Chemical Stimulation of the Intracranial Dura Induces Enhanced Responses to Facial Stimulation in Brain Stem Trigeminal Neurons

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Chemical stimulation of the intracranial dura induces enhanced responses to facial stimulation in brain stem trigeminal neurons. J. Neurophysiol. 79: 964–982, 1998. Chemical activation and sensitization of trigeminal primary afferent neurons innervating the intracranial meninges have been postulated as possible causes of certain headaches. This sensitization, however, cannot explain the extracranial hypersensitivity that often accompanies headache. The goal of this study was to test the hypothesis that chemical activation and sensitization of meningeal sensory neurons can lead to activation and sensitization of central trigeminal neurons that receive convergent input from the dura and skin. This hypothesis was investigated by recording changes in the responsiveness of 23 [16 wide-dynamic range (WDR), 5 high threshold (HT), and 2 low threshold (LT)] dura-sensitive neurons in nucleus caudalis to mechanical stimulation of their dural receptive fields and to mechanical and thermal stimulation of their cutaneous receptive fields after local application of inflammatory mediators or acidic agents to the dura. Responses to brief chemical stimulation were recorded in 70% of the neurons; most were short, lasting the duration of the stimulus only. Twenty minutes after chemical stimulation of the dura, the following changes occurred: 1) 95% of the neurons showed significant increases in sensitivity to mechanical indentation of the dura: their thresholds to dural indentation changed from 1.57 to 0.49 g ( means, \( P < 0.0001 \) ), and the response magnitude to identical stimuli increased by two- to fourfold; 2) 80% of the neurons showed significant increases in cutaneous mecanosensitivity: their responses to brush and pressure increased 2.5– \( (P < 0.05) \) and 1.6-fold \( (P < 0.05) \), respectively; 3) 75% of the neurons showed a significant increase in cutaneous thermosensitivity: their thresholds to slow heating of the skin changed from 43.7 ± 0.7°C to 40.3 ± 0.7°C \( (P < 0.005) \) and to slow cooling from 23.7 ± 3.3°C to 29.2 ± 1.8°C \( (P < 0.05) \); 4) dural receptive fields expanded within 30 min and cutaneous receptive fields within 2–4 h; and 5) ongoing activity developed in WDR and HT but not in LT neurons. Application of lidocaine to the dura abolished the response to dural stimulation but had minimal effect on the increased responses to cutaneous stimulation (suggesting involvement of a central mechanism in maintaining the sensitized state). Antidromic activation (current of <30 \( \mu \)A) of dura-sensitive neurons revealed projections to the hypothalamus, thalamus, and midbrain. These findings suggest that chemical activation and sensitization of dura-sensitive peripheral nociceptors could lead to enhanced responses in central neurons and that this central sensitization therefore could result in extracranial tenderness (mechanical and thermal allodynia) in the absence of extracranial pathology. The projection targets of these neurons suggest a possible role in mediating the autonomic, endocrine, and affective symptoms that accompany headaches.

**INTRODUCTION**

For the past 50 years, the prevailing theory of migraine has held that the pain results from an abnormal dilatation of intracranial blood vessels and the consequent mechanical excitation of sensory fibers innervating them (Graham and Wolff 1938). This theory, however, has not been validated due to lack of evidence for greater vasodilatation during headache attacks than during headache-free periods (Olesen et al. 1990; Zwetsloot et al. 1992). In recent years, alternative theories have proposed a chemical mode of activation of meningeal perivascular sensory fibers (Moskowitz et al. 1988). According to these theories, ions, protons, and inflammatory agents that activate and sensitize peripheral nociceptors (Handwerker and Reeh 1991; Steen et al. 1992, 1995) are released in the vicinity of sensory fibers innervating the dura after an episode of cortical spreading depression (Lauritzen 1994) or neurogenic inflammation (Goadsby and Edvinsson 1993; Moskowitz and Macfarlane 1993). Although the cause of the initial release of these chemicals is unknown, a general notion has been developed recently suggesting that temporary exposure of perivascular fibers to chemical agents alters their sensitivity to mechanical stimuli and leads to the sensation of head pain. In support of this idea, we showed that the application of acidic and inflammatory agents to the dura enabled peripheral fibers innervating the dura (Strassman et al. 1996) to respond to mechanical stimuli that initially evoked minimal or no response and proposed that this chemically mediated sensitization can explain the hypersensitivity of migraineurs to changes in intracranial pressure (e.g., during coughing). While sensitization of peripheral fibers alone could account for intracranial hypersensitivity, a central component is needed to account for the hypersensitivity that can develop during some headaches in the temporal, pericocular, intraoral, and maxillary regions of the head and face (Drummond 1987; Langemark and Olesen 1987). Based on previous studies (Beck and Handwerker 1974; Davis et al. 1993; Fjallbrant and Iggo 1961; Handwerker and Reeh 1991; Kessler et al. 1992; Khan et al. 1992; Kumazawa et al. 1987b; Lang et al. 1990; Mizumura et al. 1987; Neugebauer et al. 1989) and on the notion that both peripheral nociceptors and nociceptive dorsal horn neurons contribute to the development of allodynia, hyperalgesia, and persistent pain after inflammation (reviewed in Willis 1992), we hypothesized that the chemically induced excitation of the dural primary afferents produces central changes (in medullary dorsal horn neurons receiving input from the dura, skin, hair follicles, cornea, and teeth) that could account for the extracranial hypersensitivity. We also hypothesized that to mediate the autonomic symptoms that can accompany headache, these dura-sensitive neurons project to sensory and limbic nuclei in the brain. Previous studies have focused on the mechanism that underlies the referred pain.
SENSITIZATION OF VC NEURONS BY DURAL STIMULATION

of intracranial origin by examining the distribution (Hoskin et al. 1996; Kaube et al. 1993; Nozaki et al. 1992; Strassman et al. 1994) and the response properties (Davis and Dostrovsky 1986, 1988a,b; Lambert et al. 1992; Strassman et al. 1986) of dura-sensitive neurons in the upper cervical and medullary dorsal horn. Because central changes and sensitization were not examined in these studies, the possible role of central trigeminal neurons in the altered sensitivity of intra- and extracranial tissues during and after chemical dural irritation remains unknown. Hence the present study examines changes that occur in intracranial and extracranial sensitivities of brain stem trigeminal neurons after a brief exposure of the dura to inflammatory agents and how these changes are affected by local anesthetics. This study also examines whether neurons that are activated by dural stimulation project to the thalamus, hypothalamus, or other nuclei in the brain stem and thus might be involved in mediating the sensory, affective, and endocrine changes that occur during headache.

