example of an oral-sensitive HT-THT neuron is illustrated in Fig. 10 (left). This hypothalamic projecting neuron was recorded in the most dorsomedial portion of lamina V and exhibited an oral receptive field that included the tongue, hard palate, and the upper lip (A). It responded more vigorously to noxious mechanical stimuli of the hard palate than the tongue (B). The mechanical response profiles of 19 oral-sensitive neurons are illustrated in C. Their mean (± SE) firing rates to brush, pressure, pinch, and crush were 4.7 ± 1.6, 12.2 ± 3.0, 30.6 ± 4.6, and 37.2 ± 4.2 spikes/s, respectively. The heat response profiles of eight oral-sensitive neurons are illustrated in D. Their mean (± SE) firing rates to 39, 41, 46, 50, and 55°C were 0.1 ± 0.1, 0.3 ± 0.3, 4.1 ± 1.5, 17.9 ± 6.3, and 27.6 ± 8.6 spikes/s, respectively. About 15% of the oral-sensitive THT neurons also responded to innocuous cold stimuli (data not shown).

CORNEA-SENSITIVE NEURONS. Thirteen neurons responded to mechanical stimulation of the cornea. They were classified as HT in four cases, WDR in seven cases, and LT in two cases. All THT neurons were recorded in lamina V at the level of caudal Vc (5 units) and rostral C1 (4 units). Of the remaining four neurons, three were recorded in the LRF, and the recording location of one unit was not identified. Although no attempt was made to map the corneal receptive fields of these neurons, they seemed to respond to stimulation of all four corneal quadrants. In all but one case, the cutaneous receptive field extended over the periorbital skin; they included the ophthalmic and maxillary skin in all cases, and the mandibular skin in less than half of the cases. An example of a cornea-sensitive THT neuron is illustrated in Fig. 10 (middle). This hypothalamic projecting neuron was recorded in the most lateral region of lamina V, and its receptive field included the cornea and the periorbital skin (A). It responded preferentially to noxious mechanical stimuli of the ophthalmic skin and most vigorously to mechanical and chemical (nicotine) stimulation of the cornea (B). The mechanical response profiles of 10 cornea-sensitive neurons are illustrated in C. Their mean firing rates to brush, pressure, pinch, and crush were 10.8 ± 5.8, 31.0 ± 10.0, 41.7 ± 7.2, and 39.6 ± 6.5 spikes/s, respectively. The heat response profiles of nine cornea-sensitive neurons are illustrated in D. Their mean firing rates to 39, 41, 46, 50, and 55°C were 0.1 ± 0.1, 0.8 ± 0.5, 3.4 ± 1.5, 7.8 ± 1.6, and 15.1 ± 3.5 spikes/s, respectively. In five cases, 1 M nicotine was applied to the cornea for 30 s. The neuronal responses in all cases were vigorous.

DURA-SENSITIVE NEURONS. Ten THT neurons responded to electrical and mechanical stimulation of the dura mater over-lying the transverse sinus. The majority (8 units) were recorded in the most lateral region of lamina V at the level of Vc, and only two neurons were recorded in laminae I–III or in C1. The dural receptive fields of these neurons were usually small and restricted to the transverse or superior sagittal sinuses. All dura-sensitive neurons also exhibited cutaneous receptive fields (which varied in size). Regardless of the cutaneous receptive field size, maximal neuronal responses were most commonly elicited from the periorbital skin region. Eight of the 10 dura-sensitive neurons were physiologically characterized based on their responses to cutaneous stimulation: all were classified as WDR. An example of a dura-sensitive WDR-THT neuron is illustrated in Fig. 10 (right). This hypothalamic projecting neuron (A1) was recorded in the most lateral region of lamina V (A2). It exhibited ophthalmic receptive fields on the skin (A3) and the dura (A4). It responded preferentially to noxious mechanical stimulation of the ophthalmic skin and to dural brushing (B). The mechanical response profiles of eight dura-sensitive neurons are illustrated in D. Their mean firing rates to 39, 41, 46, 50, and 55°C were 4.0 ± 3.0, 5.8 ± 2.5, 16.0 ± 4.1, 33.8 ± 7.3, and 33.0 ± 10 spikes/s, respectively.

LRF-RHT NEURONS. Eighteen hypothalamic projecting neurons were recorded in the LRF. Their recording sites were in general more medial and slightly more ventral than the recording sites of the THT neurons in the ventrolateral area of lamina V (Fig. 3). Their most distinct property was their cutaneous receptive fields. They included orofacial and extracephalic skin areas in all cases (Fig. 4). In 11 cases, receptive fields extended over the entire body. An example of a HT LRF-RHT neuron is illustrated in Fig. 11. This hypothalamic projecting neuron exhibited a whole body receptive field. Like most LRF-RHT neurons in this study, it responded more vigorously to mechanical stimulation of ipsilateral orofacial organs (i.e., tongue, cornea, skin) compared with mechanical stimulation of contralateral orofacial organs or any of the extracephalic regions (i.e., paws and tail). Figure 12 illustrates the responses of all 18 LRF neurons to mechanical stimulation of their facial (i.e., trigeminal, Fig. 12A) and extracephalic (i.e., non-trigeminal, Fig. 12B) receptive fields. The means (±95% confidence interval) of the responses to brush, pressure, pinch, and crush are shown in Fig. 12C. Responses induced by trigeminal receptive fields stimuli were significantly greater than the responses to the respective stimuli of the non-trigeminal receptive fields (P values given in the right column of the table). These findings indicate that LRF neurons responded to brush and pressure of their trigeminal but not non-trigeminal receptive fields and that pinch and crush induced larger responses when applied to their trigeminal than to non-trigeminal receptive field.

COMPARISONS OF THE RESPONSE PROFILES OF C1-THT, VC-THT, AND LRF-RHT NEURONS. The response profiles of C1-THT, Vc-THT, and LRF-RHT were compared. These data are presented in Fig. 13, where the response profiles of all HT, WDR, and LT neurons recorded in each of the indicated areas were grouped. Within all three areas, responses to pressure were significantly larger than responses to brush, and responses to pinch were significantly larger than responses to pressure.
FIG. 10. Response properties of oral-sensitive (left), cornea-sensitive (middle), and dura-sensitive (right) THT neurons. A’s: locations of low-threshold points for antidromic activation, recording sites, and receptive fields. B’s: peristimulus time histograms illustrating responses to mechanical stimulation of the tongue and hard palate, mechanical and chemical stimulation of the cornea and periorbital skin, and mechanical stimulation of the dura and periorbital skin. Numbers in parentheses depict mean response (spikes/s) to each stimulus. C’s and D’s: stimulus response curves illustrating the response profiles of all oral-, cornea-, and dura-sensitive neurons to mechanical and thermal stimulation of the lips, periorbital skin, and skin innervated by the ophthalmic branch of the trigeminal nerve, respectively. Thick lines denote means.
FIG. 11. Response properties of an LRF-RHT neuron classified as HT. Because this neuron exhibited a whole-body receptive field, innocuous and noxious mechanical stimulation were applied to all indicated skin areas on both sides of the body. The peristimulus time histograms show the responses to brush, pressure, pinch, and crush. Horizontal lines depict stimulus duration and numbers in parentheses indicate means of responses. Recording site is shown in the circle within the rat drawing. Note that the neuron responded only to noxious stimuli and that the responses to stimulation of ipsilateral orofacial organs and skin areas were more vigorous than the responses to stimulation of extracephalic skin areas or the contralateral head.
Among the three groups, the magnitude of the responses to brush (e.g., C1-THT brush vs. Vc-THT brush vs. LRF-RHT brush), pressure, pinch, and crush were not different (Fig. 13A). The mean ± 95% confidence interval of the responses are shown in the tables on the right. The trends of increased response magnitudes with increased stimulus intensity were also significant for all three groups (P values shown in the row marked “correlation”).

