Mechanical Response Properties of A and C Primary Afferent Neurons Innervating the Rat Intracranial Dura

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Levy, Dan, and Andrew M. Strassman. Mechanical response properties of A and C primary afferent neurons innervating the rat intracranial dura. J Neurophysiol 88: 3021–3031, 2002; 10.1152/jn.00029.2002. The intracranial dura receives a small-fiber sensory innervation from the trigeminal ganglion that is thought to be involved in some types of headaches, including migraine. Mechanical response properties of dural afferent neurons were examined to investigate variation across the population in the properties of threshold, slope, adaptation, and incidence of mechanosensitivity. Dural afferent neurons were recorded in the trigeminal ganglion of urethane-anesthetized rats and were identified by their constant-latency response to dural shock. Neurons were classified as fast A or slow A, slow C, or fast C based on response latency to dural shock. Mechanical receptive fields were identified by stroking or indenting the outer surface of the dura. Stimulus-response curves were obtained from responses to 2-s constant-force indenting stimuli of graded intensities delivered to the dural receptive field with a servo force-controlled mechanical stimulator. The slow A population had the highest percentage of mechanosensitive units (97%) as well as the highest slopes and the lowest thresholds. Thus by all three criteria, the slow As had the highest mechanosensitivity. Conversely, the fast A population had the lowest mechanosensitivity in that it had the lowest percentage of mechanosensitive units (66%), the lowest slopes, and the highest thresholds. The C population was intermediate with respect to all three properties but was much more similar to the slow As than to the fast As. All three fiber classes showed a negative correlation between slope and threshold. The majority of neurons showed a slowly adapting response to a maintained 2-s stimulus. Adapting neurons could be subdivided based on whether the fitted exponential curve decayed to zero or to a nonzero plateau; the latter group contained the most sensitive neurons in that they had the lowest thresholds and highest slopes. Nonadapting neurons generally had lower initial firing rates than adapting neurons. Fast A neurons exhibited greater and more rapid adaptation than slow A and C neurons. Neurons with the lowest slopes, regardless of CV, had relatively rapid adaptation. The more slowly conducting portion of the C population was distinguished from the other C neurons by a number of properties: more mechanically insensitive neurons, higher thresholds, and more nonadapting neurons. These differences in mechanical response properties may be related in part to differences in membrane currents involved in impulse generation that have been described in subpopulations of dorsal root ganglion cells.

INTRODUCTION

Major blood vessels of the intracranial meninges receive a predominantly small-fiber sensory innervation from the trigeminal ganglion as well as the upper cervical dorsal root ganglia (Feindel et al. 1960; Keller et al. 1985; Mayberg et al. 1984; O’Connor and van der Kooy 1986; Penfield and McNaughton 1940). Stimulation of meningeal blood vessels in neurosurgical patients can evoke painful sensations that are typically referred to a region of the trigeminal dermatome (Fay 1935; Feindel et al. 1960; Ray and Wolff 1940) and are abolished by lesion or blockade of the trigeminal nerve or ganglion (Penfield and McNaughton 1940). Pain is the only sensation that can be evoked by stimulation of the intracranial meninges, regardless of whether the stimulus is electrical, mechanical, thermal, or chemical (Ray and Wolff 1940). Because meningeal blood vessels are the only intracranial sites from which pain can be evoked, headaches that accompany intracranial pathologies (e.g., meningitis, subarachnoid hemorrhage, tumor) are thought to result from meningeal stimulation and consequent activation of meningeal sensory fibers (Wolff 1963). Migraine headache, although not accompanied by any detectable pathology, shares certain clinical features with headaches of intracranial origin, and has also been postulated to result from activation of the meningeal sensory innervation (reviewed in Strassman and Raymond 1997).

Mechanosensitivity in particular seems to be important in intracranial headaches as well as migraine. Both are characterized by an extreme sensitivity to actions such as coughing, straining, or sudden head movement (Blau and Dexter 1981), which would all be expected to produce a transient alteration in the distribution of mechanical forces within the intracranial space. In addition, the throbbing quality that is characteristic of migraine has typically been attributed to arterial pulsation, which might produce pain either through stretching/displacement of peri-arterial tissue or through the generation of pressure pulses that propagate throughout the closed intracranial space. The headache that develops after lumbar puncture (post dural puncture headache) has a strict positional dependence that is indicative of a gravity-induced tension or displacement of intracranial tissue (Wolff 1963). Each of these clinical phenomena point to the presence of mechanosensitive neural elements within the intracranial space that can, under some circumstances, contribute to clinically occurring headache.

