Primary Afferent Neurons Innervating Guinea Pig Dura

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Bove, Geoffrey M. and Michael A. Moskowitz. Primary Afferent Neurons Innervating Guinea Pig Dura. J. Neurophysiol. 77: 299–308, 1997. We made recordings from filaments of guinea pig nasociliary nerve to study response properties of afferent axons innervating the anterior superior sagittal sinus and surrounding dura mater. We analyzed 38 units in 14 experiments. Units were initially located with the use of mechanical stimuli, and were then characterized by their conduction velocity and sensitivities to mechanical, thermal, and chemical stimuli. Single-unit recordings revealed innervation of dura and superior sagittal sinus by slowly conducting axons, mostly in the unmyelinated range. The receptive fields were 1–30 mm², and typically had one to three punctate spots of highest sensitivity. All units tested responded to topical application of chemical agents. Ninety-seven percent of units responded to 10⁻⁵ M capsaicin, 79% responded to a mixture of inflammatory mediators, and 37% responded to an acidic buffer (pH 5). These data underline the importance of chemical sensitivity in intracranial sensation. Heat and cold stimuli evoked responses in 56 and 41% of units tested, respectively. Although the response patterns during heating were typical of polymodal nociceptors innervating other tissues, the thresholds were lower for other tissues (32.3–42°C). Cooling led to a phasic discharge, with thresholds between 25 and 32°C. Although units had different combinations of responses to mechanical, chemical, and thermal stimuli, when grouped by their sensitivities the groups did not differ regarding mechanical thresholds or presence of ongoing activity. This suggests that meningeal primary afferents are relatively homogeneous. Sensitivities of these units are in general consistent with nociceptors, although the thermal thresholds differ. These data provide the first detailed report of response properties of intracranial primary afferent units, likely to be involved in transmission of nociception and possibly mediation of intracranial pain.

INTRODUCTION

Intracranial blood vessels and meninges are innervated by the trigeminal nerve, and together have been termed the trigeminovascular system (Moskowitz 1984). The trigeminovascular system has been implicated in intracranial pain, and has been the topic of intense anatomic and pharmacological investigation (for reviews see Moskowitz et al. 1988, 1995). However, physiological sensitivities of trigeminal afferent neurons have not been directly studied, leaving a gap in the understanding of nociceptive processing from intracranial structures.

Classic experiments by Ray and Wolff (1940) and Penfield and McNaughton (1940) first demonstrated that meninges and intracranial blood vessels were sensitive. In those experiments on conscious humans, noxious mechanical and electrical stimulation of dura, major arteries, and venous sinuses elicited pain. Subsequent anatomic investigations demonstrated that intracranial blood vessels and connective tissues are innervated by a plexus of small-caliber axons that contain substance P and calcitonin gene-related peptide and have undifferentiated terminals (Andres et al. 1987; Dürring et al. 1990; Edvinsson et al. 1981; Liu-Chen et al. 1983, 1984; Meißlinger et al. 1993), much like skin and deep tissues (Kruger et al. 1989; Uddman et al. 1986). Intracranial pain is thought to be initiated from stimulation of these structures, because stimulation of similar afferent fibers innervating skin and deep tissues is known to lead to pain (Schmelz et al. 1994; Simone et al. 1994).

Functional data on intracranial primary afferent neurons are lacking. Extracellular recordings from trigeminal ganglion neurons have been reported, but the results were preliminary (Dostrovsky et al. 1990). Recordings from second-order trigeminal neurons in the nucleus caudalis revealed wide-dynamic range and nociceptive-specific neurons that responded to electrical stimulation of dural blood vessels and electrical and mechanical stimulation of face and/or cornea (Davis and Dostrovsky 1986; Strassman et al. 1986, 1994). Subsequent studies implied nociceptive properties of primary afferents by demonstrating responses of trigeminal nuclear neurons to mechanical and chemical stimuli of the superior sagittal sinus (Davis and Dostrovsky 1988a,b). Dorsal horn neurons respond to noxious stimulation of spinal dura and other spinal structures (Gillette et al. 1993), and there has been one report of primary afferent nociceptors innervating the spinal pia and ventral roots (Jänig and Koltenburg 1991). These data suggest that similar nociceptors may innervate intracranial connective tissues. If so, their properties may be essential to our understanding of factors that can initiate intracranial pain. The current experiments were carried out to define properties of guinea pig intracranial trigeminal primary afferent axons.