Within all three groups, responses to noxious heat stimuli (i.e., 50 and 55°C) were significantly larger than responses to innocuous heat (i.e., 39 and 41°C), and responses to 55°C were significantly larger than the responses to 46°C (Fig. 13B). Among the three groups, the magnitudes of the responses to any of the heat stimuli were not significantly different. The trends of increased response magnitudes with increased heat stimulus intensity were also significant for all three groups.

Within all three groups, responses to noxious cold stimuli were larger than responses to innocuous cold stimuli, and the response magnitudes of C1-THT and Vc-THT to the noxious cold were larger than the response magnitudes of LRF-RHT neurons (Fig. 13C). Because of the small sample sizes, most of these values did not reach statistical significance. Nevertheless, the trends of increased response magnitudes with increased cold stimulus intensity were significant for all three groups.

Axonal mapping

ANTIDROMIC MAPPING IN THE CONTRALATERAL MIDBRAIN AND Diencephalon. Anatomical (Burstein et al. 1987; Cliffer et al. 1991) and physiological (Burstein et al. 1991; Dado et al. 1994a; Katter et al. 1996a) studies indicated that most spino-hypothalamic tract axons reach the hypothalamus through the posterior thalamus and the supraoptic decussation. To determine whether THT and RHT neurons also share this anatomical approach, we attempted to map their axonal routes between the midbrain and the hypothalamus. In 72 cases (54 THT and 18 RHT), neurons that were antidromically activated from the contralateral hypothalamus were also antidromically activated with currents of ≈50 μA from the contralateral midbrain and posterior diencephalon. Figure 14 illustrates an experiment in which a neuron was antidromically activated from low-threshold points in the contralateral midbrain, contralateral caudal diencephalon, and contralateral hypothalamus. The neuron was initially activated antidromically from a lowest threshold point in the contralateral lateral hypothalamus (point a). The lesion marking the location of this point was found just medial to the optic tract, within the supraoptic decussation. The antidromic latency from this point was 2.7 ms (Fig. 14C), and the minimum current required to activate the neuron was 18 μA. When the stimulating electrode was moved in the medial, lateral, dorsal, or ventral directions, higher currents were required to activate the neuron antidromically. Prior to the removal of the first stimulating electrode from the lowest threshold point in the hypothalamus, a second stimulating electrode was used to search for a low-threshold point for antidromic activation of that same neuron from the midbrain (point d). This point was found between the periaqueductal gray and the superior colliculus (Fig. 14B). The antidromic latency from this point was 1.4 ms (Fig. 14C), and the minimum current required to activate the neuron was 11 μA. The first stimulating electrode was then moved 1.5 mm posteriorly, and 11 electrode penetrations were made across the mediolateral extent of the contralateral posterior hypothalamus and thalamus. At this level, the low-threshold point was also located between the internal capsule and the optic tract (point b). The antidromic latency from this point was 2.0 ms, and the minimum current required to activate the neuron was 14 μA. At the level of the

FIG. 12. Comparisons between the response magnitudes of LRF-RHT neurons to mechanical stimulation of their trigeminal and non-trigeminal receptive fields. A and B: plots of stimulus-response curves illustrating the response profiles of LRF-RHT neurons to mechanical stimulation of their trigeminal and non-trigeminal receptive fields. C: mean ± 95% confidence interval of the responses to brush, pressure, pinch, and crush stimuli applied to trigeminal and non-trigeminal receptive fields. The P values show that LRF neurons respond to brush and pressure of their trigeminal but not non-trigeminal receptive fields and that pinch and crush induce larger responses when applied to trigeminal compared with non-trigeminal receptive fields. Trend analysis (Spearman rank correlation) showed a significant correlation between the stimulus intensity and the response magnitude.

Among the three groups, the magnitude of the responses to brush (e.g., C1-THT brush vs. Vc-THT brush vs. LRF-RHT brush), pressure, pinch, and crush were not different (Fig. 13A). The mean ± 95% confidence interval of the responses are shown in the tables on the right. The trends of increased response magnitudes with increased stimulus intensity were also significant for all three groups (P values shown in the row marked “correlation”).

Within all three groups, responses to noxious heat stimuli (i.e., 50 and 55°C) were significantly larger than responses to innocuous heat (i.e., 39 and 41°C), and responses to 55°C were significantly larger than the responses to 46°C (Fig. 13B). Among the three groups, the magnitudes of the responses to any of the heat stimuli were not significantly different. The trends of increased response magnitudes with increased heat stimulus intensity were also significant for all three groups.

Within all three groups, responses to noxious cold stimuli were larger than responses to innocuous cold stimuli, and the response magnitudes of C1-THT and Vc-THT to the noxious cold were larger than the response magnitudes of LRF-RHT neurons (Fig. 13C). Because of the small sample sizes, most of these values did not reach statistical significance. Nevertheless, the trends of increased response magnitudes with increased cold stimulus intensity were significant for all three groups.
posterior commissure (3.5 mm posterior to point a), eight electrode penetrations were made contralaterally. At this level, the lowest threshold point for antidromic activation was located in the substantia nigra pars compacta (point c; 1.6 ms, 9 μA). That the same neuron was antidromically activated from each of the lowest threshold points (a–c), was confirmed by colliding the antidromic spikes evoked at each of these points with the antidromic spikes evoked from the second stimulating electrode in the midbrain (Fig. 14C, d-a, d-b, and d-c). Based on the recorded latencies from each point and their distances from the recording site, we estimated that the conduction velocity of this axon was 6.2 m/s to point a, 7.7 ms to point b, and 8.3 ms to point c.

In 21 cases, adequate attempts were made to determine whether the axons of THT and RHT neurons that entered the contralateral hypothalamus terminated there or continued anteriorly to the basal ganglia or medially to the ipsilateral side. An example of such an experiment is shown in Fig. 14. In this case, the lowest threshold point for antidromic activation (point a in the contralateral SOD) was surrounded dorsally, ventrally,
lateral, medially, and anteriorly by 248 points from which higher currents were required to activate the neuron. Forty-three percent of the neurons tested (6 THT and 3 RHT) could not be activated by low currents from more anterior or more medial points, suggesting that their axons terminated in the contralateral hypothalamus.