Peripheral and central neurons in the meningeal sensory pathway have been identified by recording unit responses to...
electrical stimulation of the intracranial dura in the trigeminal nerve (Bove and Moskowitz 1997) or ganglion (Strassman et al. 1996), medullary and upper cervical dorsal horn (Burstein et al. 1998; Davis and Dostrovsky 1986, 1988a; Kaube et al. 1992; Lambert et al. 1991; Strassman et al. 1986), and the ventrobasal thalamus (Davis and Dostrovsky 1988b; Zagami and Lambert 1990). These studies found neuronal responses to punctate probing and other forms of mechanical stimulation applied to the intracranial dura, primarily at sites on or near the dural venous sinuses or middle meningeal artery.

Our initial study of dural afferents used von Frey hairs for measurement of mechanical response thresholds and demonstrated that these thresholds could be lowered by dural application of inflammatory mediators (Strassman et al. 1996). In subsequent experiments, to obtain more precise measurements of mechanosensitivity, we have used a force servo-controlled mechanical stimulator in place of the von Frey hairs for dural stimulation. Using this stimulator to study both threshold and suprathreshold components of sensitization, we observed sensitizing effects on these two response components occurring separately in individual neurons (Levy and Strassman 2002). The two patterns of sensitization seemed to be occurring in separate subpopulations in that the neurons also showed some differences in their conduction velocities (CVs) and their baseline stimulus-response properties (threshold and stimulus-response slope).

The present study was carried out to examine in more detail how much these and other baseline response properties vary across the entire population of dural afferents to provide a stronger basis for the identification of potential subpopulations. For this purpose, we have examined the baseline mechanical response properties of slope, threshold, and adaptation, and the relationship of these properties to each other, and to CV, in a much larger sample of dural afferents (including those for which slope and threshold were described in Levy and Strassman 2002). This information is fundamental to our ongoing efforts to identify potential subpopulations among the dural afferents and for understanding the different patterns of modulatory effects exhibited by different groups of neurons.

**Methods**

**Surgery and electrophysiological recording**

Experiments were carried out on male Sprague-Dawley rats (330–450 g). Data was obtained from 302 rats, of which 117 were tested with the mechanical stimulator (see following text). The experimental protocol was approved by the institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical center. Rats were anesthetized with urethane (1.8 g/kg ip, Sigma-Aldrich, St Louis MO) and were maintained under anesthesia throughout the experiment with supplemental injections as needed to suppress blink reflexes. Rats were killed at the end of the experiment by anesthetic overdose or intravenous bolus injection of 1 M KCl.

Anesthetized rats were placed in a stereotactic head-holder. A craniotomy was made to expose the left transverse sinus and then the adjacent dura extending ~2 mm rostral and caudal to the sinus (overlying the cerebral and cerebellar cortices, respectively). The transverse sinus was exposed from the midline laterally ±4 mm. In some experiments, the exposure was extended to include the entire sinus, a length of ~8 mm (as measured along the curved surface of the sinus, extending first laterally and then ventrally around the lateral convexity of the cerebral/cerebellar cortex, to a point just dorsal to the auditory canal).

The exposed dura was bathed with a modified synthetic interstitial fluid (SIF) consisting of (in mM) 135 NaCl, 5 KCl, 1 MgCl₂, 5 CaCl₂, 10 glucose, and 10 HEPES at pH 7.2.

A second craniotomy was made more anteriorly to allow platinum-coated tungsten microelectrodes to be advanced through the forebrain into the left trigeminal ganglion, at ~2 mm caudal to Bregma, 2–2.5 mm lateral, and 9.5–10 mm below the cortical surface. Single-shot electrical search stimuli (0.5 ms, 5 mA, 0.5 Hz) were delivered through bipolar stimulating electrodes (1- to 1.5-mm separation) to the outer dural surface of the transverse sinus, usually at 3–5 mm from the midline, which is the region where most of the dural projecting axons join the sinus in their course from the underlying tentorium. Single-unit recordings were made from dural afferent neurons in the trigeminal ganglion that were identified by their constant-latency response to the electrical search stimulus (Strassman et al. 1996). Response thresholds and latencies were mapped at multiple sites to identify the sites associated with the shortest latency response (Strassman and Raymond 1999). The response latency at this site was used to calculate CV, based on a conduction distance to the trigeminal ganglion of 12.5 mm. Neurons were classified as either C units (CV ≤ 1.5 m/s), slow A units (1.5 < CV < 5 m/s), or fast A units (CV > 5 m/s). We previously referred to these latter two groups as slow and fast Aβ units (Strassman and Raymond 1999; Strassman et al. 1996), but we are now omitting the “B” because the fast A group includes a number of units that are more properly classified as Aβ, according to the CV criteria of some investigators (see Discussion). Action potentials were processed with a real-time waveform discriminator (SPS-8701, Signal Processing Systems, Prospect, South Australia, Australia) and acquired on- and off-line analysis with the Discovery data-acquisition program (DataWave Technologies, Longmont, CO).