With the use of classical teased fiber techniques, recordings were made from filaments of the nasociliary nerve (NCN). Mechanical search stimuli were used on the dura mater overlying and surrounding the superior sagittal sinus to evoke unitary activity. Once isolated, units were characterized with the use of mechanical, thermal, and noxious chemical stimuli. These experiments revealed a population of slowly conducting units with response properties closely resembling nociceptors characterized in other body areas. Activation of these units is likely to provide the first signals in the pathway leading to intracranial pain.

Some of these data have appeared in preliminary abstract form (Bove and Moskowitz 1995).

METHODS

The experiments described herein were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care.
The experiments were carried out on 25 Hartley guinea pigs (450–650 g) of either sex. Guinea pigs were deeply anesthetized with urethan (1.5 mg/kg ip), ketamine (44 mg/kg im), and xylazine (5 mg/kg im). When the animal was areflexive to pinch of the upper lip by serrated forceps, it was intubated for ventilation and placed on a heated water pad (Gaymar). Respiration was monitored (Multinex, Datascpe) and maintained at 40 breaths per minute with room air supplemented to 60% O₂. Anesthesia was monitored throughout the experiment by periodic assessment of reflex contraction in response to pinch of the upper lip. Supplemental doses of urethan (0.25 mg/kg), ketamine (8 mg/kg), and xylazine (1 mg/kg) were given as indicated. The right eye was coated with petrolatum and the left lid was sealed shut with cyanoacrylate. The animal was placed in a custom-designed surgical head holder that allowed multiaxial rotation (Kopf, No. 323), and a midline incision was made to expose the skull. With the use of a dental burr, the skull was thinned in a rectangle 6–7 mm wide, from 2–5 mm posterior of the nasofrontal suture to 5–8 mm posterior of the coronal suture. The thinned bone was removed with fine forceps, exposing the dura mater covering the olfactory bulbs and part of the frontal lobes. The craniotomy was covered with cotton soaked in 0.01 M phosphate-buffered saline (PBS, 35°C, pH 7.4). Cranial periosteum was then reflected past the orbital margin to access the retroorbital space. The ocular muscles were reflected and the eyeball was removed. The venous plexus covering the nerve was ligated with 10/0 Ethilon suture (Ethicon) and reflected, revealing the NCN. The superior surface of the NCN was covered from its origin from the frontal nerve, leaving its inferior attachments and blood supply intact. The perineurium was removed from 3–4 mm of the NCN with sharpened forceps. Skin flaps were secured to a metal frame, and the pool between the orbital margin and the lateral skin was filled with 35°C mineral oil. Filaments (10–15 μm diam) were teased from the nerve and placed over a gold bipolar electrode for recording. The cotton covering the craniotomy was reflected and the dura was mechanically stimulated with the use of a glass rod with a 1-mm round tip (see below). When unitary activity could be evoked in response to this stimulus, characterization began. Filaments containing two units were also characterized if the waveforms differed enough to allow discrimination (see below). The dura remained covered with PBS-soaked cotton while filaments were being prepared and during most of the procedures to prevent drying. This cotton also allowed superfusion of the dura, used both for chemical application and rinsing. PBS, which never elicited affrent activity, could be pulled through this cotton with the use of vacuum, and was used for rinsing the tissue between stimuli. The experimental setup is illustrated in Fig. 1. At the end of the experiment, the animal was killed with an intracardiac overdose of ketamine.