ANTIDROMIC MAPPING IN THE CONTRALATERAL AND IPSILATERAL HYPOTHALAMUS. Fourteen THT and 7 RHT neurons that were initially activated antidromically from the contralateral hypothalamus were also tested for their projections to the ipsilateral hypothalamus. Eight THT (57%) and 4 RHT (57%) neurons were antidromically activated with currents of ≤30 μA from low-threshold points in the ipsilateral hypothalamus (it was not within the scope of this study to determine how far caudal these axons descend). Figure 15 illustrates an example of a case in which the neuron was antidromically activated from low-threshold points in the contralateral midbrain, contralateral hypothalamus, and ipsilateral hypothalamus. The neuron was initially activated from a single low-threshold point located 1.3 mm caudal to Bregma, within the contralateral hypothalamus (point f). All antidromic spikes induced from the contralateral hypothalamus at this level reached the neuron within 3.3 ms. Following the identification of a low-threshold point in the midbrain (point a), the stimulating electrode was moved 1 mm anteriorly (0.3 mm caudal to Bregma), and nine individual electrode penetrations were made across the mediolateral extent of the preoptic area, bilaterally. The neuron could not be activated antidromically from any one of the 180 tested points at this level, suggesting that the axon does not project more anteriorly. The electrode was therefore moved back to the anterior hypothalamus (1.3 mm caudal to Bregma), and another set of electrode penetrations was made in the ipsilateral hypothalamus. A low-threshold point for antidromic activation was located in the SOD (point g), suggesting that the axon crossed the midline within the optic chiasm. All antidromic spikes induced from the ipsilateral hypothalamus at this level needed the same time to reach the neuron (4.2 ms). The stimulating electrode was repositioned, and 12 individual electrode penetrations were made at the level of the ventromedial hypothalamic nucleus (2.3 mm caudal to Bregma). At this plane (on the ipsilateral side), two low-threshold points were found. One was located in the optic tract and the other in the lateral hypothalamus, just ventral to zona incerta (ZI). The antidromic spikes induced from these low-threshold points differed in their latencies; the spike induced from the optic tract (point h) reached the neuron in 4.6 ms, and the spike induced from the lateral hypothalamus needed 6.0 ms (point i). Because these low-threshold points were separated by points from which higher currents were required to induce antidromic spikes of similar latencies and because the long latencies could not be induced from point h nor the short latencies from point i, we concluded that the parent axon in the ipsilateral optic tract issued a collateral branch in the lateral hypothalamus. When the stimulating electrode was moved back to the contralateral side, additional low-threshold points were found in the contralateral SOD (point d) and contralateral lateral hypothalamus (point e). Since the antidromic spikes induced from these low-threshold points differed (2.8 ms from point d, and 4.4 ms from point e), we again concluded that the parent axon in the contralateral SOD issued a collateral branch in the lateral hypothalamus. By tracking the axon backward on the contralateral side, it was also possible to activate this neuron antidromically from low-threshold points in the subthalamic nucleus (point c) and the central tegmental field (point b, deep mesencephalic nucleus (DPME)) of the midbrain.

The latencies for antidromic activation of this neuron were longer in the contralateral hypothalamus (2.8–3.3 ms) than in the contralateral midbrain (2.0–2.2 ms) and longer in the ipsilateral hypothalamus (4.2–4.6 ms) than in the contralateral hypothalamus. Assuming that the axonal path in the midbrain and diencephalon is relatively straight, the distance between the most caudal and most rostral points in the midbrain (points a and c) was 3.6 mm. The distance between caudal and rostral points in the contralateral hypothalamus (points c and f) was 3.3 mm, and the distance between the rostral contralateral hypothalamus (point f) and caudal ipsilateral hypothalamus (point h) was 4.3 mm. The estimated conduction velocities of this neuron were ~18 m/s in the contralateral midbrain and ~3.0 m/s in the contralateral hypothalamus, reflecting a sixfold decrease in conduction velocity between the midbrain and the hypothalamus. A further decrease in conduction velocity occurred between the parent axon in the SOD and the collateral branches in the lateral hypothalamus. Based on the latency shifts and the calculated distances between the parent axon and the most distal point from which the branches could be activated, we estimated that the conduction velocity of the collateral branches was 0.3–0.4 m/s. These calculations indicate that the parent axon of this neuron crossed the midline in the brain stem, ascended to the hypothalamus on the contralateral side, crossed the midline again in the SOD, and descended in the ipsilateral hypothalamus.

The experiment illustrated in Fig. 15 also shows that both the ascending and descending sections of the parent axon issue collateral branches in the hypothalamus. In 10 other cases, parent axons of THT and RHT neurons seemed to issue branch in the hypothalamus. These branches were found in

FIG. 14. Example of a neuron that projected only to the contralateral hypothalamus and the method for antidromic mapping of its axon in the midbrain and posterior diencephalon. A: dorsal view of the horizontal plane of the contralateral diencephalon (the position of this rectangle in relation to the rat’s brain is shown in D). The positions of 33 individual electrode penetrations made at 5 anterior-posterior levels are shown inside the rectangle. The lowest threshold points for antidromic activation are indicated by the letters a–d. The smallest antidromic current required to activate the neuron from each penetration is represented by a symbol (inset). Distances from midline are indicated at the top and distances from Bregma on the left. B: line drawings illustrating the locations of the electrode penetrations made at the 5 anterior-posterior levels (transverse view) and the locations of the lowest threshold points at each level. C: digitized oscillographic traces of antidromic spikes elicited from points a–d demonstrate the axonal mapping technique (left). Note that 1 mm rostral to point a, the axon could not be activated by a current of 500 μA delivered in 128 locations. cp, cerebral peduncle; DPME, deep mesencephalic nucleus; ml, medial lemniscus; MTT, mammillothalamic tract; OC, optic chiasm; PC, posterior commissure; PO, preoptic area or posterior thalamus; SNC, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SON, supraoptic nucleus; Stn, subthalamic nucleus; VBC, ventrobasal complex; 3V, 3rd ventricle.
the lateral hypothalamus, perifornical region, dorsomedial hypothalamus, suprachiasmatic nucleus, and supraoptic nucleus.

**ANTIDROMIC MAPPING IN THE BASAL GANGLIA.** Previous anatomical studies (Burstein and Giesler 1989; Burstein and Potebtic 1993; Burstein et al. 1987; Cliffer et al. 1991; Malick and Burstein 1998; Yasui et al. 1987) indicated that some spinal cord neurons, including those located in C1 and Ve, project to forebrain areas positioned anterior to the hypothalamus (i.e., nucleus accumbens, globus pallidus, substantia innominata, septal nuclei, bed nucleus stria terminalis). Although systematic attempts were not made to explore these anterior areas thoroughly, we occasionally (3 cases) activated neurons in C1 and LRF from forebrain nuclei such as the caudate-putamen, globus pallidus, ventral pallidum, and the substantia innominata. Figure 16 illustrates antidromic activation of a C1-THT neuron from several basal ganglia regions. This lamina V-WDR neuron was initially activated antidromically from the supraoptic nucleus in the hypothalamus (point g). In addition, it was also possible to activate this neuron antidromically from the contralateral basa ganglia and midbrain (Fig. 16, A and B). At the level of the posterior commissure, only one low-threshold point was found (point k, 3.0 ms). This point was just dorsal to the medial lemniscus, within the deep mesencephalic/cenral tegmental field. At the two levels of the diencephalon (1.7 and 0.7 mm caudal to Bregma), several low-threshold points were found (Fig. 16B). At the level of mid-hypothalamus (1.7 mm caudal to Bregma), one low-threshold point was located in the supraoptic decussation (point j, 4.1 ms), and the other two were located in the internal capsule (point h, 3.8 ms) and globus pallidus (point i, 8.0 ms). At the level of the rostral hypothalamus (0.7 mm caudal to Bregma), one low-threshold point was located in the supraoptic nucleus (point g, 4.2 ms), and the others in the caudate-putamen (point c, 4.4 ms), substantia innominata (point d, 5.2 ms), and globus pallidus (point e, 6.6 ms; point f, 7.0 ms). At the level of the anterior commissure (0.3 mm anterior to Bregma), only one low-threshold point was found (point b, 4.6 ms). This point was located in the most ventral and lateral area of the caudate-putamen. The most anterior low-threshold point from which the neuron was antidromically activated was found at the level of nucleus accumbens (1.3 mm anterior to Bregma). This point (a) was located just lateral to nucleus accumbens, within the caudate-putamen. Based on the latencies induced from low-threshold, points a, b, c, g, h, j, and k, and their distances from the recording site, it seems that the parent axon may have bifurcated in two directions between the midbrain and the hypothalamus. One branch seems to approach the supraoptic nucleus through the supraoptic decussation (points k, j, and g), while the other seems to approach the ventrolateral caudate-putamen region through the internal capsule (points h, c, b, and a). In addition, since the latencies induced from points d, e, f, and i were longer than the latencies induced from more anterior levels, it is possible that small collateral branches were activated at these points. The two smaller branches (h to i and c to d, e and f) may have emanated from the branch that headed to the caudate-putamen.