**Mechanical stimulation and data analysis**

Mechanical receptive fields of dural afferents were mapped by stroking the dura with blunt forceps and indenting it with von Frey monofilaments (0.03–6.9 g, exerting 38–510 kPa; Stoelting, Chicago, IL). The 510-kPa filament was the highest intensity used to avoid damaging the dura or causing subarachnoid bleeding. For quantitative determination of mechanical stimulus-response functions, graded stimuli were applied to the dural surface at the lowest threshold site with a servo force-controlled mechanical stimulator (Series 300B Dual Mode Servo System, Aurora Scientific, Aurora, Ontario) (Khalsa et al. 1997; Levy and Strassman 2002). A flat-ended cylindrical plastic probe was attached to the tip of the stimulator arm. Because the dural surface is curved, the stimulator was mounted on a universal joint to allow the probe angle to be made perpendicular to the dural surface at the stimulation site. One of three probe diameters (0.5, 0.8, or 1.1 mm) was selected for each neuron, depending on the sensitivity of the neuron. The smallest probe was used unless the neuron’s baseline threshold was so low that responses were evoked even at the stimulator’s minimum setting of 2 mN. In this case, to deliver subthreshold stimuli, one of the larger probes was used (resulting in lower stimulus pressures). Stimulus intensity is reported in units of force/area (kPa, where 1 kPa = 1 mN/mm²). Only one probe was used for each neuron.

Each stimulus trial consisted of a graded series of square-wave stimuli (100-ms rise time, 2-s width, 10-s inter-stimulus interval) delivered in ascending order (Fig. 1). The response to each mechanical stimulus was calculated by subtracting the spontaneous firing rate from the mean firing rate during the stimulus. The spontaneous firing rate for each trial was calculated from the 10-s interval preceding the first stimulus of that trial. The majority of units had low (<0.5 Hz), or no spontaneous firing.

An initial series of stimulus trials were delivered in which stimulus intensity was systematically varied to determine response threshold, which was defined as the lowest intensity that evoked a response of one to two spikes. The baseline stimulus-response curve was then
in 10 200-ms bins, was also fitted to a first-order exponential decay, according to the equation $y = y_0 + Ae^{-kt}$ (Origin 6.0, Microcal Software). The fit was considered significant if $P < 0.05$.

ANOVA with the Fisher PLSD post hoc test was used for comparisons between fiber classes. Slope and threshold showed highly non-normal distributions, so log values were used for statistical comparisons of slope and threshold. Comparisons between the fiber classes in the distributions of slope and threshold were made by inspecting the population histograms and comparing the proportion of neurons with values above or below selected criterion values ($\chi^2$). Values for slope and threshold are reported as median $\pm$ inter-quartile range (IQR). Ward’s method of hierarchical cluster analysis was performed with slope, threshold, and CV as cluster variables, using JMP 3.1 (SAS Institute). Other statistical analyses were carried out with StatView 5.0 (SAS Institute).

RESULTS

Incidence of mechanosensitivity

A total of 454 units were recorded from the trigeminal ganglion that exhibited a constant-latency response to dural shock (Strassman et al. 1996) and were sufficiently well-isolated to allow recording of responses to mechanical stimulation. Units were subdivided into three fiber classes, based on the conduction velocity between the trigeminal ganglion and the electrical stimulation site on the dura: fast A ($>5$ m/s), slow A ($1.5 < CV < 5$ m/s), and C ($=\leq1.5$ m/s). Mechanical receptive fields were located by stroking the outer surface of the dura with a blunt probe and indenting it with von Frey hairs. Receptive fields were primarily on the dura overlying or immediately adjacent to the ipsilateral transverse sinus (Strassman and Raymond 1999; Strassman et al. 1996).

A mechanical receptive field could not be found for 29% (133/454) of the units that responded to dural shock. This percentage was smaller in experiments in which the entire length of the transverse sinus was exposed (20%, 54/265) as compared with experiments in which only the medial half of the sinus was exposed (42%, 79/189) (see METHODS). Thus doubling the area of the exposed dura reduced the percentage of units with no identifiable mechanical receptive field by approximately one-half.

The percentage of these mechanically insensitive units differed in the three fiber classes (Fig. 2, including only experiments with a complete exposure of the transverse sinus; $\chi^2$ test for proportion of mechanically insensitive units: slow A $< C$, $P = 0.0077$; C $<$ fast A, $P = 0.01$). This percentage was close to 0 among the slow A’s (3%, 2/59), but it increased sharply among neurons with CVs $>5$ m/s (33%, 33/99). This difference in the percentage of mechanically insensitive units was the basis for subdividing the A-fiber population at this CV (as was done originally in Strassman et al. 1996 for a smaller sample of 45 units, which are included in Fig. 2). The percentage of mechanically insensitive units was also somewhat higher among C units (18%, 19/107) as compared with slow A units, particularly among units $<1$ m/s.