**Experimental protocol**

After identification of a mechanically sensitive receptive field (RF), electrical stimulation suprathreshold for c fiber activation (0.01–0.1 ms, 30–60 V) was delivered to the most sensitive spot of the RF through a platinum bipolar electrode, with the use of a Grass S48 stimulator linked to an isolation unit. The stimulation intensity was set at the lowest intensity that produced zero conduction failures. Only units with a fixed latency to repeated stimulation (variation ≤ 0.4 ms) were accepted for further study (Fig. 2). The conduction velocity was determined with the use of this latency and the distance between the stimulating and recording electrodes. Collision methods could not be used in this experiment because of severe space constraints. The majority of filaments studied contained only one axon from which an action potential could be reliably evoked electrically, and the data acquisition program used (see below) facilitated adequate on-line visual comparison of electrically and mechanically evoked potentials. When the isolation of a unit was questioned, the program also allowed off-line statistical comparison of these potentials (Forster and Handwerker 1990).

In cases where more than one fiber was studied simultaneously, each waveform had to be clearly discernable by the investigator and the software. Filaments with more than two active fibers were exported to a graphing program (GraFit) and expressed as mean action failures. Only units with a fixed latency to repeated stimulation (Fig. 2) were accepted for further study (Forster and Handwerker 1990). The waveform times were exported to a graphing program (GraFit) and expressed as interval histograms.

**Mechanical stimulation**

Mechanical stimuli were applied with the use of a glass rod and with a set of von Frey-type filaments. The glass rod had a rounded tip (~1 mm diam), exerted a pressure of 150–300 kPa obtained by measuring the mass equivalent during a similar application on a scale covered by a 5-mm layer of silicone polymer (to more
...was warmed to 35°C. Between these groups, the data are presented together.

The chemosensitivity of the receptors was determined with the use of, in order, 35°C isotonic and acidic PBS (pH 5.0), a mixture of inflammatory mediators (INFM), and 10⁻³ M capsaicin. The INFM included bradykinin, histamine, serotonin, and prostaglandin E1 (Davis et al. 1993), all at concentrations of 10⁻³ M (Kessler et al. 1992) and dissolved in PBS. Aliquots of this mixture were stored at −70°C until immediately before use, and the mixture was warmed to 35°C before application. Solutions were applied topically to the RF with a small soaked cotton ball. If there was no response after 2 min, the solution was reapplied. If there was no response to topical application of all solutions, they were individually injected subdurally (0.1–0.2 ml) close to the RF. Because capsaicin can inactivate small-fiber afferents, and because the typical response was intense and had a short latency, it was usually rinsed off after <30 s. At the end of each stimulus, the area was superfused with 5–10 ml 35°C PBS. No other stimulus was presented for 4 min after rinsing, or less if the afferent activity had subsided or stabilized. After capsaicin stimulation, the cotton covering the dura was replaced and ≥20 min were allowed before another unit was characterized.

For each unit, we measured the latency of the response and the response frequency. Total response time was assessed if it fell within the protocol outlined above, but often outlasted the time allowed (2 min). Latency was defined as the time between the stimulus and either the first action potential (for units with no ongoing activity), or an increased rate on the basis of the instantaneous frequency histogram (for units with ongoing activity). The first 10 s of the response were used to determine response frequency because the responses usually lasted ≥10 s and the peak responses were contained within this interval.

RESULTS

Data presented are from 38 units recorded from 14 animals. Numerous other units were recorded from but were not stable enough to allow sufficient characterization for inclusion in the study. The mean conduction velocity for all units was 0.9 ± 0.7 (SD) m/s (n = 38, Fig. 3). Most fibers had conduction velocities ≥1.5 m/s (c fiber); five fibers had conduction velocities between 1.5 and 4.3 m/s (Aδ-fibers). However, because there were no apparent differences between these groups, the data are presented together.