METHODS

Surgical preparation

Thirty-five male (20 cases) and female (15 cases) Sprague-Dawley rats weighing 350–700 g were anesthetized with urethan (1.2 g/kg ip) and treated with atropine (0.04 mg ip). A metal tube was inserted into the trachea for artificial ventilation, and the rat was mounted in a stereotaxic apparatus. End-tidal CO2 was kept at 3.5–4.5%. Core temperature was kept at 37°C. The laminar process of C1 and the ventral portion of the occipital bone were removed to allow the introduction of a recording electrode into nucleus caudalis. Large portions of the frontal and parietal bones were removed on both sides to allow mapping of the dural receptive field in the first part of the experiment and the introduction of the antidromic stimulating electrode in the second part. Usually, the bone was removed from bregma (rostrally) to the cerebellum (caudally) and 4 mm from the midline, bilaterally (as shown in Fig. 8). The dura overlying the dorsal surface of the brain was covered with warm saline. Rats were paralyzed with either gallamine triethiodide (0.5 g/kg ip injections) or 1:1 combination of doxacurium chloride and pipecuronium bromide (0.5 mg/kg iv) and artificially ventilated (room air).

Neuronal identification

Single-unit activity was recorded in the dorsal horn 0–2.5 mm caudal to the obex with stainless steel epoxy-coated microelectrodes (8–12 MΩ). To identify dura-sensitive neurons, the recording microelectrode was advanced into the dorsal horn while single shocks (0.8 ms, 0.5–4.0 mA, 1 Hz) were delivered repeatedly through a bipolar stimulating electrode placed on the dura overlying the ipsilateral transverse sinus (Fig. 1A). These stimulus parameters were capable of activating both Aδ and C fibers that innervate the dura (Strassman et al. 1996). Suprathreshold single-shock stimuli typically evoked an early burst of action potentials, which began at latencies of 5–9 ms (thought to be initiated by Aδ fibers), followed by a number of later action potentials the earliest latencies of which were ~18 ms (thought to be initiated by C fibers). On the basis of measurements of the distance between the dural stimulation site and the trigeminal ganglion (12.5 mm) and between the trigeminal ganglion and the central recording site (15–17 mm), it was calculated that the latencies of these early and late discharges corresponded approximately to that expected for action potentials initiated by Aδ (2.0 m/s) and C fibers (0.5–2.0 m/s), respectively. These calculations assume a straight line between the trigeminal ganglion and nucleus caudalis (Vc); without a loop through the trigeminal subnuclei oralis and interpolaris.

Receptive fields mapping

After determining whether the neuron received Aδ- or C-fiber input, stimulation was stopped for 10 min to allow the neuron to recover from the possible sensitizing effects of the search stimuli. The dural receptive field was then mapped with electrical and...
Mechanical and thermal stimulation

After the receptive fields were mapped, the response properties of the neurons were studied by determining their mechanical and thermal thresholds and by classifying them according to their responsiveness to graded intensities of mechanical and thermal stimuli. Measurements of neuronal responses to these quantitative sensory stimuli usually began 45 min after the last electrical stimulus was delivered to the dura. Mechanical thresholds for dural stimulation were determined with a series of calibrated von Frey hairs (Stoelting, tip shape: flat and round, diameter range: 0.15–0.38 mm) applied to the most sensitive part of the dural receptive field in ascending order (0.080, 0.176, 0.217, 0.445, 0.745, 0.976, 2.35, and 4.19 g). Each von Frey hair was applied to the dura once for 5 s. The response magnitude was determined by dividing the total number of spikes by the number of seconds the dura was indented (i.e., mean spikes/second). The threshold value was considered the lowest strength von Frey hair that elicited at least one burst of spikes in ≥50% of the trials.

Responses to mechanical stimulation of the skin were determined by applying brief (10 s) innocuous and noxious stimuli to the most sensitive portion of the cutaneous receptive field. Innocuous stimuli consisted of slowly passing a soft bristled brush across the cutaneous receptive field (one 5-s brush stroke from caudal to rostral and one 5-s brush stroke from rostral to caudal) and pressure applied with a loose arterial clip. Noxious stimuli consisted of pinch with a strong arterial clip and squeeze with nonserrated forceps. More intense or prolonged stimuli were not used to avoid inducing prolonged changes in spontaneous neuronal discharge or response properties. Neurons classified as low threshold (LT) responded maximally or exclusively to innocuous stimulation. Neurons classified as wide-dynamic range (WDR) responded to brushing but responded at higher frequencies to noxious stimulation. Neurons designated as high threshold (HT) did not respond to brushing but responded to more intense mechanical stimuli (pressure, pinch, and squeeze) of their cutaneous receptive fields (Dado et al. 1994b; Palecek et al. 1992). After the responses to mechanical stimuli were determined, the thermal thresholds were assessed. The cutaneous receptive fields were determined by slowly heating (35–50°C at a rate of 1.0°C/s) or cooling (35–0°C at a rate of 1.5°C/s) the cutaneous receptive field with a 9 × 9 mm contact thermal stimulator (Yale University). Thermally conductive paste was applied to the skin and the stimulated surface was maintained at 35°C during the periods between stimuli. Temperatures at which neuronal activity increased by 50% over baseline (i.e., mean number of spikes/second during the 30-s period before the initiation of the stimulus) were considered as thresholds. Thermal responses were determined by rapidly (10°C/s) heating (to 37, 39, 41, 43, 45, 47, 49, and 51°C) or cooling (to 33, 31, 29, 27, 25, 23, 21, 19, 17, 15, 13, 11, and 0°C) the skin in steps of increasing intensity (Burstein et al. 1991). The interstimulus interval between these steps was 180 s. To avoid sensitizing the cutaneous receptive field, thermal stimulation was also brief (10 s). To calculate the response magnitude to each stimulus, the mean ongoing activity occurring before the onset of the first stimulus (10 s for mechanical, 30 s for thermal, 100 s for chemical) was subtracted from the mean firing frequency that occurred throughout the duration of each stimulus. Action potentials were amplified, sent to a window discriminator, collected by computer, analyzed quantitatively by Neurospike software (PSTH software), and presented as peristimulus time histograms (500-ms binwidth).

Chemical stimulation and sensitization

Potentially sensitizing chemicals were applied topically to the dura for a period of 2–5 min. These chemicals included the inflammatory soup (IS; histamine, serotonin, bradykinin—10−3 M, prostaglandin E2—10−5 M, at pH 5.0) (adapted from Steen et al. 1995), and low pH (pH 4.7) phosphate buffer (PB) (Steen et al. 1992, 1995). When both chemicals were used, the dura was exposed to the acid PB (2 min) first, rinsed with saline (10 min), and then exposed to the IS (2 min). Responses to chemical stimuli were considered positive if the mean firing frequency during the 2-min period in which chemical agents were applied to the dura increased by ≥50% over the mean background activity occurring 100 s before the stimulus. To prevent the chemicals from spreading outside the dural receptive field, the position of the head was adjusted so that the dorsal surface of the dura was perfectly horizontal, a wall of agar was built around the craniotomy, and a small piece of gelatin sponge soaked in the chemicals was then placed on the dura.

To determine the effects of chemical irritation of the dura on the responsiveness of the dura-sensitive neurons in Vc, dural and cutaneous receptive fields were mapped again, and all sensory stimuli described above were repeated. The sizes of the dural and cutaneous receptive fields were examined at 30 and 60 min in the first 15 experiments and at 60, 120, 180, and 240 min in the last 8 experiments. Thresholds to mechanical stimulation of the dura were tested every 10 min for the first hour after the application of the IS or the PB and then every hour until the end of the experiment. Cutaneous thermal thresholds usually were examined 15–30 min after the chemical irritation of the dura, and the responses to innocuous and noxious cutaneous mechanical stimuli were examined 15, 30, and 60 min after dural stimulation and then every hour until the end of the experiment. To minimize the possibility that our repeated stimuli could cause a change in the neuron’s responsiveness, stimuli that were least likely to produce lasting changes were applied first (i.e., mechanical stimulation of the dura and the skin). Thresholds to thermal stimulation of the skin always were determined last.