Stimulus-response relationships: slope and threshold

Responses to 2-s constant-force stimuli were used to obtain stimulus-response curves of 120 mechanosensitive units (19 fast A, 47 slow A, and 54 C). The plots in Fig. 3 show that, in each fiber class, some units had relatively low thresholds and

![Figure 1](http://jn.physiology.org/)
steep slopes, whereas others had high-thresholds and flat slopes. The plots also illustrate that the fast A population had the highest proportion of units with high thresholds and flat slopes, whereas the slow A population had the lowest proportion of such units. These relationships are illustrated more quantitatively in Fig. 4, which shows the distribution of thresholds and slopes for each of the three fiber classes.

The three fiber classes differed significantly in the distribution of slopes and, to a lesser extent, thresholds (Fig. 4, A and B). Fast A units had the lowest slopes, while slow A’s had the highest (0.18 ± 0.15, 0.25 ± 0.24, and 0.44 ± 0.30 Hz/kPa, median ± IQR for fast A, slow A, and C, respectively; slow A > C, P < 0.005; C > fast A, P < 0.001, by ANOVA of log slope). The majority (74%) of fast A units had slopes <0.06 Hz/kPa, whereas only 11% of slow A and 30% of C units had slopes below this level (P < 0.05, χ²). Conversely, the majority of slow A (81%), but not fast A (21%) or C units (46%), had slopes >0.12 Hz/kPa (P < 0.0005, χ²).

The relationship among the three fiber classes was reversed with respect to the distribution of thresholds: slow A units had the lowest thresholds, whereas fast A’s had the highest (41 ± 119, 10 ± 16, and 16 ± 34 kPa, median ± IQR for fast A, slow A, and C, respectively; fast A > C > slow A, P < 0.05, by ANOVA of log threshold). The slow A population had a significantly higher percentage of units with thresholds <30 kPa (87%) than the fast A (47%) and C (64%) populations (P < 0.01, χ²).

Figure 4C illustrates that there was a strong tendency for units with lower thresholds to have higher slopes and that this negative correlation was present within each of the three fiber classes (correlation coefficient for log slope versus log threshold, r = −0.856; P < 0.0001). Thus insofar as high slopes and low thresholds can both be regarded as indications of high sensitivity, units that exhibited high sensitivity with respect to one parameter also tended to have high sensitivity with respect to the other. In the plots of slope versus threshold (Fig. 4C), the most sensitive units occupy the upper left quadrant while the least sensitive units occupy the lower right quadrant. The slow A population had the highest sensitivity overall, while the fast A population had the lowest.

**Adaptation**

Adaptation was studied by examining discharge rate (averaged in 200-ms bins) during a maintained 2-s stimulus, using stimulus intensities of three to four times threshold.
The majority of units (108/136, 79%) showed an adapting response pattern in which discharge rate was highest during the initial 200–400 ms of the stimulus and then declined (e.g., Fig. 5, A and B). The remainder of units showed a nonadapting response pattern in which firing rate was maintained or, in a few cells, showed a slight increase during the 2-s stimulus (e.g., Fig. 5C). Nonadapting responses were especially common among the slowest C units (7/15, or

**Fig. 4.** Histograms of slope (A) and threshold (B) and log plots of slope vs. threshold (C) for fast A, slow A, and C units. C, bottom: C units with CV >0.8 m/s are represented (○) to show that the subgroup of C units with high thresholds (>50 kPa) have low CVs.

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**Fig. 5.** Examples of different adaptation patterns, illustrated in histograms of discharge rate during single trials of the 2-s stimulus (bin-width: 200 ms). A: adaptation with exponential decay to 0. B: adaptation with exponential decay to a positive plateau (the value of \( y_0 \) in the equation \( y = y_0 + Ae^{-t/t} \)). C: nonadapting responses. t, time constant of decay.
47%, of units with CV <0.45 m/s). Nonadapting neurons had relatively low peak firing rates, compared with adapting neurons (Fig. 5); peak firing rates >20 Hz were only found in adapting neurons, at response onset.

In the majority of adapting units (88/108, 81%), the response was well-fit by a first-order exponential decay ($P < 0.01$). A subset of the neurons with exponential adaptation (31/88, 35%) exhibited a response pattern in which the fitted exponential curve did not decay to zero but rather decayed to a positive plateau (the value of $y_0$ in the equation $y = y_0 + Ae^{-\alpha t}$). Such responses might be described as having both an adapting and a nonadapting component (e.g., Fig. 5B). Neurons with this response pattern had the highest sensitivity in that they had higher slopes and lower thresholds than other units (slope: $0.31 \pm 0.53$ vs. $0.14 \pm 0.34$ Hz/kPa; threshold: $10.2 \pm 15.4$ vs. $18.3 \pm 33.1$ kPa; median $\pm$ IQR: $P < 0.05$ for $t$ tests on log slope and log threshold). Slow As had the highest percentage of units with this response pattern (35% vs. 17% of Cs and 11% of fast As).