Physiological characterization

RESPONSE TO MECHANICAL STIMULI. Of 38 units, 34 were located with the use of the search stimulus of 150–300 kPa. All these units had RFs located on the ipsilateral dura mater overlying the frontal lobes and olfactory bulbs. One unit also had an RF on the contralateral dura, 2–3 mm from the superior sagittal sinus. RFs consisted of one to three punctate “hot spots” surrounded by an area of much higher mechanical threshold. The majority of the hot spots were overlying or within 2–3 mm of the superior sagittal sinus, but hot

![Graph](http://jn.physiology.org/)

FIG. 3. Conduction velocities of 38 afferent units calculated from electrical stimulation of the RF 8–20 mm from the recording site. Mean = 0.88 m/s.
spots also were found on the path of the NCN and on the blood vessel separating the frontal lobe from the olfactory bulb. The total RF area ranged from 1 to 30 mm$^2$, and the edge of the RF usually extended no more than 2 mm from an individual hot spot (determined with the use of the most pressure judged by the experimenter not to break through the dura). If the RF extended to the medial or posterior edge of the craniotomy, the craniotomy was extended to surround the RF by an area with no mechanical response. There were no apparent changes in RF topography during characterization. Of the 38 units, 4 did not respond to the initial mechanical search pattern, but were located during heating of the dura during characterization of a different unit. None of these four units responded to mechanical stimulation during their subsequent characterization.

Mechanical stimulation led to immediate, reproducible, and slowly to moderately adapting discharges with instantaneous frequencies as high as 266 Hz ($n = 30, 109 \pm 67$ Hz, mean $\pm$ SD). All of the units studied responded in a graded fashion to increasing pressure on the dura, and most had slowly adapting responses to maintained stimulation (Fig. 4). Discharge ceased abruptly with stimulus removal. The threshold for mechanical activation was obtained for 25 units. In many cases the recording electrode was too close to the RF to allow proper use of the filaments. The mean threshold of the units was $25 \pm 28$ (SD) kPa, ranging from 3.4 to 166 kPa (Fig. 5). Units could not be separated into groups by their mechanical threshold. For a given unit with multiple hot spots, each hot spot had similar mechanical response properties. The threshold between hot spots was always much greater, and was not routinely assessed. A comparison of thresholds of first-found units with those characterized later failed to show a significant difference.

CHEMICAL SENSITIVITIES. Acid, INFM, and capsaicin were applied to the RFs of 30, 28, and 29 units, respectively (Table 1). Four units did not respond to topical application of the solutions, but responded to subdural injections. All three stimuli were applied to 26 units. Response latencies were often difficult to assess because of ongoing discharge. Of 31 units, 11 (37%) responded to acid, with latencies ranging from 0 to 67 s ($n = 8, 18 \pm 22$ s, mean $\pm$ SD) and discharge rates ranging from 1.3 to 6.1 Hz ($n = 7, 3.7 \pm 1.8$ Hz, mean $\pm$ SD; Fig. 6A). Although the discharge of one unit lasted >2 min, the others stopped within 20–50 s. Discharge could usually be elicited again by reapplication, although this was not done routinely. Because others have shown that acid stimulation of nociceptors produces a non-adapting response (Steen et al. 1995), it is possible that the observed adaptation was due to endogenous buffering at the RF (Steen and Reeh 1993). The INFM provoked a response in 22 of 28 units (79%), with latencies ranging from 1 to 27 s ($n = 21, 8.3 \pm 6.4$ s, mean $\pm$ SD) and discharge rates ranging from 2.2 to 37 Hz ($n = 21, 8.6 \pm 4.2$ Hz, mean $\pm$ SD; Fig. 6B). Although the threshold to adapt was similar to that observed with acid stimulation, the discharge rates were significantly higher and the responses often lasted for >2 min. Capsaicin elicited a response from 28 of 29 units (97%), with latencies ranging from 0 to 21 s ($n = 22, 4.8 \pm 5.5$ Hz, mean $\pm$ SD) and discharge rates ranging from 1.5 to 35 Hz ($n = 22, 15.5 \pm 7.6$ Hz, mean $\pm$ SD; Fig. 6C). The responses were more rapidly adapting and shorter-lasting than those following acid or INFM, usually ceasing before the solution was rinsed. In most units all three stimuli were applied. Figure 6D shows the response of one unit to all three stimuli; the response frequencies were least for acid and greatest for capsaicin, which was a typical observation. The response to acid was never as robust as the response to capsaicin, although in one case the response was similar to INFM. The response was less robust for INFM than for capsaicin, with two stronger and two similar responses.