Lidocaine application

To determine the contribution of the incoming impulses from the dura (Strassman et al. 1996) to the changes that occurred in the responses of cutaneous neurons, we anesthetized the dural primary afferent fibers and then tested the responsiveness of five central neurons to stimulation of their cutaneous receptive fields and their ongoing activity. The peripheral branches of the dural primary afferent neu-
rons were anesthetized by applying gel foam soaked with 5% lidocaine to the dural area that was exposed to the inflammatory soup. This concentration was used because lower (1–2%) concentrations of lidocaine failed to anesthetize the dura. Inability to induce neuronal responses by stimulating the dura with suprathreshold mechanical stimuli was used to confirm that the dural primary afferent fibers were anesthetized. The ability to induce neuronal responses by stimulating the cutaneous receptive field was used to confirm that the trigeminal ganglion was not affected and that the anesthesia had not spread outside the dura. The interval between the application of the IS and the lidocaine was 1 h in two cases and 2 h in three cases. After the application of lidocaine, mechanical threshold to dural indentation, cutaneous receptive field size, the response profile of the neuron to mechanical stimulation, and the thresholds to thermal stimuli were determined at 30-min intervals for 2 h.

**Antidromic stimulation and anatomic analysis**

Finally, the projection targets of the dura-sensitive neurons were determined by using the same antidromic microstimulation mapping technique that was described in Burstein et al. (1991), Dado et al. (1994a), and Fields et al. (1995). In brief, one unipolar stimulating electrode was moved systematically through the midbrain and an antidromic stimulus (cathodal pulse 500 μA, 200 μs, 10 Hz) was delivered until a point was found at which the antidromic threshold was ≈30 μA. A second stimulation electrode then was lowered into the diencephalon and systematically moved from medial to lateral (300-μm intervals), from dorsal to ventral (200-μm intervals) and from anterior to posterior (500-μm intervals) to determine whether the axon continued to the hypothalamus or thalamus (or both). When several low-threshold points were found in the diencephalon, antidromic spikes elicited from these points were collided with antidromic spikes elicited from the midbrain to ensure that all spikes were generated by the same neuron.

At the conclusion of each experiment, the recording site and the low-threshold point(s) for antidromic activation were marked with electrolytic lesions (anodal DC of 25 μA for 20 s). The distances between the low-threshold points and the recording site were measured for calculating conduction velocities (CV). Rats were perfused with 1% potassium ferrocyanide in 10% formalin. The brain and brain stem were removed, fixed, and reacted for Prussian blue stain of ferric ions. The tissue was cut transversely (50 μm) on a freezing microtome and counterstained with Neutral red. Non-stained and Nissl-stained sections containing the lesions were examined with dark- and bright-field illumination, respectively. Locations of lesions were reconstructed by use of a camera lucida.

**Statistical analysis**

Data are presented as means ± SE for arithmetic averaging and compared statistically with the parametric Student’s *t*-test for all but the von Frey hair measurements. Von Frey hair measurements are compared with the nonparametric Wilcoxon signed-rank test.

**RESULTS**

**Neuronal identification**

Thirty-five trigeminal brain stem neurons that responded to electrical stimulation of the dura (Fig. 1) were identified. In 23 cases, physiological baseline was established and the neurons were tested for sensitization, in 8 cases, sensitization was not tested, and in 4 cases, the initial level of ongoing activity was so high that it was not possible to study the
neurons further. Because the focus of this study was to determine the changes that occur in central trigeminal neurons after chemical irritation of the dura, only the last 23 neurons are described in detail. Of these 23, 17 neurons (14 WDR and 3 HT) that exhibited a reproducible burst of discharges at 5–15 ms and a unitary discharge at 18–60 ms were classified as receiving Aδ- and C-fiber inputs, 1 neuron (HT) exhibited only the early discharge and was classified as receiving only Aδ-fiber input, and 3 neurons (2 WDR and 1 HT) exhibited only the late (18–60 ms) discharge and were classified as receiving C-fiber input only.

Once a dura-sensitive neuron was identified, it was necessary to determine how stable the sensory properties of the neuron were over time without the chemical stimulation. In three experiments, the threshold to mechanical indentation of the dura, the response profile to innocuous and noxious mechanical stimulation of the skin, the thresholds to heating the cutaneous receptive fields, and the spontaneous activity rate were examined every 30 min for a 2-h period (5 min interstimulus interval). Figure 2 illustrates the results of one of these experiments. As demonstrated by the figure, the neuron maintained its sensory properties throughout the 2-h period in spite of the repetitive stimulation. Five series of innocuous and noxious mechanical stimulation of the skin induced in this neuron similar responses (Fig. 2A), its thresholds to mechanical indentation of the dura remained at 2.35 g (Fig. 2B), its threshold to heating of the skin remained at 47–48°C (Fig. 2C), and its ongoing activity remained <1 spike/s (Fig. 2D). In six additional experiments, stimulation of the dura and the skin were repeated two to three times before the chemical irritation of the dura. In these cases, responses to brush, pressure, and pinch varied minimally. Thresholds to dural indentation and rates of ongoing activity did not change. Thresholds to hot and cold stimulation did not change in 70% of the cases and varied by 1–2°C in 30% of the cases. The response properties of all 23 neurons described in this study were consistent. In seven cases, responses to repeated mechanical and thermal stimulation were inconsistent. These later cases were not studied further for lack of a baseline value.

Chemosensitivity

In the 23 cases in which chemical stimuli were applied topically to the dural receptive field, 15 neurons were exposed to both low-pH PB (pH 4.7) and IS (histamine, serotonin, bradykinin—10−5 M, prostaglandin E2—10−7 M, at pH 5.0), 4 neurons were exposed only to IS, and 4 neurons were exposed only to the low-pH PB (Table 1). Each chemical usually was applied to the dorsal surface of the dura for a 2-min period. Seventy percent (16/23) of the neurons were chemosensitive; 6 responded to the IS only, 3 responded to the PB only, and 7 responded to both. Figure 3 illustrates four neurons that exhibited different patterns of response to the chemical stimuli. As indicated in the figure, topical application of a soup of inflammatory agents (Fig. 3, A and D) or low-pH PB (Fig. 3, B and C) induced an immediate response in 16 chemosensitive neurons. In 10 cases, responses were short, lasting the duration of the stimulus only (Fig. 3, A and B), in 4 cases, they were long, lasting the stimulus by ~10 min (Fig. 3C), and in 2 cases, responses were characterized by long periods of bursting and short periods of pause in activity (Fig. 3D). Usually, neurons exhibiting oscillating responses after the chemical stimulation of the dura had a tendency to show oscillations in ongoing discharge before the dural stimulation (see Fig. 3D).