Fast A units generally showed greater and more rapid adaptation than slow A and C units. No fast As were nonadapting (as compared with 21% of slow As and 26% of Cs). Adaptation was complete (i.e., firing had declined to 0) by the final 400 ms of the 2-s stimulus in 53% (10/19) of fast A units but only 12% (6/52) of slow A and 11% (7/65) of C units. A subset of fast A units showed a particularly rapid adaptation pattern, in which all firing occurred during the first 200 ms of the stimulus (e.g., Fig. 5A, 1st graph). Such a rapidly adapting response was exhibited by 32% of fast A units, but no slow A or C units. These rapidly adapting units were among the neurons with the lowest mechanosensitivity, in that they had low slopes (<0.025 Hz/kPa) and high thresholds (>20 kPa). They mainly occupied the upper end of the fast A range (>9 ms).

Although this extremely rapid adaptation was only found among fast As, the association between low slopes and adaptation held across fiber classes. Almost all units (16/17) with low slopes of <0.05 Hz/kPa, regardless of fiber class, had relatively short adaptation time constants (<0.5 s). This low-slope group included eight fast A, four slow A, and five C units.

To better quantify the degree of adaptation, an AI was calculated for each neuron, defined as the proportion of total firing that occurred during the initial 400 ms of the 2-s stimulus (Fig. 6A). Thus a rapidly adapting response in which all firing occurs in the first 400 ms has an AI of 1.0, whereas a nonadapting response in which firing is evenly maintained throughout the stimulus has an AI of 0.2 (=400 ms/2 s). The ■ in Fig. 6A represent the units whose responses were well-fit by a first-order exponential decay. The time constant of decay for those units with an exponential decay is shown in Fig. 6B. Figure 6 shows that fast A units had larger AIs and shorter time constants than slow A and C units (mean AI of 0.64 ± 0.32 for fast A vs. 0.37 ± 0.15 for slow A ($P < 0.0001$) and 0.35 ± 0.17 for C units ($P < 0.0001$); mean time constant of 0.19 ± 0.20 s for fast A vs. 0.52 ± 0.34 s for slow A ($P < 0.0005$) and 0.67 ± 0.33 s for C units ($P < 0.0001$)). Figure 6A also shows that the AI was generally larger for exponential than for nonexponential units.

Cluster analysis

The preceding analyses first grouped the neurons according to fiber class (CV) and then compared the groups. However, there was considerable heterogeneity within each fiber class. To further investigate the presence of subpopulations, a cluster analysis was carried out using CV, slope, and threshold as cluster variables. The neurons were divided into six clusters as illustrated in the plot of threshold versus CV shown in Fig. 7. Cluster 3 consisted of predominantly fast A units with high-thresholds (and correspondingly low slopes). Clusters 1 and 5 were predominantly slow A units with intermediate and low thresholds, respectively. Clusters 2, 4, and 6 were predominantly C units with high, intermediate, and low thresholds, respectively.

The clusters showed no systematic differences in AI but did show some trends in the distribution of different adaptation patterns. Clusters 1, 3, and 5 were predominantly adapting neurons. Most of the neurons that showed adaptation to a nonzero plateau were in clusters 1 and 5. Clusters 2, 4, and 6 contained both adapting and nonadapting neurons.

The clusters were also examined to determine the distribution of a subset of neurons that had been classified in a previous study based on the pattern of PKA-dependent mechanical sensitization (Levy and Strassman 2002). That study described two groups of neurons that showed sensitizing effects predominantly on either their threshold (TH group, $n = 15$) or suprathreshold (STH group, $n = 10$) responses. That study also
found that the TH and STH neurons also showed some differences in their CVs and baseline mechanical response properties. Consistent with those findings, the TH and STH neurons showed a differential cluster distribution, in that 80% of the STH neurons were in clusters 1 and 4, while 80% of the TH neurons were in clusters 2, 3, 5, and 6.

**DISCUSSION**

Dural afferents were examined to investigate the degree of variation across the population, and the relation to CV, of the mechanical response properties of threshold, slope, adaptation, and incidence of mechanosensitivity. These properties showed a large variation that was partly related to conduction velocity. In general, fast A neurons differed markedly from slow A and C neurons, whereas the differences between the slow A and C populations were more subtle. The slow A population had the highest percentage of mechanosensitive units as well as the highest slopes and the lowest thresholds. Thus by all three of these criteria, the slow As had the highest mechanosensitivity. Conversely, the fast A population had the lowest mechanosensitivity in that it had the lowest percentage of mechanosensitive units, the lowest slopes and the highest thresholds. The C-fiber population was intermediate with respect to all three properties but was much more similar to the slow As than to the fast As. All three fiber classes showed a strong negative correlation between slope and threshold.