THERMAL SENSITIVITIES. The temperature of the dura before stimulation was 32–34°C, depending on the ambient temperature. Intracranial temperature was measured in two animals at four sites beyond the margins of the craniotomy. In these animals, intracranial temperature ranged from 36 to 38°C at all sites.

Of 33 units, 18 (55%) responded to heat. The thresholds ranged from 32.3 to 42°C ($n = 11, 39.3 \pm 2.7$°C, mean $\pm$ SD). In 10 units, thresholds to repeated stimuli were obtained. In seven of these units, thresholds stayed constant even when the interstimulus interval was 30 s, although two thresholds decreased and one increased. The discharge frequency during a stimulus tended to be less with repeated stimuli, but the discharge pattern was retained (Fig. 7A). The response frequency in general increased with the tem-
perature, and firing ceased with the stimulus (Fig. 7B). In a few units the RF was heated past 45°C; this did not increase the firing rate and usually led to a bursting discharge pattern.

Of 32 units, 13 (41%) responded to cold. Because the cooling method led to a rapid decrease in temperature, thresholds were difficult to obtain, and were estimated to be between 25 and 32°C. In one unit, three progressively lower cold thresholds were obtained (31.6, 31.1, and 30.7°C; Fig. 7C). The discharges were restricted to the initial few seconds of cooling and paralleled the rate of change of temperature rather than the static temperature (Fig. 7, C and D). No units responded during rewarming, regardless of the rate (Fig. 7, C and D).

RESPONSE COMBINATIONS. All stimuli were presented to 26 units, leading to 12 different response combinations (Table 2). The one unit with only heat sensitivity was excluded from this analysis (see DISCUSSION). Attempts were made to categorize the units on the basis of the presence or absence of mechanical, chemical, and thermal sensitivities (this allowed the inclusion of 2 units excluded from Table 2). This reduced the number of categories to three: mechanical, chemical, and thermal (n = 18); mechanical and chemical (n = 6); and chemical and thermal (n = 3). However, these groups did not differ in mechanical thresholds or ongoing discharge. No units were found that responded to only mechanical stimuli or to thermal but not chemical stimuli. The presence of chemical and thermal sensitivities was not related to the size of the RF.

ONGOING ACTIVITY. Ongoing activity (activity in the absence of applied stimulation) was present in 26 units (71%). The discharge was always irregular, with rates ranging from 0.12 to 3.8 Hz (1.2 ± 0.2 Hz, mean ± SD). Units without ongoing activity when isolated did not develop it during characterization, but if present, the rates often changed after stimulation. After initial mechanical testing, ongoing activity usually decreased. Heating and INFM led to increased ongoing activity from a few units, but this was not a consistent finding. The presence of ongoing activity was not predictive of a response to thermal or chemical stimuli. No units exhibited any phasic ongoing discharge, which could have been related to heart rate or breathing.

The ongoing activity rates of the first units characterized during an experiment were compared with the rates of subsequently characterized units. Although a higher proportion of subsequently characterized units had ongoing activity, the difference was not significant (z = 0.46, P = 0.6). However, the subsequently characterized units did have higher ongoing rates (t = 1.7, P = 0.07).

FIG. 6. Representative sensitivities of meningeal units to chemical stimuli. Arrows: time of application. A: response to acid solution, latency 12 s; B: response to inflammatory mediators (INFM) on different unit, latency 9 s; C: response to capsaicin, latency 1 s; D: responses to all 3 stimuli on 1 unit.

DISCUSSION

The data demonstrate that the mechanosensitive afferent units innervating the dura are largely homogeneous, not being easily separable into functional classes on the basis of their response properties. All units had some type of noxious chemical sensitivity, but not all were mechanically or thermally sensitive. The combinations of sensitivities are consistent with properties of polymodal nociceptors, and may help define “noxious” for intracranial tissues.