Changes in neuronal responses induced by chemical irritation of the dura

INCREASED SENSITIVITY TO MECHANICAL STIMULATION OF THE DURAL RECEPTIVE FIELD. The effect of chemical stimulation of the dura on dural mechanosensitivity was examined in 23 neurons (16 WDR, 5 HT, and 2 LT) by indenting the dura with von Frey hairs. In most cases, dural indentation with von Frey hairs induced a rapidly adapting response consisting of a 2- to 5-s burst of spikes (Fig. 4A). Responses to dural indentation with varied forces were examined before topical application of chemical agents to the dura and 10–60 min after the chemical agents were removed (Table 1 and Fig. 4A, left vs. right columns). Before the chemical stimulation, mechanical indentation of the dura with forces of ~4.19 g induced responses in 20 neurons (87%). After the chemical stimulation of the dura, the neurons showed a significant increase in their sensitivity to dural indentation (Fig. 5A) as the minimum force (threshold) required to activate them dropped ~70%, from 1.57 to 0.49 g (means, unpaired Wilcoxon signed-rank test, P < 0.0001). Similar changes in the mechanosensitivity of the neurons to dural stimulation were found when HT, WDR, and LT neurons were analyzed separately. Mechanical thresholds dropped from 1.6 to 0.2 g (means) for HT (87.5%), from 1.55 to 0.6 g for WDR (61%), and from 1.54 to 0.2 g for LT neurons (87%). An example of these changes is illustrated in Fig. 4A. In this case, the threshold to mechanical stimulation of the dura was 2.35 g (Fig. 4A, left) before the application of low-pH PB to the dura, and then dropped to 0.217 g (Fig. 4A, right) 20 min after the chemical irritation. Finally, the three mechanoinsensitive neurons became mechano-sensitive after the chemical stimulation of the dura.

This intracranial hypersensitivity usually developed gradually within 20–30 min (Fig. 5B). It lasted up to 1 h in 12 cases (10 WDR, 1 HT, and 1 LT), up to 2 h in 5 cases (3 WDR, 2 HT, and 1 LT), up to 3 h in 4 cases (2 WDR and 2 HT), and longer than 5 h in 2 cases (1 WDR and 1 HT). An example of a short- and a long-lasting mechanical hypersensitivity to dural indentation is illustrated in Fig. 5B. After the application of IS to the dura for 2 min, the threshold of one neuron (WDR, ○) dropped from 2.35 to 0.217 g in 30 min. The mechanical threshold returned to 2.35 g within 90 min. The threshold of the second neuron (HT, ▲) dropped from 0.745 to 0.217 g within 30 min and remained at 0.217 g for 7 h.

In addition to the drop in threshold, the intracranial hypersensitivity also was reflected by the increase in the magnitude of the responses to the dural indentation (Fig. 5C). The response magnitude was determined by dividing the total number of spikes by the number of seconds the dura was indented (i.e., mean spikes/second). Complete data were recorded for these stimulus-response curves in 16 cases (11 WDR, 3 HT, and 2 LT). As indicated, chemical stimulation lowered the threshold and increased the response to supra-threshold stimuli two- to fourfold.
TABLE 1. Summary of all experiments in which chemical stimulation was applied to the dura

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NR, no response during chemical stimulus; R, response during chemical stimulus; blank boxes, not tested; Vc, nucleus caudalis; Vi, nucleus interpolaris; lat, lateral lesions that cannot be assigned to a particular lamina. VHF, von Frey hair; IS, inflammatory soup; PB, phosphate buffer. * Brush (Br), pressure (Pr), pinch (Pi), and squeeze (Squ) values are in mean spikes per second; heat and cold are in degrees Celsius.

INCREASED SENSITIVITY TO MECHANICAL STIMULATION OF THE CUTANEOUS RECEPTIVE FIELD. The effect of chemical stimulation of the dura on cutaneous mechanosensitivity was examined in 20 neurons (5 HT, 14 WDR, and 1 LT). Responses to innocuous and noxious mechanical stimulation of the cutaneous receptive field were examined before topical application of the chemical agents to the dura and 15–30 min after the chemical agents were removed. After the dural stimulation, the neurons showed significant increases in their responses to brush (2.5-fold, P < 0.05) and pressure (1.6-fold, P < 0.05) with no significant changes in their responses to pinch or squeeze (Table 1, Fig. 6). However, when this analysis was made separately for HT (A) and WDR (C) neurons (Fig. 6), one difference was found. Although HT neurons showed significant increases in their responses to brush (50-fold, P < 0.05), pressure (1.7-fold, P < 0.05), and pinch (1.5-fold, P < 0.05), WDR neurons showed significant increases in their responses to brush (2.4-fold, P < 0.05), and pressure (1.6-fold, P < 0.05) only. An example of the changes that occurred in the cutaneous mechanosensitivity of one WDR neuron is illustrated in Fig. 4B. In this case, the neuron responded maximally to noxious chemical stimulation of the skin (Fig. 4B, left) before the application of low-pH PB to the dura. However, 25 min after the chemical irritation its response to brushing the cutaneous receptive field increased fourfold, becoming as large as its response to noxious stimulation (Fig. 4B, right). As a result of the increase in the responses to innocuous and noxious mechanical stimulation, the classification of 11 neurons was changed: 1 HT and 6 WDR neurons were changed to LT and 4 HT neurons were changed to WDR. Of the remaining nine neurons, five WDR units responded more vigorously after the chemical irritation of the dura although their classification did not change, and the responses of three WDR and one LT units did not change. Thus 4/20 neurons (20%) did not exhibit extracranial cutaneous hypersensitivity to mechanical stimulation.

INCREASED SENSITIVITY TO THERMAL STIMULATION OF THE CUTANEOUS RECEPTIVE FIELD. The effect of chemical stimulation of the dura on thermosensitivity was examined in 12 neurons (Table 1). Responses to slow heating (1°C/s) of the cutaneous receptive field were examined before topical application of chemical agents to the dura and 20–30 min after the chemical agents were removed. After the chemical stimulation of the dura, the neurons showed a significant increase in their sensitivity to heating as their thresholds dropped from 43.7 ± 0.7°C (mean ± SE) to 40.3 ± 0.7°C (P < 0.005, Student’s paired t-test). Figure 7A (○) shows the changes in thresholds to heat stimulation of all 12 neurons. In nine cases, heat thresholds dropped, in two cases, they were unchanged, and in one case, the neuron did not respond to heat before or after the chemical irritation of the dura. An example of a change in the threshold to hot stimulation is illustrated in Fig. 4C. Initially, the thermal threshold of this neuron to slowly heating the cutaneous receptive field from 35 to 45°C was 42°C (Fig. 4C, left). However, 30 min after the chemical irritation of the dura, the threshold to heat dropped to 39°C and a threefold increase in neuronal activity was registered (Fig. 4C, right). Figure 7B illustrates an example of the effect of dural irritation on the magnitude of the response to heat stimulation of the skin. Two series of heat stimuli were applied to the skin before the chemical irritation of the dura (open circles). The first was used to determine the baseline, and the second was used to show that the first series did not sensitize the skin (stimulation lasted for 10 s only). Twenty minutes after the application of the IS to the dura, another series of heat stimuli was applied (filled squares). This series shows the increased response to identical thermal stimuli.