Fast A neurons exhibited greater and more rapid adaptation than slow A and C neurons. Neurons with the lowest slopes, regardless of CV, had relatively rapid adaptation. Neurons with both an adapting and a nonadapting response component (adaptation to a non-0 plateau) were the most sensitive neurons in that they had the lowest thresholds and highest slopes. The more slowly conducting portion of the C population was distinguished from the other C neurons by a number of properties: more mechanically insensitive neurons, higher thresholds, and more nonadapting neurons.

In a previous study of mechanical sensitization in dural afferents, we found different patterns of sensitization occurring in different neurons after application of cAMP (Levy and Strassman 2002). Some neurons showed primarily a lowered response threshold, whereas other neurons showed primarily an increase in their suprathreshold responses. These two effects appeared to be occurring in different subpopulations because the two groups also showed some differences in baseline mechanical response properties and CV. In the present study, which included a much larger sample of neurons, cluster analysis was carried out to further examine the heterogeneity across the population with respect to CV, slope, and threshold. The two subsets of neurons that had been defined in the previous study based on their patterns of sensitization showed a differential cluster distribution, further supporting the possibility that they constitute different subpopulations.

**Subdivision of the A-fiber population**

The 5-m/s cutoff used in this study for subdividing the A fibers was made initially based on the observation of a sharp increase in the percentage of mechanically insensitive neurons among neurons with CVs >5 m/s (Strassman et al. 1996). This observation was confirmed and extended in the present study to include differences in other mechanical response properties as well. A number of other studies of primary afferent nociceptors have also observed distinctive properties among the most slowly conducting portion of the Aδ population that prompted them to treat these neurons as a separate group from the faster conducting Aβ fibers (Campbell and Meyer 1986; Hoheisel and Mense 1987; Ikeda et al. 1997; Liang and Terashima 1993; Liang et al. 1995; Lynn et al. 1995). In previous studies (Strassman and Raymond 1999; Strassman et al. 1996), we considered the fastest dural fibers to fall within the upper limit of the Aδ range (22–25 m/s) as commonly defined for other primary afferent populations in the rat (e.g., Leem et al. 1993). However, some studies have found that Aβ conduction velocities in the rat are slower than in other species, and have demarcated the Aδ/Aβ border as low as 12–15 m/s (Lynn and Carpenter 1982; Waddell et al. 1989). Some variation between studies may be attributable to age differences (Birren and Wall 1956) as well as differences between distal and proximal portions of individual nerve fibers (Harper and Lawson 1985; Waddell et al. 1989). In addition, trigeminal primary afferents may have somewhat slower CVs than their spinal analogs, although we know of no study that has reported the CVs of Aβ fibers or defined the Aδ/Aβ border for the rat trigeminal system. Consequently, we have not attempted to define this border for the dural afferents and instead simply note that the slow A group includes the slower conducting portion of the Aδ population, whereas the fast A group contains the faster Aδ fibers as well as a number of fibers that would be classified as Aβ according to the CV criteria of some investigators.
Comparisons with afferents from other tissues

The present study found a negative correlation between slope and threshold. This correlation has not been calculated in previous studies of primary afferent mechanosensitivity. However, a few such studies have shown a similar trend by dividing their neuronal sample into groups of different thresholds and comparing the response properties of the groups. In studies of cutaneous and mucosal mechanonociceptive afferents, it was found that neurons with moderate thresholds had higher response rates and better encoding of stimulus intensity than those with higher thresholds (Burgess and Perl 1967; Cooper et al. 1991). Although not explicitly noted, a similar trend can also be seen in the mechanical stimulus-response curves illustrated in a number of other studies of cutaneous and visceral afferents. Garell et al. (1996) divided cutaneous nociceptors into two groups according to threshold and plotted the group-averaged stimulus-response curves separately. Graphical analysis of the plots shows that the low-threshold group had very steep initial slopes (A fibers: 0.5 Hz/g, C fibers: 0.2 Hz/g), with saturation at higher intensities (>20 g), while the high-threshold group had much lower slopes (0.05 and 0.04 Hz/g for A and C fibers, respectively). A similar relationship can be seen in the individual stimulus-response curves of pelvic nerve afferents responding to distension of the colon or the urinary bladder (Sengupta and Gebhart 1994a,b). These were also divided into low- and high-threshold groups. Although the authors found no difference in the mean slopes, graphical analysis of the plots nonetheless reveals a striking difference between the two groups in the distribution of slopes. The low-threshold group contained neurons with steep initial slopes (1–3 Hz/mmHg) that then saturated (similar to the low-threshold cutaneous afferents of Garell et al. 1996) as well as neurons with flatter slopes. The neurons in the high-threshold group almost all had flat slopes (<0.7 Hz/mmHg). Thus the relationship between slope and threshold exhibited by dural afferents can be discerned in mechanosensitive afferents of other tissues as well.