These results suggest a predominant c fiber innervation of the meninges. Although this correlates with anatomic studies (Andres et al. 1987), it is inconsistent with recordings made

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### Table 1. Ongoing discharge and responses of meningeal afferent units to chemical and thermal stimuli

<table>
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<tr>
<th></th>
<th>Capsaicin</th>
<th>INFM</th>
<th>Acid</th>
<th>Heat</th>
<th>Cold</th>
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<tr>
<td>Number</td>
<td>27/38 (71)</td>
<td>28/29 (97)</td>
<td>22/28 (79)</td>
<td>11/30 (37)</td>
<td>19/34 (56)</td>
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Numerator is number positive, denominator is number tested. Values in parentheses are percentages. INFM, inflammatory mediators.
from the trigeminal ganglion and nucleus, where the mean conduction velocities were higher, predominantly in the Aδ-range (Davis and Dostrovsky 1988a; Dostrovsky et al. 1990). Thinly myelinated fibers taper, especially in their last 20 mm (Duclaux et al. 1976; Morrison 1973), and conduction velocities of the most distal 2–3 mm of a c fiber can be as low as 0.05 m/s (G. M. Bove and A. R. Light, unpublished data). Also, the peripheral projection of individual pseudounipolar neurons is often smaller in diameter than the central projection (Duclaux et al. 1976). Both of these possibilities are consistent with the present results, since the recordings were made from the most distal and thinnest part of the peripheral axon. Therefore it is possible that the reported conduction velocities are not representative of the entire neuron, and that the overall conduction velocities of trigeminal neurons innervating the meninges are higher.

Although the total RF areas were up to 30 mm², it is likely that the receptive structures were in close proximity to the hot spots. The increased pressure required for a response from stimulation of the surrounding area probably reflects the increased distance from the receptive structure rather than the terminal arborization of the neuron. A plexus of small-fiber axons was visualized surrounding the superior sagittal sinus and in the dura (Andres et al. 1987; Düring et al. 1990), but the terminal arborizations of single neurons, and therefore their anatomically potential RF(s), have not been visualized. Although we identified a surrounding mechanically insensitive zone for each unit studies, it is possible that an insensitive part of the axon projected across this zone to lie under the intact bone. Therefore the full RF may have gone undetected with the use of the methods employed.

Because mechanically insensitive nociceptors have been reported for other tissues (Häbler et al. 1988; Handwerker et al. 1991; Meyer et al. 1991), the mechanical search pattern used in our experiments largely precluded identification of this type of afferent if present in the meninges. However, four mechanically insensitive, thermally sensitive units were found, three by their ongoing discharge when the filament was placed on the electrode and one serendipitously during characterization of another unit. Three of these four units were sensitive to INFM and capsaicin, and it is likely that these units were truly mechanically insensitive. The one heat-only unit may have responded to direct heating of the axon, because this can lead to a response identical to that observed with heating a terminal (Zimmermann and Sanders 1982). This was indirectly supported by the lack of mechanical or chemical sensitivity (this was the only unit that did not respond to topical capsaicin), because these stimuli will not elicit discharge when applied to axons (Zimmermann and Sanders 1982). Purely chemosensitive units, described in other tissues (Häbler et al. 1988; Handwerker et al. 1991; Meyer et al. 1991), were not identified. Many filaments had viable axons other than the single unit being tested. The chemical stimuli were applied to the entire exposed dura.

### Table 2. Sensitivity combinations for 25 meningeal afferent units

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<tr>
<th>Number</th>
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<th>INFM</th>
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<th>Heat</th>
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All units had all stimuli applied. Solid circles: responses. Open circles: lack of response. INFM, inflammatory mediators. *Two units in this group were not mechanically sensitive.
and therefore it seems likely that if there was a large population of purely chemically sensitive units at least one would have been identified.