In 11 cases, responses to slow cooling (1.5°C/s) of the cutaneous receptive field were examined before topical application of chemical agents to the dura and 20–30 min...
after the chemical agents were removed (Table 1). After the chemical stimulation of the dura, the neurons showed a significant increase in their sensitivity to cold stimulation as their thresholds changed from $23.7 \pm 3.3^\circ C$ (mean $\pm$ SE) to $29.2 \pm 1.8^\circ C$ ($P < 0.05$, Student’s paired t-test). Figure 7A (●) shows the changes in threshold to cold stimulation of the 9/11 (82%) neurons that responded to the cold stimuli. As shown, 7/9 neurons (77%) became more sensitive to the cold stimulus after the chemical irritation of the dura, one neuron became less sensitive, and the sensitivity of one neuron did not change. An example of a change in the threshold to cold stimulation is illustrated in Fig. 4D. Initially, the thermal threshold of this neuron to slowly cooling its cutaneous receptive field from 35 to 0°C was 21°C (Fig. 4D, left). However, 35 min after the chemical irritation of the dura, the threshold to cold changed from 21 to 33°C and the magnitude of the response increased (Fig. 4D, right).

**ONGOING ACTIVITY.** The ongoing activity of the neurons following the topical application of the IS or the low-pH PB to the dura was recorded in 19 cases (13 WDR, 4 HT, and 2 LT). The ongoing activity rate of 13/19 neurons (68%) increased after the chemical stimulation of the dura. Before chemical stimulation, 14 (74%) of the neurons had little or no ongoing activity (0–3 spikes/s) and 5 neurons (26%) had an ongoing activity rate of 8–28 spikes/s. Of the first group, 9/14 neurons (64%) exhibited an increased rate of ongoing activity and 5/14 neurons (36%) did not. Of the second group, 4/5 neurons (80%) exhibited an increased rate of ongoing activity and one neuron did not. Among the different classes of neurons, 77% (10/13) of the WDR neurons and 75% (3/4) of the HT neurons developed increased spontaneous activity. In contrast, the two LT neurons did not.

**RECEPTIVE FIELDS.** The extracranial and intracranial excitatory receptive fields of the 23 neurons that were tested for sensitization are illustrated in Fig. 8. Intracranial receptive fields were generally small. They spanned 1–2 mm in most cases and 2–6 mm in a few cases. They were restricted to the dura overlying the transverse sinus in 8 cases, included the transverse sinus and adjacent areas in 11 cases, were anterior or posterior to the transverse sinus in 3 cases, and included the superior sagittal sinus in 2 cases. All dura-sensitive neurons had cutaneous receptive fields. Cutaneous receptive fields included skin areas innervated by one ($n = 10$), two ($n = 7$), or all three ($n = 6$) divisions of the trigeminal nerve. In general, the most common receptive field site (95%) was found on areas innervated by the ophthalmic branch of the trigeminal nerve (V1). In 9 cases, cutaneous receptive fields were restricted to the territory of V1, and in 21 cases they were found to be most sensitive within the territory of V1. Nevertheless, nearly 60% (14/23) of the dura-sensitive neurons exhibited receptive fields on skin areas innervated by the maxillary (V2) and/or the mandibular (V3) branches.

After the application of the IS or the low-pH PB to the dura, intracranial (dural) receptive fields expanded in 10/15 cases, and extracranial (cutaneous) receptive fields expanded in 4/8 cases. The expanded areas of the receptive fields are illustrated by the light gray color in Fig. 8. Although expanded dural receptive fields were recorded in 3/5 HT neurons, 2/2 LT neurons, and 5/16 WDR neurons, expanded cutaneous receptive fields were recorded only in WDR neurons (see row 3, columns 1–4 in Fig. 8). And although dural receptive fields expanded within 30 min from the time chemical irritants were applied to the dura, the cutaneous receptive fields required 2–4 h before changes were observed. Expanded cutaneous receptive fields always were accompanied by sustained sensitization to mechanical and heat stimulation. In the first 15 experiments, cutaneous receptive fields were mapped only at 30 and 60 min after the application of the chemical irritants to the dura; this may explain the failure to observe expanded receptive fields in these neurons.

**Lidocaine effects**

In five cases in which intracranial and extracranial sensitization lasted for 2–5 h, successful attempts were made to anesthetize the primary afferent fibers that innervate the dura. In these cases, 5–10% lidocaine was applied to the external surface of the dura one (2 cases) or two (3 cases) hours after the sensitization was initiated by the chemical stimulation. Figure 9 illustrates the effects of anesthetizing the dura on the sensitivity of one neuron. In the illustrated case, the neuron had an ongoing activity rate of ~2 spikes/s,
FIG. 4. Development of intracranial and extracranial hypersensitivity after chemical irritation of the dura. Comparisons of physiological responses of a dura-sensitive neuron in lamina V of nucleus caudalis (Vc) that projects to the hypothalamus (top row). Responses of the neuron to a graded increase in the intensity of mechanical indentation of the dura (A), mechanical stimulation of the skin (B), and slowly heating (C) and cooling (D) the skin are shown before (left) and after (right) the irritation of the dura with the low pH buffer. Black area in the hypothalamus depicts low-threshold point for antidromic activation, black dot in the brain stem depicts recording site, black areas on the skin and dura depict sizes and locations of receptive fields before the chemical irritation of the dura, and gray area on the dura depicts the expanded receptive field after the chemical irritation. Numbers above lines in A indicate forces of von Frey hairs, boxes in A depict the mechanical threshold, and numbers under lines in B indicate mean number of spikes per second in response to each stimulus. Arrowheads in C and D show the temperature at which a response occurred. Note the drop in the mechanical threshold of the dural receptive field, the exaggerated response to brushing the skin, and the drop in the thresholds for heating and cooling the skin. HYP, hypothalamus; Br, brush; Pr, pressure; Pi, pinch; Cr, squeeze; VBC, ventrobasal complex.

a mechanical threshold to dural stimulation of 0.976 g, and a low rate of response to brushing its cutaneous receptive field. After a 2-min application of low-pH PB to the dura, the ongoing activity increased to ~30 spikes/s, the threshold to mechanical stimulation of the dura dropped to 0.217 g, and the response to brushing the skin increased fivefold. One hour later, lidocaine was applied topically to the dura. Fifteen minutes later, suprathreshold electrical and mechanical stimulation of the dura failed to induce a neuronal response, the ongoing activity rate remained elevated although it dropped 17%, and the response to brush resembled that of the sensitized state. The neuronal responses and the ongoing activity remained elevated 30, 60, 90, and 120 min after the dura was anesthetized. In the other cases in which lidocaine was applied to the dura after the establishment of a sensitized state, similar results were recorded (Table 1, last 5 rows). The responses to brush that increased sevenfold ($P < 0.05$) after the chemical stimulation of the dura, remained elevated...
(fivefold) after the lidocaine application to the dura. Statistical comparisons between the three conditions shows that after the lidocaine application to the dura, the responses to brush differed \((P < 0.005)\) from the responses to brush before but not after the chemical stimulation of the dura.