**ANTIDROMIC ACTIVATION SITES OF THT AND LRF-RHT NEURONS.** Figure 17 illustrates the locations of 197 low-threshold points for antidromic activation of 54 THT (A) and 18 LRF (B) neurons. Regardless of the location of the recording site (A and B) or the physiological classification (C) of the neurons, all axons seemed to follow the same path. At the level of the red nucleus, in the rostral midbrain, 32 low-threshold points for antidromic activation of the ascending axons of 15 THT and 12 RHT neurons were found in a poorly defined area between the central gray, medial lemniscus, substantia nigra, and the superior colliculus. Although uncommon, individual axons could also be found in the ventral region of the superior colliculus (SC), the substantia nigra pars compacta (SNC), and the red nucleus. At the level of the posterior commissure, most axons (of both populations) had the tendency to shift ventrally, toward the cerebral peduncle. In the 13 experiments (i.e., individual neurons) in which the location of the axon was determined at this level, five low-threshold points were found in the posterior thalamus, three in the medial lemniscus, seven in the sub-thalamus, and three in the cerebral peduncle. Occasionally low-threshold points were found in the substantia nigra and the anterior pretectal nucleus (APT). Rostral to the posterior commissure, within the caudal diencephalon, 73 low-threshold points for antidromic activation of the ascending axons of 36 trigeminal and 18 non-trigeminal neurons were found in the internal capsule (6), on the border between the internal capsule and optic tract (7), supraoptic decussation (41), lateral hypothalamus (13), perifornical area (2), dorsomedial hypothalamus (1), zona incerta (2), and ventroposterior medial thalamic nucleus (1). In the rostral diencephalon, the ascending axons of 15 THT and 17 RHT neurons were activated antidromically from 44 low-threshold points that were located in the supraoptic decussation (29), internal capsule (2), supraoptic nucleus (3), suprachiasmatic nucleus (1), lateral hypothalamus (2), globus pallidus (3), caudate putamen (1), substantia innominata (2), and the basal nucleus of Meyenert (1). Anterior to the hypothalamus, low-threshold points were found in the ventrolateral area of caudate putamen (2) and in the globus pallidus (1). On the ipsilateral side, 27 low-threshold points for antidromic activation of nine THT and four RHT neurons were found between the anterior hypothalamus and the midbrain. These points were located in the optic chiasm (3), suprachias-
morphic nucleus (2), supraoptic decussation (9), optic tract (4), lateral hypothalamus (4), internal capsule (1), cerebral peduncle (2), and medial to the medial geniculate nucleus (2). Photomicrographs of lesions made at low-threshold points in the basal ganglia, hypothalamus, and midbrain are shown in Fig. 2.

CONDUCTION VELOCITIES. In 58 cases (38 THT and 20 RHT), conduction velocities (CVs) were determined at several points along the axonal path (Fig. 18A). The estimated mean CV between the recording sites and the midbrain in the hypothalamus was 7.3 ± 0.3 m/s, with a range of 1.2–15.0 m/s (Fig. 18I). The estimated CVs between the recording sites and the midbrain and between the midbrain and caudal hypothalamus were 8.3 ± 0.4 and 7.2 ± 0.7 m/s, respectively (Fig. 18, II and III). Between the caudal and rostral hypothalamus, conduction of THT and RHT axons averaged 3.4 ± 1.4 and 6.1 ± 2.1 m/s, respectively (Fig. 18, IV and V). These data indicate that the axons of THT but not RHT neurons slowed by 50% as they passed through the hypothalamus (P = 0.001; t test); pointing to the possibility of collateral branches.

CURRENT SPREAD. The effective spread of the antidromic stimulation currents in the hypothalamus was estimated by examining the relationship between stimulus intensity and distance from the axon (as defined by the lowest threshold point for antidromic activation). The plots in Fig. 18, I and II, summarize threshold readings from 66 electrode penetrations containing lowest threshold points (i.e., ≤50 μA) in the hypothalamus. As indicated by the plots, there was a direct relationship between the distance from the axon and the threshold for antidromic activation. At 200, 400, 600, and 800 μm from the axon, the mean effective current spread was 40, 105, 197, and 225 μA. The threshold for antidromic activation of 90% of the neurons described in this study was ≤30 μA. Although the mean effective spread of current ≤30 μA was smaller than 200 μm (Fig. 18/I), it occasionally (2/66) reached as far as 400 μm and rarely (1/66) beyond this distance (Fig. 18/I). Throughout the study, a current of 500 μA was used in the initial search for hypothalamic projection neurons. The maximum effective spread of this current was only 1,500 μm (Fig. 18/I). These data show that the current-distance relationship within the hypothalamus depends on the intensity of the current; low currents can effectively activate axons at distances that suggest a spread of 10–20 μm/μA and high currents at distances that suggest a spread of 3–5 μm/μA.

DISCUSSION

This study describes the response properties of two ascending somatosensory pathways that convey primarily nociceptive information to the hypothalamus: the trigeminal-hypothalamic tract, which conveys sensory signals that arise in facial skin, cornea, vibrissae, oral mucosa, and intracrani al dura, and the reticulohypothalamic tract, which relays sensory signals from the entire body via the spinal cord and TBNC. To our knowledge, this is the first evidence that sensory information that originates in these orofacial organs reaches the hypothalamus directly. It is also the first study to document the kind of sensory information that RHT neurons in the LRF carry. We consider the THT a direct pathway because it receives direct input from trigeminal primary afferent fibers. We consider the LRF-RHT an indirect pathway because it does not receive direct input from peripheral fibers, rather the input that drives its neurons originates in laminae I and V throughout the length of the spinal and medullary dorsal horn. The findings that nociceptive signals that arise in orofacial organs reach the hypothalamus through both the THT and the RHT suggest that highly prioritized signals regarding orofacial pain are transferred in parallel channels to ensure that this critical information reaches the hypothalamus; a brain area that regulates homeostasis and other humoral responses required for the survival of the organism.