Although other studies of primary afferent mechanosensitivity have not subdivided the A population by CV as we have, it is nonetheless possible to discern parallels with a number of our findings on differences between fiber classes. A study of pelvic nerve afferents showed differences between fiber classes in the incidence of mechanosensitivity that are similar to our findings in dural afferents (Sengupta and Gebhart 1994b). Although that study did not subdivide the A population, it showed histograms of the conduction velocities of mechanically sensitive and insensitive fibers, that allow calculation of the incidence of mechanosensitivity in the three fiber classes that we used in the present study. From these histograms it can be calculated that the percentage of mechanically insensitive neurons was ~15% in the slow A population and 40% in the fast A and C populations. Thus the higher incidence of mechanosensitivity in slow A neurons is apparently not unique to the dural afferent population. This is also true of our finding of a higher percentage of mechanically insensitive units among the most slowly conducting C neurons because a similar trend is present in human cutaneous C fibers (Weidner et al. 1999).

Studies of mechanical stimulus-response functions of cutaneous nociceptors have found that A fibers have higher slopes than C fibers (Garell et al. 1996; Slugg et al. 2000). This is only partly true for the dural afferents in that the slow A’s have somewhat higher slopes than C fibers, but the fast A’s have lower slopes. Our finding of a subgroup of fast A fibers with low mechanosensitivity does not appear to have a parallel in studies of other primary afferent populations.

A number of previous studies have examined relationships between mechanical stimulus-response properties (slope or threshold) and adaptation and, in some cases, found associations that are similar to those found in the present study. Among pulmonary mechanoreceptive afferents (Knowlton and Larrabee 1946), as well as cutaneous mechanical nociceptors (Burgess and Perl 1967), neurons with rapid adaptation tend to have high thresholds as was also found for dural afferents in the present study. In cutaneous nociceptors, neurons with an augmenting response pattern have high thresholds (Garell et al. 1996) similar to our finding in dural afferents. Similarly, a study of mechanosensitive colonic afferents found that non-adapting neurons have high thresholds (Blumberg et al. 1983). That study also found that the steepest slopes were exhibited by those neurons that had a combined dynamic and static response pattern, which is similar to our finding of high sensitivity in dural afferents that have combined adapting and nonadapting response components (i.e., neurons with exponential decay to a non-0 plateau).

Mechanisms determining mechanical response properties

Mechanical response properties might be influenced by the characteristics of the mechanical transduction process as well as the process of impulse generation by voltage-gated currents. There is currently no information about how the properties of mechanical transduction elements might differ among different groups of nociceptive or small-diameter sensory neurons.

However, a number of voltage-gated currents are differentially expressed within subpopulations of sensory neurons, including currents that are thought to affect impulse generation or the capacity for repetitive firing. Currents that show such differential expression within populations of small-diameter sensory neurons include the tetrodotoxin-resistant (TTX-R) voltage-gated sodium currents (Akopian et al. 1996; Cardenas et al. 1997; Gold et al. 1996a; Tate et al. 1998), the hyperpolarization-activated cation current $I_h$ (Scroggs et al. 1994; Petruska et al. 2000), a number of transiently activated (I_A) or sustained (I_K) outward voltage-gated K+ currents (Cardenas et al. 1995; Gold et al. 1996b; Petruska et al. 2000), and Ca2+- activated K+ currents (Christian et al. 1994).

The differential expression of I_A and I_h is particularly striking because these two currents are thought to have opposite effects (enhancement and suppression, respectively) on the capacity for repetitive firing (Ingram and Williams 1996; Rudy 1988). According to a classification system for dorsal root ganglion cells based on membrane currents, type 4 cells have a large $I_h$ with no $I_A$, whereas type 2 cells have a large $I_A$ with no $I_h$ (Cardenas et al. 1995; Petruska et al. 2000). Because slope and adaptation both depend in part on a neuron’s ability to fire repetitively, the segregation of these two currents in distinct neuronal populations raises the possibility that these populations might also differ in their sensory response properties. A similar segregation might contribute to differences in slope and adaptation found between in different populations of dural afferents.
However, slope was strongly correlated with threshold in the dural afferents. Because threshold does not depend on the capacity for repetitive firing, it seems unlikely that this capacity could be the predominant factor in the determination of slope, although it might be a contributing factor in some neurons. Possibly repetitive firing capacity becomes important in determining slope only when that capacity reaches extremely low levels (such as might occur in neurons with very large \( I_A \) and no \( I_N \)), in which case it might tend to produce both slow slopes as well as rapid adaptation. This would be consistent with the association found between these two characteristics in the present study.