**Location of recording sites**

Recording sites were identified for 34 dura-sensitive neurons in the trigeminal Vc and first cervical segment (C1). Figure 10 illustrates the recording sites of 22 neurons that were sensitized (black dots), 4 neurons in which the spontaneous activity rate was too high to allow further examination (stars), and 8 neurons that were characterized physiologically but not tested for sensitization (gray dots). The location of one dura-sensitive neuron that was sensitized was not recovered. Examples of lesions at recording sites in laminae I-II and IV are illustrated in Fig. 11, G and H. As shown in Fig. 10, all sensitized neurons were recorded in the ventrolateral part of C1 dorsal horn and Vc. Along this area, 1/23 neurons was recorded at the level of Vc/Vi transition zone, 4/23 neurons (22%) were found in the rostral half of Vc (0–1 mm caudal to Vc/Vi transition zone), 13/23 neurons (57%) were located in the caudal half of Vc (1–2 mm caudal to Vc/Vi transition zone), 3/23 neurons (16%) were recorded in the C1/Vc transition zone (1.75–2.25 mm caudal to Vc/Vi transition zone), and 1/23 neurons was in C1. Within the different laminae, the locations of lesions marking the recording sites of the sensitized neurons were assigned to laminae I-II in 3 cases, laminae III-IV in 1 case, and lamina V in 12 cases. One lesion included the laminae II/III border, two lesions included the laminae IV/V border, and three lesions included the most medial portion of laminae I-II (ventrolaterally), the most ventrolateral area of laminae III-IV, and the nearby corner of lamina V. These six lesions were not assigned to any particular lamina. The neurons that were not tested for the sensitizing effects of the inflammatory mediators or the PB were located in laminae I-II and V of C1 and in the transition zone between C1 and Vc.

**Projection sites**

In 14/20 attempts (70%), dura-sensitive neurons were antidromically activated from the diencephalon and the course of their axons was mapped (Burstein et al. 1990; Fields et al. 1995). Of these, seven neurons were exposed...
only, two from the hypothalamus only, two from both the thalamus and hypothalamus, and two from the parabrachial area. Of the seven neurons that were not tested for sensitization, three were activated antidromically from the hypothalamus only, three from both the hypothalamus and deep mesencephalic area in the midbrain, and one from the hypothalamus and thalamus. Figure 12A illustrates the antidromic activation of a sensitized dura-sensitive neuron and the mapping of its axon in the hypothalamus and the thalamus. Low-threshold points for antidromic activation of all 14 neurons are illustrated in Fig. 12B. They were found in the ventro-posterior medial (VPM) and parafascicular (PF) thalamic nuclei, lateral preoptic, anterior, lateral, periforsimal and caudal hypothalamic areas, and the pontine parabrachial region. Most axons reached the caudal diencephalon through the central tegmental field (a poorly defined area between the central gray and the medial geniculate nucleus) and the cerebral peduncle. Those projecting to the thalamus issued collateral branches that crossed zona incerta. Those projecting to the hypothalamus continued with the supraoptic decussation. Photomicrographs illustrating locations of low-threshold points in the hypothalamus, VPM, PF, zona incerta, internal capsule, and central tegmental field are shown in Fig. 11 (A—F).

**Discussion**

In this study, brief exposure of the dura to chemical agents associated with inflammation caused central dura-sensitive neurons to become more sensitive to mechanical stimulation of the dura and to mechanical and thermal stimulation of the skin, to increase their dural and cutaneous receptive field size, and to develop ongoing activity. These findings suggest that the occurrence of extracranial tenderness in headaches that have an intracranial component (evidence for intracran-
IAL COMPONENTS ARE STRONGEST FOR MIGRAINE AND POSSIBLY ALSO CLUSTER HEADACHE) DOESN’T NECESSARILY REQUIRE THE PRESENCE OF AN EXTRACRANIAL PATHOLOGY AND INSTEAD COULD RESULT FROM CENTRAL SENSITIZATION. BECAUSE THE HYPERSENSITIVITY TO CUTANEOUS STIMULATION REMAINED AFTER BLOCKING THE INPUT FROM THE DURA BY LOCAL ANESTHETICS, THESE FINDINGS FURTHER SUGGEST THAT THE SENSITIZATION OF THE CENTRAL NEURONS IS NOT A MERE REFLECTION OF INCREASED AFFERENT INPUT FROM THE DURA BUT THAT CENTRAL MECHANISMS MAY PARTICIPATE IN THE SENSITIZATION PROCESS.

high-threshold mechanosensitive nociceptors (Steen et al. 1992, 1995), and, furthermore, can have synergistic effects on the action of some of the agents included in the IS (Steen et al. 1995).

Theoretically, there are several alternative ways by which changes in the response properties of the central trigeminal neurons could have been induced. 1) To identify the neurons, electrical search stimuli were delivered to the dorsal surface of the dura at a rate of 1 Hz. This rate is usually sufficient to cause wind-up in sensory spinal cord dorsal horn neurons (Cook et al. 1987). To minimize the risk of causing wind-up in the recorded neurons, search stimuli were delivered for a short period and stopped every few minutes. After a neuron was found to respond to the electrical stimulation, the stimulation was turned off and spontaneous activity was recorded in the absence of stimulation. In most cases, the spontaneous activity decreased during the following 5–10 min and then stabilized. 2) Because neurons were stimulated repeatedly during several hours, changes in neuronal activity could have occurred as a result of the repetitive stimulation of the receptive fields. To test this possibility, brief innocuous and noxious stimulation were applied repeatedly to the dura and the skin during several hours. As shown in Fig. 2, when chemical irritants were not applied to the dura, responses properties and spontaneous activity rates of the neurons remained stable in spite of the repeated stimulation. Moreover, in eight of nine experiments in which thermal stimulation was not used at all, the neurons showed changes after chemical irritation of the dura that were similar to the changes recorded in the neurons that were exposed to the thermal stimulation. 3) Because in many cases, cutaneous receptive fields were in proximity to the surgical exposure of the dura and because trigeminal neurons receive convergent input from regions proximal to the surgical exposure of the brain stem, it is possible that some degree of sensitization was induced by the surgery. In fact, in several cases, neurons seemed sensitized at the beginning of the recording session, as their baseline response profile and spontaneous activity

FIG. 9. Lidocaine effects. A: recording site. B: intracranial receptive field. C: extracranial receptive field. D and E: responses to chemical stimulation of the dura with low-pH PB. G and H: responses to mechanical stimulation of the dura and the skin 20 min after the irritation of the dura. I: responses to local anesthetics (5% lidocaine) applied to the dura. J and K: responses to mechanical stimulation of the dura and the skin 1 h after the dura was anesthetized. Black area in C indicates most sensitive region of the receptive field and gray area indicates less-sensitive regions. Numbers above lines (D, G, and H) indicate forces of mechanical stimuli. Numbers in parentheses below lines indicate the mean number of spikes/s. Note that in spite of blocking the dural inputs, the spontaneous activity rate and the hypersensitivity to brushing the skin remained elevated.
rate resembled that of neurons that had been exposed to the chemical irritation of the dura. These neurons were not studied further because any additional sensitization seemed to be masked. In the 23 cases presented in the study, changes induced by the surgery did not mask the ability to detect further changes after the application of IS or PB to the dura.