Although the properties of the THT are broadly similar to those described previously for the SHT, a number of fundamentally new findings have emerged from the present study: 1) neurons classified as LT by their response to mechanical stimulation also respond to innocuous and noxious intensities of thermal stimulation. Their markedly different stimulus-response curves during the static and dynamic phases of the thermal stimuli suggest a novel pattern of convergent inputs from thermonensitive and nociceptive primary afferent neurons (see following text). Such thermal responses have not been described for other populations of LT neurons at spinal or trigeminal levels. 2) Somatotopy is expressed in some laminae V WDR neurons as a gradation of sensitivity within a large receptive field such that the most sensitive part of the receptive field corresponds to the neuron’s position along the dorsomedial-to-ventrolateral axis. 3) Dorsal horn neurons that project directly to the forebrain, which have been described previously only in anatomical studies, have been physiologically characterized and shown to convey nociceptive information. It has further been shown that the axons of these neurons descend by a different route than SHT/THT neurons, through the internal capsule rather than through the supraoptic decussation. 4) Neurons in laminae V of Vc can be distinguished physiologically from neurons in the adjacent LRF in that lamina V neurons have trigeminal-only receptive fields while LRF receptive fields include both trigeminal and extra-trigeminal regions.

Responses to cutaneous orofacial stimuli

Although the response properties of THT and RHT neurons to mechanical and thermal stimulation of the orofacial skin
FIG. 17. A and B: summary of the locations of 197 lesions marking lowest threshold points for antidromic activation of 54 THT and 18 LRF-RHT neurons. C: summary of the locations of 186 lesions marking lowest threshold points for antidromic activation of 64 neurons that were classified according to their responses to innocuous and noxious mechanical stimulation of the facial skin. Rostral sections are at top, caudal sections at bottom, and contralateral is to the right. In general, latencies for antidromic activation from the contralateral midbrain were shorter than latencies from the contralateral hypothalamus, and latencies from the contralateral hypothalamus were shorter than latencies from the ipsilateral hypothalamus. Note that all neurons followed the same path to the hypothalamus. Acb, nucleus accumbens; APT, anterior pretectal nucleus; cp, cerebral peduncle; HDB, horizontal limb diagonal band of Broca; MGN, medial geniculate nucleus; RN, red nucleus; SCN, suprachiasmatic nucleus; SI, substantia innominata; SN, substantia nigra; Sn, septal nuclei; VDB, ventral limb diagonal band of Broca; VM, ventromedial thalamic nucleus; VP, ventral pallidum; VPM, ventroposterior medial thalamic nucleus.
FIG. 18.  A: histograms showing the conduction velocities (means ± SE) of THT and LRF-RHT neurons between the recording sites and the hypothalamus (I), recording sites and midbrain (II), midbrain and caudal hypothalamus (III), and caudal and rostral hypothalamus (IV) of all neurons (left), of THT neurons (middle), and of LRF-RHT neurons (right). V: plots of mean (±SE) conduction velocities. Note that only THT axons slowed significantly (>50%) as they passed through the hypothalamus. B: current spread. I: plots of antidromic threshold versus distance from lowest threshold points (indicated by 0 on the abscissa) in the hypothalamus. II: same data shown in A for currents ±50 μA. III: relationship between the mean antidromic threshold (±SE) and the lowest threshold point for antidromic activation. CH, caudal hypothalamus; MB, midbrain; RH, rostral hypothalamus; RS, recording site.
appear similar, they differed in their anatomical locations and receptive field properties, suggesting that different inputs govern their responses. They will therefore be discussed separately.

Thirty-eight percent of all THT neurons were classified as HT. They responded exclusively to noxious mechanical and thermal stimulation and were capable of reliably encoding the intensity of the noxious stimulus. Based on the trend in their responses to pressure, pinch, and crush (but not to brush) and to 46, 50, and 55°C (but not to 39 and 41°C), it is reasonable to propose that their peripheral inputs originate in cutaneous nociceptors that innervate the hairy skin. In primates, these nociceptors include type I and II A-mechanoheat (AMH) and C-mechanoheat (CMH) fibers; AMH and CMH fibers are also termed HT mechanoreceptors and C polymodal nociceptors, respectively (Bessou and Perl 1969; Perl 1969). The early burst of activity, the slow adaptation rate, the monotonic increase in responses to the increased intensities of both mechanical and thermal stimuli, and the plateau in the responses to the most intense (heat) stimuli suggest a CMH input (Garell et al. 1996; LaMotte and Campbell 1978; Meyer and Campbell 1981), while the graded responses to the increased intensities of the mechanical stimuli (i.e., the lack of plateau), and the relatively high heat threshold (47.4°C) point to type II AMH input (Treede et al. 1995). Type II AMH nociceptors respond to lower heat stimuli (median threshold 46°C) than type I AMH (50°C) (Treede et al. 1995), and higher heat stimuli than CMH nociceptors (43–44°C) (LaMotte and Campbell 1978), 40–41°C (Tillman et al. 1995a,b; Weidner et al. 1999). The “lack of” or poor responses to cold stimuli suggest that THT-HT neurons receive most of their peripheral inputs from mechanohot nociceptors that do not usually respond to cold stimuli (Simone and Kajander 1997).

Forty-two percent of all THT neurons were classified as WDR. They responded preferentially to noxious mechanical stimuli and almost exclusively to noxious heat stimuli. Their responses to innocuous mechanical stimuli, and the graded fashion of their responses to noxious stimuli suggest that the peripheral inputs they receive originate in both mechanoreceptors and nociceptors. Clues to the source of their nociceptive input are provided by their different responses to heat. WDR neurons responded incrementally to the dynamic (i.e., reached their peak during the dynamic phase of the heat stimulus and static (i.e., reached their peak during the later static phase of the heat stimulus) phases of the heat stimulus. The incremented response to the static phase of the increased heat stimulus (between 45 and 55°C) and the heat threshold (43.6°C) suggest an input from CMH (LaMotte and Campbell 1978) and potentially from type I AMH (Treede et al. 1995), while the graded responses to the dynamic phase could be mediated by signals that originate in type II AMH (Sumino et al. 1973; Treede et al. 1995) and potentially CMH (Meyer and Campbell 1981) nociceptors. The incremented responses to the static phase of the increased cold stimuli (between 20 and 0°C) suggest that THT-WDR neurons can encode the intensity of the noxious cold stimuli and that their so-called static response to cold is mediated by nociceptors. Because the responses of WDR neurons to thermal stimuli of 35–20°C were not examined routinely, we cannot rule out or provide sufficient evidence for a potential influence of cold receptors on the initiation of their response to cold.

Twenty percent of all THT neurons were classified as LT. Their responses to the dynamic innocuous mechanical stimuli (brush) were significantly larger than their responses to the noxious mechanical stimuli (P < 0.05). Traditionally, these response profiles are believed to be mediated by the activation of LT mechanoreceptors (LTM) that provide information about texture and shape (reviewed in Johnson 1983). Most LT neurons also responded to deflection of a single vibrissa or hair follicle; stimuli that are considerably more gentle than brushing the skin. In the rat, several classes of rapidly and slowly adapting mechanoreceptors that innervate the vibrissae, guard hairs and F-line upper lip hair are believed to provide the sensory information required for recognition of objects in the environment (Jacquin et al. 1986a,b). Based on the mechanical response properties of LT neurons, we must conclude that a part of their input originates in LT mechanoreceptors that innervate the hairy skin. We are somewhat puzzled, however, by the thermal response properties of these THT-LT neurons. As shown in Figs. 8 and 9, the responses of LT neurons increased incrementally during the steady-state phase of the noxious heat and cold stimuli. Since nociceptors are the only receptors that respond in a graded fashion to increased intensities of noxious thermal stimuli, we have interpreted these data as suggesting that the static responses of THT-LT neurons are influenced by inputs that originate in nociceptors. A possible site of interaction between primary afferent nociceptors and second-order LT neurons is lamina V of the dorsal horn, as this lamina receives direct input from cutaneous nociceptors and contained most (8/11) LT-THT neurons. Based on the mechanical response profile, however, it is not reasonable to propose that their inputs originate in cutaneous CMH and AMH nociceptors. Rather, an input from nociceptors that are heat and/or cold sensitive, but mechanically insensitive is proposed. Heat nociceptors have been found in both humans and animals (Baumann et al. 1991; Georgopoulos 1976; Treede et al. 1998; Weidner et al. 1999; Welk et al. 1984). In humans, they constitute a class of C-fiber nociceptors that is clearly distinct from CMH nociceptors; they are mechanically insensitive, conduct slower, and exhibit higher heat thresholds than CMH fibers (Weidner et al. 1999). In primates, they consist of type II A-fiber nociceptors that are mechanically insensitive (Treede et al. 1998).