The majority of units had ongoing activity when isolated. Although consistent with some reports (Bahns et al. 1986a; Blumberg et al. 1983; Haupt et al. 1983), lower ongoing activity rates have been reported in other visceral tissues (Bahns et al. 1986b, 1987; Kumazawa and Mizumura 1980b; Kumazawa et al. 1987; Sengupta and Gebhart 1994). It is possible that the surgical exposure used in these experiments evoked an inflammatory response in the dura. During inflammation, nociceptors develop ongoing discharge (Cohen and Perl 1988; Grigg et al. 1986; Perl et al. 1976; Schaible and Schmidt 1985), a possible indicator of sensitization (Berberich et al. 1988; Schaible and Grubb 1993). Repeated mechanical, chemical, and thermal noxious stimuli are known to sensitize nociceptive afferents (Cohen and Perl 1988; Reeh 1994; Reeh et al. 1987; Thalhammer and LaMotte 1982). Most units had their RFs repeatedly stimulated during characterization, especially second and later units that had also been exposed to stimuli from characterizing earlier units. Comparing the ongoing rates of first- and later-found units demonstrated a difference, supporting some sensitizing effect due to the experimental protocol. Although not fully characterized, units innervating undisturbed tissue are nociceptors.

All of the mechanosensitive units responded to at least one chemical stimulus. Similarly high proportions of afferent units from viscera (Adelson et al. 1996; Bahns et al. 1986b; Kumazawa and Mizumura 1980a,b; Nishi et al. 1977), skin (Davis et al. 1993), and deep somatic tissues (Bove and Light 1995) are chemosensitive. Davis et al. (1993) reported that 100% of mechanically sensitive afferent units responded to application of an algesic mixture like the one used in these experiments. The percentage of acid-sensitive units is comparable with cutaneous afferent units in vitro (Steen et al. 1992, 1995). Additionally, there have been reports of 100% sensitivity of nociceptors in skin to capsaicin (Szolcsányi 1987; Szolcsányi et al. 1988). This points to a similarity of the present units to nociceptors innervating other tissues, and also argues for the importance of chemical sensitivity to intracranial sensation.

In general, the responses increased with successive chemical stimuli, which were always presented in the order acid, INFM, and capsaicin. This observation may represent the relative strengths of these agents in depolarization, but could also have been due to a cumulative sensitizing effect of repeated stimuli. However, the order in which the agents were applied was meant to minimize sensitization and possible damage to the terminal, not to address the issue of sensitization, and no conclusion can be made regarding any cumulative effect of one or more stimulus modalities on a subsequent response.

Are these units nociceptors?

Nociceptors are commonly defined as neural elements that can encode damaging or potentially damaging stimuli and discriminate these from innocuous stimuli (Lewis 1942; Merskey and Bogduk 1994; Sherrington 1906). Cervero (1994) contends that noxious “should be defined on the tissue innervated and not in absolute terms,” and Kumazawa (1990) adds that there are nociceptors that respond to stimuli in both innocuous and noxious ranges. Although noxious is not well established for intracranial tissues, psychophysical data from human studies (Feindel et al. 1960; Penfield and McNaughton 1940; Ray and Wolff 1940) demonstrated that pain was the only sensation that arose from mechanical, thermal, or electrical stimuli of intracranial structures. Those studies, although not quantifying the stimuli used, gave insight into what is noxious for these tissues.

With these concepts in mind, there is direct evidence from our experiments suggesting that most if not all of these units are nociceptors. 1) Most of the units responded in a graded fashion to pressures that caused visible tissue damage (bleeding, sometimes tearing of dura), fulfilling this criterion of “nociceptor” (see above). 2) Most of the units responded to capsaicin, which primarily excites nociceptors (reviewed by Holzer 1995). 3) Most of the units responded to the INFM, which contained substances released by tissue damage. Similar preparations elicit responses from up to 85% of polymodal nociceptors (Reeh 1994) and cause pain in humans and pseudoaffective behavior in animals (reviewed by Reeh and Kress 1995). Additionally, many units responded to acid, part of inflammatory milieu (reviewed by Steen et al. 1995). These observations, coupled with the lack of other evokable sensations from the dura, support the hypothesis that these units are nociceptors.