Because topical application of inflammatory agents to the dura could activate and sensitize Aδ and C fibers that innervate the dura (Strassman et al. 1996), it is likely that the development of hypersensitivity in the central trigeminal neurons was initiated by the incoming impulses. Alterations in the response properties of central trigeminal neurons by application of the inflammatory agents to the dura include increased responsiveness to innocuous mechanical stimulation of the skin, hypersensitivity to hot and cold cutaneous stimulation, and expanded cutaneous receptive fields. Because the primary afferent fibers that innervate the cutaneous receptive fields presumably are not affected by the local application of the IS to the dura, it is likely that this cutaneous hypersensitivity is mediated by the altered state of the central neurons (Neugebauer and Schaible 1988, 1990; Schaible et al. 1987; Simone et al. 1991). As the altered state of the central neurons can outlast the altered state of the peripheral neurons by several hours, the question remains whether the maintenance of these changes is completely or partially dependent on the incoming signals from the dural primary afferents. In cases in which the dural primary afferent fibers were anesthetized (as evidenced by the inability to induce neuronal responses to mechanical or electrical stimulation of the dura), elevation in spontaneous activity decreased by only 14–50% and cutaneous hypersensitivity to mechanical stimuli remained elevated. Although the partial decrease of spontaneous activity suggests that at least part of the ongoing activity in the central neurons is dependent on the ongoing peripheral input from the dura, the inability of the anesthesia to bring the neuron back to its initial rate of activity or to reduce the cutaneous hypersensitivity suggests that some aspects of the central changes are independent of the afferent inputs from the sensitized nociceptors (Hu et al. 1992; Thompson et al. 1990; Murase and Randic 1984; Woolf 1987). In agreement with this, Cook et al. (1987) and Woolf (1983) showed that even a brief input from primary afferents (especially unmyelinated) can alter the response properties of dorsal horn neurons for an extended period of time by shifting their membrane potential toward a more depolarized state.

Because evidence is now present for sensitization of both the peripheral and the central neurons in the development of sensitization in the trigeminovascular pathway, it is possible that both contribute to the common experience of exaggerated intracranial hypersensitivity during headache (Blau and Dexter 1981) as well as the throbbing nature of the pain. Because the smallest force capable of inducing activity in the altered central

![Fig. 10. Summary of the locations of lesions marking the recording sites of 34 dura-sensitive neurons in the upper cervical dorsal horn and in nucleus caudalis. Black dots, 22/23 neurons that were sensitized (1 lesion was not recovered); stars, 4 neurons that were not studied further because of the initially high level of ongoing activity; and gray dot, 8 neurons that responded to electrical stimulation of the dura only. Although it was not always possible to assign the lesions to a particular lamina, they were found mostly in the area that receives the heaviest projections from the ophthalmic branch of the trigeminal nerve.](http://jn.physiology.org/)

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neurons (0.08 g) did not produce a visible indentation of the sinus, it is not unreasonable to speculate that under conditions of extreme hypersensitivity, regular heart rate pulsation that momentarily changes the pressure on the dura can be sufficient to activate the sensitized dura-responsive peripheral nociceptors and consequently the central neurons. Theoretically, the pressure on the dura during normal pulsation can be induced by small brain movements that press the dura against the bone or by the small changes in intracranial pressure. Because the intracranial space is closed, any mechanical change is propagated throughout this space, and, consequently, the arterial pressure pulse can be seen in any recording of intracranial pressure (Daley et al. 1995). These hypotheses, however, could not be tested in the current study because of the craniotomy. When a craniotomy is performed, the brain cannot press the dura against the bone.

Unlike the intracranial hypersensitivity, sensitization of central neurons could contribute to the symptoms of extra-

FIG. 11. Photomicrographs illustrating lesions (•) of low-threshold points for antidromic activation (A–F) of 5 neurons and recording sites (G and H) of 2 neurons. A: lateral hypothalamus. B: thalamic ventro-posterior medial (VPM) nucleus. C: thalamic parafascicular (PF) nucleus. D: zona incerta. E: internal capsule. F: midbrain central tegmental field. G: recording site in lateral lamina V at the level of caudal nucleus caudalis. H: recording site in superficial layers of Vc (laminae I–II) at the level of Vc/trigeminal nucleus interpolaris (Vi) transition zone. In G, lines mark the approximate borders of lamina V and the ventrolateral border of laminae I–II. Round hole marks the recording site (lesion in this case is larger and not stained with blue because recording was made with tungsten electrode). Lines in H mark the border between Vi and Vc. Although laminar borders in Vc become less apparent at this level, dark-field view of this lesion revealed its location in laminae I–II (this lesion is $125 \mu m$ from the lateral end of lamina I). CP, cerebral peduncle; ctf, central tegmental field; IC, internal capsule; HYP, hypothalamus; OT, optic tract; PAG, periaqueductal gray; PO, posterior thalamic nucleus; VL, ventrolateral thalamic nucleus; ZI, zona incerta. Scale bar = 1,000 $\mu m$ for A–D, and F; 630 $\mu m$ for E; 700 $\mu m$ for G; and 570 $\mu m$ for H.
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cranial hypersensitivity that accompany some headaches. Because the mechanical hypersensitivity of WDR neurons was limited to brush and pressure but not to more intense mechanical stimulation of the skin and because the responses to brush often exceeded the responses to the noxious stimuli, it is possible that the WDR neurons play a role in the development of allodynia. Because the mechanical hypersensitivity of HT neurons included brush, pressure, and pinch (innocuous and noxious stimulation), it is possible that the HT neurons play a role in both allodynic and hyperalgesic changes in cutaneous mechanical sensitivity during headache. In fact, patients suffering certain headaches often report that touching their hair (unpublished observations) or applying innocuous pressure to periorcular regions is painful during the attack (Drummond 1987; Langemark and Olesen 1987; Langemark et al. 1989). Regarding the hypersensitivity to heat and cold, such changes in sensation are supported by a recent study (Langemark et al. 1989) reporting that hot pain thresholds were significantly lower (control subjects: 45°C, headache patients: 41°C) and cold pain thresholds significantly higher (control subjects: 15°C, headache patients: 22°C) in the temporal region of patients reporting high levels of headache on the examination day. Consistent with the notion that cold stimulation of extracranial structures can worsen the pain, it is known that some headache patients avoid cold food such as ice cream as it induces pain in the site usually affected by their regular headache (Drummond and Lance 1984; Raskin and Knittle 1976).

The present study suggests that sensitization of central neurons could contribute to the thermal hypersensitivity. Similar conclusions regarding the role of central neurons in hot and cold hyperalgesia were expressed previously by Frithtorfer and Lindblum (1984), LaMotte et al. (1991), Ochoa (1992), and Meyer et al. (1994).