More than 50% of the LT-THT neurons, however, started to respond when the adapting temperature (35°C) changed by only 2°C (i.e., 37°C in the heating direction and 33°C in the cooling direction). Since nociceptors are unlikely to respond to such small changes in skin temperature, we must propose that the initiation of the thermal responses of LT-THT neurons is influenced by activation of cold and warm receptors. A detailed examination of the responses during the dynamic phase of the heat stimuli shows that the discharge rate of LT-THT neurons increases in a near-linear fashion from 39 to 46°C, and then decreases. This response profile is characteristic of C-warm receptors (Duclaux and Kenshalo 1980; Hensel and Iggo 1971; Hensel and Keshalho 1969; Konietzny and Hensel 1977; Kumazawa and Perl 1977; LaMotte and Campbell 1978). Because some LT-THT neurons started to respond when the temperature was lowered from 35 to 33°C, it is tempting to consider input from cold receptors as well. A detailed examination of the responses during the dynamic phase of the cold stimuli shows that the discharge rate of LT-THT neurons
decreases in a near-linear fashion from 20 to −10°C. This response profile is characteristic of cold receptors (Dubner et al. 1975; Duclaux et al. 1980; Dykes 1975; Hellon et al. 1975; Hensel and Igo 1971; Igo 1969; Kenshala and Duclaux 1977; Poulos and Lende 1970a,b). For lack of information about the response properties of these neurons to innocuous cold stimuli (35–20°C), however, we cannot provide conclusive evidence for this proposal.

Responses to stimulation of specific orofacial organs

Twenty-four percent of all THT neurons responded to stimulation of the oral mucosa, tongue, or lips. They were located in the dorsomedial region of laminae III, IV, and V of C1 and Vc; the principal termination area of primary afferent neurons that innervate the tongue, hard palate, and lips (Arvidsson et al. 1992, 1995; Marfurt 1981; Shigenaga et al. 1986). This distribution matches the distribution of c-fos immunoreactive neurons following stimulation of the tongue and lips (Carstens et al. 1995; Strassman and Vos 1993) and the recording sites of neurons responsive to noxious stimuli of other oral structures (Shigenaga et al. 1976). In spite of the prominent presence of oral-sensitive neurons in lamina I (Carstens et al. 1995, 1998; Dostrovsky and Hellen 1978; Hutchison et al. 1997; Strassman and Vos 1993), we did not find them. Three factors could contribute to our inability to record from oral-sensitive neurons in lamina I: the use of relatively low-impedance electrodes and their possible bias toward large neurons, the small sample size (only 8 lamina I-THT neurons were recorded dorsomedially) and the absence of lamina I-THT neurons that are oral sensitive.

Most (85%) oral-sensitive THT neurons encoded the intensity of the noxious mechanical and thermal stimuli, over half (54%) encoded light mechanical stimuli, and a few (15%) encoded innocuous cold. These response properties of oral-sensitive THT neurons could be driven by inputs they receive from nociceptors (Hayashi 1985; Jacquin et al. 1986a; Light et al. 1992; Sumino et al. 1973), mechanoreceptors (Hensel and Zotterman 1951; Poulos and Lende 1970a,b), and cold receptors (Dubner et al. 1975; Heinz et al. 1990; Hensel and Wurster 1970; Hensel and Zotterman 1951; Poulos and Lende 1970a,b) that innervate the tongue and the lips. Similar oral-sensitive nociceptive (WDR and HT), mechanoreceptive (LT), and thermo-responsive Vc neurons have been previously described (Carstens et al. 1998; Hoffman et al. 1981; Hu et al. 1981; Hutchison et al. 1997; McHaffie et al. 1994; Price et al. 1976; Renezhan et al. 1986), many of which project to the thalamus and brain stem. It is therefore likely that oral-sensitive Vc neurons convey sensory information to the hypothalamus which is similar to the information they convey to other areas of the brain.

Sixteen percent of all THT neurons were classified as cornea-sensitive. Since activation of the cornea by mechanical, thermal, or chemical stimuli produces predominantly a sensation of pain in humans (Beuerman and Tanelian 1979; Kenshala 1960; Lele and Weddell 1959) and vocalization and escape behaviors indicative of pain in animals (Gerard 1923), cornea-sensitive neurons were considered nociceptive. Given the many tasks of this study, however, it was not possible to activate all classes of corneal receptors or to differentiate between cornea alone and cornea plus conjunctiva stimulation for all but the mechanical stimuli. Therefore the identification of cornea-sensitive neurons was based only on their responses to cornea brush. The application of a brush stimulus to the cornea is usually the most effective way for identifying cornea-sensitive neurons as it activates corneal Aδ mechanosensitive receptors (Belmonte et al. 1991; Lele and Weddell 1959; MacIver and Tanelian 1993a,b; Tanelian and Beuerman 1984) and Aδ- and C-polymodal nociceptors (Belmonte and Giraldes 1981; Belmonte et al. 1991; Gallar et al. 1993); receptors that are most easily excited by a moving stimulus rather than by static corneal indentation (Belmonte and Giraldes 1981; Mosso and Kruger 1973).

All cornea-sensitive THT neurons were recorded in ventrolateral lamina V at the level of caudal Vc and rostral C1, a gray matter area that receives input from corneal nociceptors and periorbital receptors (Marfurt 1981; Marfurt and Del Toro 1987; Panneton and Burton 1981; Shigenaga et al. 1986) and contains one of the two groups of cornea-sensitive TBNC neurons (Bereiter et al. 1994; Lu et al. 1993; Marfurt and Del Toro 1987; Strassman et al. 1993). While in this study all cornea-sensitive THT neurons were found in lamina V, in previous studies they were found mainly in lamina I (Carstens et al. 1998; Hu et al. 1981; Meng et al. 1997; Nagano et al. 1975; Nishida and Yokota 1986; Pozo and Cervero 1993). Their absence from lamina I in this study was a likely result of not recording there; a sample bias that often occurs in electrophysiological experiments in which single-unit recording techniques are used. The presence of cornea-sensitive neurons in lamina V is not surprising, however, as it was demonstrated using c-fos (Strassman et al. 1993) and single-unit recordings (Nagano et al. 1975) in the rat.

About 19% of all THT neurons responded to electrical and mechanical stimulation of the intracranial dura. Since pain is the only sensation that can be evoked by stimulating the sinuses in the human, regardless of whether the stimulus is electrical, mechanical, or chemical (Ray and Wolff 1940), we have considered these neurons nociceptive. In fact, based on their responses to cutaneous stimulation, most dura-sensitive THT units were classified as nociceptive neurons capable of processing somatosensory signals that originate in both the intracranial dura and extracranial (mainly ophthalmic) skin. Their response properties suggest that the inputs they receive originate in meningeal C and Aδ fibers that respond to mechanical, thermal, and chemical stimulation (Bove and Moshkovitz 1997; Strassman et al. 1996), and cutaneous nociceptors (discussed in the preceding text). The response properties of these neurons resemble those of dura-sensitive neurons that project to the thalamus in the cat (Davis and Dostrovsky 1988). In fact, three of the dura-sensitive THT neurons also project to the thalamus.