What is noxious for intracranial tissues?

The properties described allow speculation as to what is noxious for intracranial tissues. As mentioned above, the majority of these afferent units respond to chemical stimuli, supporting a major role of chemical stimulation in intracranial nociception.

In addition to afferent discharge, depolarization evokes the release of vasoactive peptides from nociceptors, leading to neurogenic inflammation (Jancso et al. 1967; Kenins 1981). This mechanism has been proposed to contribute to headache pathogenesis (Moskowitz 1984, 1993). The current findings of chemosensitive nociceptors innervating intracranial structures give strength to this hypothesis.

It is likely that intracranial nociceptors are mechanically activated in pathological states. Potential sources of natural mechanical stimulation to the dura include intracranial masses and increased intracranial pressure. Intracranial masses, especially those with direct contact or adhesion to the dura, produce sustained pressures, and potentially cause changes in forces during head movement. Meningitis presents with headache, and the pain is increased by neck flexion (Brudzinski’s sign), thought to mechanically irritate inflamed meninges. Intracranial pressure, normally 1–2 kPa, increases in meningitis to >2 kPa (Miller and Jubelt 1989) and in experimental meningitis increases from 0.64 to 4.9 kPa (Goitein and Shapiro 1992). Although this is lower than the reported mean activation threshold, many units were sensitive to pressures within this range (Fig. 5). Pressures applied in these experiments were focal, and caused dis-
placement and local tensile and shear forces that may be absent during increases in intracranial pressure. The importance of these individual components to the mechanical sensitivity remains unknown. Nevertheless, these data support an importance of mechanical activation to intracranial noce-

cipation.

The heat thresholds averaged 39.4°C, and were all <42°C. These thresholds are lower than those reported for cutaneous, visceral, and articular nociceptors (Bahns et al. 1986b; Guil-baud et al. 1985; Kumazawa and Mizumura 1980b; Kumazawa et al. 1987; Torebjork et al. 1984), even during in-flammation. This lower threshold is not surprising, because adverse effects, including cardiac arrhythmias and brain damage, have been reported when core temperatures exceed 41°C (Khogali and Mustafa 1987; Saper and Breder 1994). Therefore, although relatively low compared with noxious temperatures for other tissues, these temperatures are within the physiological limits for the intracranial space and may be noxious for the dura. Headache and fever coexist in a plethora of illnesses, usually infectious, most of which lead to complicated metabolic changes that may be inherently noxious. Headache is an early symptom of a heat stroke (Beller and Boyd 1975), but the cause of the headache has not been systematically evaluated. Therefore, although the data support a role for thermal activation, possibly during fever, it would be preliminary to ascribe headache accompanying fever to the increased intracranial temperature.

The responses to cooling had thresholds similar to deep nociceptors innervating veins (Klement and Arndt 1992). The responses were phasic, however, in contrast to the almost linear perceptions of pain associated with slow cooling to <10°C (Klement and Arndt 1992). Although this difference may reflect different types of receptors being stimulated, it is likely methodological, due to single-fiber recording versus the processed input from many nociceptors. The cooling thresholds are within the physiological range of core temperatures, because consciousness is maintained above 27–32°C (Cooper et al. 1964; Holdcroft 1981). However, headache is not reported with accidental hypothermia, and experimental hypothermia at these temperatures has not been performed on humans. Thus it is not known whether the cold-responsive properties of intracranial nociceptors serve any physiological function.

In summary, these data support the theory that the cranial dura is innervated by a relatively homogeneous population of afferent units that have properties similar to those of polymodal nociceptors recorded from other tissues. The chemical sensitivities and the low mechanical and thermal thresholds are consistent with stimuli that the units are potentially subjected to, and may help define noxious for intracranial structures. These units are likely to be recruited during intracranial inflammation and other pathologies, including headache, and the sensory consequence of their discharge may be pain.

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