Given the evidence for the altered state of the central neurons and the increased sensitivity to dural and cutaneous stimuli, it was not surprising to find that both dural and cutaneous receptive fields expanded. It seems that different mechanisms enabled these changes. The rapid expansion of the dural receptive field (within 30 min) could be mediated by the sensitized peripheral afferents (Strassman et al. 1996), by the recruitment of silent nociceptors (McMahon and Klotzenburg 1990a, b), and by the sensitization of the central cells (Cook et al. 1987; Grubb et al. 1993; Hoheisel and Mense 1989; Hylden et al. 1989; Laird and Cervero 1989; McMahon and Wall 1984; Schaible et al. 1987). The slower expansion of the cutaneous receptive fields (during 2–4 h) could be mediated by the sensitized central neurons as well as by alterations in supraspinal modulatory pathways induced by the afferent barrage (Ren and Dubner 1996). Although all three classes of neurons (WDR, HT, and LT) exhibited expanded dural receptive fields, only WDR neurons exhibited expanded cutaneous receptive fields. Although the sensitization of three of five HT neurons lasted for >3 h, their cutaneous receptive fields didn’t seem to expand. It is possible, however, that more time is required for receptive field expansion of HT neurons (Hylden et al. 1989). Nevertheless, because in other models of injury-induced plasticity the receptive fields of HT neurons expanded (Hu et al. 1992; Hylden et al. 1989; Neugebauer and Schaible 1990; Woolf and King 1990), it is also possible that the chemical irritation used in this study was too brief to expand the receptive fields of the more change-resistant HT neurons (Laird and Cervero 1989).

Based on the findings that most dura-sensitive neurons are located in the ventrolateral area of Vc and that their cutaneous receptive fields normally include the periorbital region (Davis and Dostrovsky 1986, 1988a, b; Lambert et al. 1992; Strassman et al. 1986), it was proposed that these dura-sensitive neurons play a role in mediating the referred pain of headache (Olesen et al. 1993). The present study, however, describes some dura-sensitive neurons with maxillary and mandibular receptive fields and some dura-sensitive neurons that expand their cutaneous receptive fields to these areas after chemical irritation of the dura. The presence of such neurons may explain the altered sensitivity in maxillary and mandibular regions during dural stimulation in awake patients (Wirth and Van Buren 1971) as well as during headache attacks (Anthony and Rasmussen 1993; Manzoni et al. 1981).

In most cases, recording sites of dura-sensitive neurons were found within the ventrolateral region of caudal Vc. The current notion is that as the homologue of the spinal dorsal horn, Vc processes most nociceptive information that arises

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FIG. 12. Projection sites of dura-sensitive neurons. A: an example of the axonal mapping of a sensitized dura-sensitive neuron. A1: line drawings illustrating the locations of penetrations that were made with a stimulating electrode at 3 anterior-posterior levels contralateral to the recording site. Drawing on top shows the 3 tracks of the stimulating electrode in the hypothalamus (0.5 mm posterior to bregma), and the locations of the 39 points from which antidromic threshold was determined. Drawing in the middle shows the 7 tracks in the thalamus (3.5 mm posterior to Bregma) and the locations of the 85 points from which antidromic threshold was determined. Drawing on bottom shows the 6 tracks in the midbrain (7.0 mm posterior to bregma) and the locations of the 82 points from which antidromic threshold was determined. Minimum current (μA) required to activate the neuron from each point within each penetration is indicated by the numbers along the tracks. Low-threshold points are marked by letters and circled in black. A2: oscillographic traces illustrating the antidromic action potentials that were elicited from each low-threshold point. Numbers in parentheses indicate the latencies for antidromic activation from each point. Parent axon of this neuron was activated antidromically from the hypothalamus (point A, 15 μA, 2.0 ms), zona incerta (point B, 12 μA, 1.4 ms), and midbrain (point H, 4 μA, 1.0 ms). At the level of the caudal diencephalon, a collateral branch was found in the thalamus (points D and E). Latencies for the antidromically induced spikes became progressively longer as the electrode moved from lateral to medial. A3: recording site in the ventrolateral area of Vc. A4: receptive fields and responses to mechanical stimulation of the dura and the skin. A, amygdala; AH, anterior hypothalamus; Aq, cerebral aqueduct; CG, central gray; CP, cerebral peduncle; DPME, deep mesencephalic nuclei; F, fornix; fr, fasciculus retroflexus; MGN, medial geniculate nucleus; ml, medial lemniscus; MT, mamillothalamic tract; NTS, nucleus of the solitary tract; PC, posterior commissure; PH, posterior hypothalamus; PVN, paraventricular hypothalamic nucleus; Px, pyramidal decussation; Snc, substantia nigra pars compacta; Snr, substantia nigra pars reticulata; SOD, supraoptic decussation; stn, subthalamic nucleus; 4n, trochlear nucleus (for other abbreviations, see previous Fig. legends).
in intracranial and extracranial organs innervated by the trigeminal nerve. Its somatotopic organization is such that the ophthalmic nerve terminates mainly in the ventrolateral region, the mandibular nerve terminates mainly in the dorsomedial region, and the maxillary nerve terminates in between (Marfurt 1981; Renehan et al. 1986). The dural stimulation site used in this study is innervated mainly by the tentorial nerve, a branch of the ophthalmic nerve (Andres et al. 1987; Penfield and McNaughton 1940). Therefore it was not surprising to find most recording sites in the ventrolateral region. In some cases, however, recording sites were found in more medial and dorsal regions of caudal Vc. Consistent with these recording sites are the locations of some receptive fields on skin areas innervated by the maxillary and mandibular nerves (Davis and Dostrovsky 1988a; Hu et al. 1981; Jacquin et al. 1989; Lucier and Egizii 1989; Mosso and Kruger 1973; Nagano et al. 1975; Renehan et al. 1986; Tabata and Karita 1991; Yokota 1975; Yokota and Nishikawa 1980) and the findings that dural stimulation induces c-fos in neurons located throughout most of the mediolateral extent of Vc. Although five neurons were recorded in the rostral Vc, only one was located in the Vc/Vi transition zone, another area activated by dural stimulation (Strassman et al. 1994). It is therefore beyond the scope of this study to comment on the role of more rostrally located neurons in the processing of intracranial information.

Finally, intracranial and extracranial exaggerated hypersensitivities are not the only symptoms associated with headache. Other symptoms include depression, intolerance, excitement, anxiety, apathy, irritability, sweating, hunger, thirst, increase in body temperature, and interrupted biological cycles such as menstrual, sleep-wake and melatonin secretion (reviewed in Olesen et al. 1993). Both the sensory and the integrated behavioral symptoms of headache suggest that the altered sensory information that originates intracranially and extracranially reaches brain areas involved in the processing of sensory, affective, endocrine, and autonomic functions. This study shows that sensitized dura-sensitive neurons in Vc project directly to the thalamus and hypothalamus and suggests that these neurons might mediate (at least in part) the altered somatic and visceral sensation and the changes in integrated behavior.

In this study, we have identified a group of neurons in the trigeminal nucleus caudalis that respond to dural stimulation, exhibit sensory plasticity that can be correlated with common headache symptoms, and project to sensory and limbic brain areas. Because these neurons may contribute to the symptoms of headache, efforts to relieve headache should take into consideration the need to block these centrally located neurons.

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