Responses of LRF-RHT neurons

All LRF-RHT neurons had large or complex receptive fields that extended beyond the innervation territory of the trigeminal nerve. They responded exclusively to noxious mechanical and thermal stimulation of extracephalic skin (e.g., paws and tail) and exclusively or preferentially to noxious mechanical and thermal stimulation of facial skin, lips and tongue and to brushing the cornea. The trends in
their responses to mechanical and thermal stimulation of the facial skin (Fig. 13) suggest that they are capable of encoding the intensity of innocuous and noxious mechanical and noxious heat but not cold. Based on their response profiles, it is reasonable to propose that the inputs that drive them originate in nociceptive-specific (HT) spinal cord dorsal horn neurons and nociceptive (HT and WDR) plus non-nociceptive (LT) neurons in the TBNC.

In the absence of clear anatomical landmarks around the recording sites that were found medial and ventral to the ventrolateral tip of nucleus caudalis, we have opted to adapt Nord’s nomenclature (Nord and Kyler 1968) and define them as LRF neurons. This broadly defined area contains the caudal part of the A1 catecholaminergic cell group (Dahlstrom and Fuxe 1964), subnucleus reticularis ventralis (Meessen and Olszewski 1949), and possibly a few subnucleus reticularis dorsalis neurons (Villanueva et al. 1988). In agreement with our findings, this area was previously found to receive direct input from nociceptive spinal cord neurons (Craig 1995; Lima et al. 1991; Menetrey et al. 1983; Tavares et al. 1993; Westlund and Craig 1996), to contain nociceptive neurons that respond to stimulation of orofacial organs such as the cornea, skin, tongue, nose, and tooth-pulp in cats, monkeys, and rats (Burton 1968; Nagano et al. 1975; Yokota et al. 1991), and to project to the hypothalamus (Cunningham and Sawchenko 1991; Loewy et al. 1981; McKellar and Loewy 1981; Sawchenko and Swanson 1981) and forebrain (Zagon et al. 1994).

SUBNUCLEUS RETICULARIS VENTRALIS (SRV). Although the recording locations of LRF-THT neurons resemble most closely those of SRV neurons, we did not consider them to be typical SRV units because of their receptive field size. The receptive fields of SRV neurons are restricted to the innervation territory of the trigeminal nerve (Burton 1968; Nagano et al. 1975; Yokota et al. 1991), while the receptive fields of LRF-THT neurons often included the entire body. Many reasons could account for this discrepancy. They include species differences, exact recording locations, and the way in which studied neurons were selected (the only neurons we studied were those projecting to the hypothalamus).

SUBNUCLEUS RETICULARIS DORSALIS (SRD). In spite of the close resemblance between the response properties of the neurons we called LRF-RHT and those found in SRD (Villanueva et al. 1988), we do not believe they were SRD units because they were recorded caudal, ventral, and lateral to the SRD and because, unlike SRD neurons, LRF neurons project to the hypothalamus.

CATECHOLAMINERGIC CELL GROUP (A1). Anatomically, catecholaminergic neurons in the caudal ventrolateral medulla are found as far dorsal as the recording locations of LRF-THT neurons. Functionally, a comparison between A1 and LRF-RHT neurons could not be completed at this point because little or no information is available regarding the responsiveness of hypothalamic-projecting A1 neurons to innocuous and noxious somatosensory stimulation of organs such as the skin, cornea, lips, and dura. Traditionally, A1 neurons have been viewed as playing an important role in the reflex control of hormonal secretion from hypothalamic neurons in response to hemodynamic, gastrointestinal, and respiratory stimuli (Cunningham and Sawchenko 1991; Randle et al. 1986; Swanson 1987; Willoughby et al. 1987); sensory information they receive through the nucleus of the solitary tract (Loewy 1990). The finding that hypothalamic projecting neurons in this region convey nociceptive information that originates in multiple organs indicates their potential involvement in the initiation of endocrine responses to noxious stimuli as well; a consideration that was brought up recently by Pan and colleagues using the Fos technique (Pan et al. 1999).

Comparisons between THT and SHT neurons

Anatomically, the THT must be considered as the rostral extension of the SHT. Together, the two tracts relay signals from all levels of the spinal cord (Burstein et al. 1990) and therefore are in a position to carry to the hypothalamus sensory information that arises in the entire body. In the past 10 years, Giesler and colleagues have studied the physiological properties of SHT neurons in the sacral (Katter et al. 1996b), lumbar (Burstein et al. 1991), thoracic (Zhang et al. 1999a), and lower cervical (Dado et al. 1994b) spinal cord. They found that nociceptive information can be conveyed to the hypothalamus by ~90% of all SHT neurons (WDR and HT) and tactile information by ~50% of the neurons (WDR and LT). These neurons were capable of processing sensory information from pelvic and abdominal organs such as the vagina, colorectal canal, and bile duct, and from sacral, lumbar, thoracic, and cervical dermatomes. It is therefore reasonable to conclude that the spinohypothalamic and the trigeminohypothalamic tracts are similar. Both SHT and THT neurons are located in the same laminae, respond mainly to noxious stimuli but also to innocuous stimuli, process somatosensory signals from cutaneous and visceral organs, and exhibit receptive fields that, in general, are restricted to no more than two to four dermatomes. The identification of C1–2 and Vc neurons that project to the hypothalamus and respond to noxious stimulation of intra- and extracranial organs and trigeminal dermatomes is therefore not surprising. A surprising finding regarding the physiological properties of THT neurons is their ability to respond to mild mechanical and thermal stimuli such as vibrisseal deflections and skin cooling or warming by <3°C. Three factors may explain the presence of these responses in THT but not in SHT neurons: the unique innervation of orofacial organs such as the tongue, lips, and nose by cold and warm receptors and the vibrissae by specialized mechanoreceptors, the application of most thermal stimuli to the hairy skin of the face in this study versus the glabrous skin of the paws in the SHT studies, and the use of slow ramps for thermal stimuli in this study versus fast ramps in the SHT studies.

Based on the complete antidromic mapping of ascending and descending axons of cervical SHT neurons (Dado et al. 1994a; Kostarczyk et al. 1997; Zhang et al. 1995), partial mapping of lumbar (Burstein et al. 1991) SHT axons, and mapping of a small number of sacral SHT axons (Katter et al. 1996a), it appears that most SHT axons ascend to the hypothalamus through the contralateral lateral funiculus and ventrolateral medulla. They then shift dorsally to create a tight bundle along the medial border of the medial geniculate nucleus. This tight bundle seems to loosen on entering the caudal diencephalon, but appears to remain in the hypothalamic region. This bundle plays an important role in the reflex control of hormonal secretion from hypothalamic neurons in response to hemodynamic, gastrointestinal, and respiratory stimuli (Cunningham and Sawchenko 1991; Randle et al. 1986; Swanson 1987; Willoughby et al. 1987); sensory information they receive
chiasm, and descend in the ipsilateral hypothalamus. As shown in Fig. 17, both THT and RHT axons seem to follow SHT axons as they infiltrate through the zona incerta, cerebral peduncle, internal capsule, and optic tract and ascend in the SOD. Unlike SHT axons, however, THT and RHT axons appear more scattered between the medial geniculate nucleus, the central gray, and the substantia nigra. This difference suggests that in the midbrain, ascending SHT axons may be partially segregated from THT and RHT axons.

Functional considerations

In only 10 experiments, we successfully followed collateral branches of parent axons from the supraoptic decussation to specific nuclei within the hypothalamus. Because anatomical studies have shown that a large number of TBNC neurons project to the hypothalamus (Malick and Burstein 1998) and that many hypothalamic nuclei contain trigeminal axons (Iwata et al. 1992; Newman et al. 1996; Ring and Ganchrow 1983), the scarcity of axonal branches in the hypothalamus suggests that the antidromic activation technique used in this study was not adequate for detecting these branches. A likely explanation for our inability to identify these axonal branches is that the stimulating electrodes were too blunt (tip diameter, 35–50 μm) and the pulse stimuli too short (200 μs) and too fast (10 Hz) to activate the small (and probably unmyelinated) axonal branches. In fact, in the 10 experiments in which collateral branches were found in the hypothalamus, sharper electrodes (tip diameter, 10–25 μm), wider stimulus pulses (duration 200–800 μs), and slower interstimulus intervals (1 Hz) were used. Collateral branches were found in the anterior, lateral, and perifornical regions and the dorsomedial, suprachiasmatic, and supraoptic nuclei.

Our findings raise the possibility that THT and RHT axons convey trigeminal sensory signals to hypothalamic neurons that regulate body temperature, food and water intake, sleep and circadian rhythms and a wide range of behaviors (Bernardis and Bellinger 1993, 1998; Kruk et al. 1983; Lin et al. 1989; Norgren 1970; Panksepp 1971; Peyron et al. 1998; Roeling et al. 1993; Saper 1995; Scammell et al. 1993; Sherin et al. 1996; Simerly et al. 1987). For example, many low-threshold points of THT and RHT axons were identified within the LH. Current understanding suggests that LH neurons play an important role in the regulation of food and water intake, arousal, and aggression (Bernardis and Bellinger 1993; Date et al. 1999; Panksepp 1971). Studies in which lesions of the LH produced inhibition of food intake, and stimulation increased food intake, gave rise to the notion that LH neurons constitute a principle output pathway that promotes feeding behavior (Hetherington and Ranson 1940; Stevenson 1970). More recently, it has been proposed that LH neurons that produce melanin-concentrating hormone or orexin (Bittencourt et al. 1992; Sakurai et al. 1998) do so by activating distinct autonomic, endocrine, and behavioral responses through widespread projections to the cerebral cortex, brain stem, and spinal cord (Elmqquist et al. 1999). Regarding water intake, a recent study found that dehydration activates LH neurons and produces a type of anorexia that can be reversed by sham drinking alone (which does not affect water balance) (Watts 1999). We propose that THT neurons, which convey signals regarding the sensation of water passing through the lips, oral cavity and esophagus, could mediate the reversal of this dehydration-induced anorexia. The locations of THT axons in the LH also correspond well with the location of orexin-positive neurons (Date et al. 1999). Because of the putative role of orexin-positive neurons in the regulation of sleep and arousal (Chenelli et al. 1999; Lin et al. 1999; Peyron et al. 1998), it is also possible that THT neurons that relay nociceptive trigeminal signals can contribute to changes in arousal states in response to pain.

Several THT axons also entered the SCN and SON. Because the SCN regulates circadian rhythms such as sleep-wake cycles, plasma cortisol levels, and body temperature (reviewed in van den Pol 1991), it is possible that the nociceptive signals that reach the SCN through the THT or RHT contribute to the disruption of normal sleep patterns in pain patients. Because the SON produces oxytocin, arginine-vasopressin, and corticotrophin-releasing hormone, which regulate labor, lactation and stress responses (as reviewed in Armstrong 1995), it is possible that nociceptive signals that reach the SON through the THT or RHT contribute to the initiation or cessation of these physiological functions by pain.

Trigeminohypothalamic tract neurons were also capable of transferring to the hypothalamus tactile information from oral and perioral organs such as the tongue, lips, and vibrissae. The physiological purposes for tactile signals reaching the hypothalamus are currently unknown and therefore a matter of speculation. They could be related to feeding, drinking, sucking, and a variety of intimate social behaviors such as kissing. For example, since rodents use the tongue, lips, and vibrissae to explore for food and water, sensory signals that provide information about the structure, size, consistency, and temperature of objects in the environment may play a role in the recognition of nutrients and the triggering (or avoidance) of feeding and drinking behaviors such as licking and chewing. In humans, it could be speculated that THT neurons capable of encoding cooling and warming sensations from the lips and tongue may be involved in the facilitation or suppression of the desire to eat foods that are colder or warmer than expected. Every-day life experience also points to the close association between the tactile sensation of dry lips and tongue and the desire to drink. Consideration can therefore be given to the idea that THT neurons may carry such sensations to hypothalamic areas that regulate osmotic and volemic states and by doing so, stimulate drinking behavior.

Forebrain projections

Because many spinal cord neurons project to forebrain nuclei located anterior to the hypothalamus (Burstein and Giesler 1989; Burstein and Potrebic 1993; Cliffer et al. 1991; Malick and Burstein 1998), numerous attempts were made by Giesler and colleagues to follow the axons of SHT neurons from the SOD to the forebrain (Burstein et al. 1991; Dado et al. 1994a; Katter et al. 1996a), but no such axons were found. This led to the conclusion that SHT axons do not approach the forebrain through the SOD. The present study confirms this conclusion. The few THT and RHT axons that were followed in this study to the caudate-putamen, globus pallidus, and substantia innominata ascended lateral to the hypothalamus, within the internal capsule. A few anatomical studies have documented
the presence of axons in the forebrain that originate in spinal (Cliffer et al. 1991; Newman et al. 1996), trigeminal (Newman et al. 1996; Yasui et al. 1987), and LRF (Loewy et al. 1981; Zagon et al. 1994) neurons; findings confirmed by injections of retrograde tracers in several forebrain areas (Burstein and Giesler 1989; Burstein and Potrebic 1993; Malick and Burstein 1998; Zagon et al. 1994). Extracellular recording in CPu and GP found two populations of neurons, those responding to innocuous stimulation of orofacial receptive fields (Carelli and West 1991; Levine et al. 1987; Lidsky et al. 1979; Schneider 1991; Schneider et al. 1982, 1985) and those responding to noxious stimulation of the entire body (Bernard et al. 1992; Chudler et al. 1993; Lin et al. 1985; Richards and Taylor 1982). Based on the involvement of CPu and GP neurons in the execution of motor functions, it was proposed that CPu and GP neurons that respond to somatosensory stimulation may participate in the coordination of complex motor responses to painful and tactile stimuli (e.g., withdrawal and orientation). Regarding the ventral pallidum/substantia innominata area, behavioral studies proposed that these nuclei play a role in the generation of autonomic and somatomotor aspects of emotional and motivational states (reviewed in Heimer et al. 1997).

In 1992, Bernard et al. suggested that neurons in this area receive nociceptive input and that this input arises in the parabrachial nucleus. Our study suggests that nociceptive input to this area can also arise in LRF-RHT neurons. Together, these nociceptive pathways may contribute to the alteration of motivational state by pain.

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