**Technical considerations relating to CV measurement, threshold determination, and sampling bias**

The measurement of CV in this study is subject to several potential sources of error as a result of being determined by stimulation of a peripheral innervation site rather than an exposed nerve. One potential source of error is in the determination of conduction distance because the route that each axon takes from the ganglion to the dura is not visualized but rather is assumed to follow a direct course via the tentorial nerve as observed in anatomical studies and by dissection. This could result in an underestimation of the CV if a given fiber took a more circuitous route. A second, potentially much larger source of error can arise from the slowing of CV that typically occurs in peripheral axonal branches within the innervated tissue (e.g., Peng et al. 1999). Fiber classification is based on measurement of the CV of the parent axon in its course to the target tissue rather than the peripheral branches within the target tissue. For this reason, electrical response latencies were determined at multiple sites across the exposed dura for each neuron to locate the stimulation site associated with the shortest latency, which was presumed to correspond to the position at which the parent axon first reached the dura from its course through the underlying tentorium (see Figs. 1 and 2 of Strassman and Raymond 1999). Failure to use the shortest-latency site for CV calculation could result in an underestimation of CV by as much as three- to fourfold (Peng et al. 1999; unpublished observations).

The determination of threshold in the present study was not done by extrapolation of the stimulus-response function to zero (e.g., Sengupta and Gebhart 1994a,b; Sengupta et al. 1990), which would correspond to the stimulus intensity that evokes an infinitesimal depolarization. Instead, threshold was defined as the stimulus intensity that evoked one to two spikes, which is similar to the definition of threshold used in von Frey hairs studies. The extrapolation method will result in the calculation of lower thresholds, and the decrease in threshold will be greater for neurons with lower slopes. This might tend to weaken the negative correlation between slope and threshold that was observed in the present study.

As a result of sampling bias, the distribution of conduction velocities in the study sample, as illustrated in the histogram in Fig. 1A, may not be an accurate representation of the true distribution in the population of dural afferents. One major source of bias is that slowly conducting neurons are far more difficult to isolate in microelectrode recordings than faster conducting neurons, owing to the smaller amplitude of their extracellularly recorded spikes. A second factor, which produces an opposite bias, is that the relatively long stimulus pulse that is used for activating slow fibers is not optimal for the identification of fast fibers (>5 m/s) because the longer stimulus artifact can partially obscure short-latency spikes. For unbiased searching, the pulse parameters should be alternated between those that are optimal for slow and fast fibers, but in practice, search parameters were more often optimized for slow fibers because of their greater likelihood of exhibiting mechanosensitivity. An alternative approach for determining the fiber spectrum of the dural innervation is by anatomical measurements of axon diameter, as has been done for the unmyelinated fibers by Messlinger et al. (1993) but has not yet been done for the myelinated fibers.

**Functional considerations**

As far as is known, the only function of the meningeal sensory innervation is nociceptive. It is not known to be involved in the mediation of any nonnociceptive reflexes or nonpainful sensations. Neurogenic inflammation, which can be evoked in the dura by the release of neuropeptides from sensory fibers (Moskowitz and Macfarlane 1993), has a protective function and is presumed to be mediated by nociceptive afferents. Although the meningeal sensory fibers can evoke vasodilation through the peripheral release of neuropeptides, these fibers do not appear to participate in the normal regulation of cerebral blood flow, since such regulation is not altered by trigeminal deafferentation (Branston et al. 1995; Suzuki et al. 1990). However, trigeminal deafferentation does abolish or attenuate the increase in cerebral blood flow that is evoked by a number of potentially harmful or pathological conditions, including meningitis (Weber et al. 1996), transient cerebral ischemia (Moskowitz et al. 1989), severe hypertension, and seizures (Sakas et al. 1989). Thus currently available evidence is consistent with the idea that the meningeal sensory innervation only becomes activated under abnormal, potentially harmful conditions.

The response thresholds of the dural afferents were mostly above physiological levels of intracranial pressure. Only ~3% of the neurons had thresholds within this range (<2.5 kPa), whereas ~18% had thresholds within the range of intracranial pressures reached in experimental meningitis (<5 kPa) (cf. discussion in Bove and Moskowitz 1997). Transient elevations of intracranial pressure to higher levels might occur during actions such as coughing or sudden head movement potentially resulting in the recruitment of a larger proportion of afferents. Meningitis as well as other pathological conditions might also be accompanied by decreased thresholds as a result of sensitization, which could potentially bring a larger proportion of the response thresholds within these pressure ranges.

It should be noted that comparisons of response thresholds with intracranial pressures are complicated by the nonphysiological nature of the experimental stimulus conditions. Response thresholds in this study were determined with a focal indenting stimulus, whereas intracranial pressures are more diffusely distributed, and would tend to compress the dura against the rigid cranium. Thus our stimulus might be expected to produce more tension and shear, but less compression, than would occur under physiological conditions within the closed cranial space.

Clinical observations suggest that sensory fibers involved in...
some types of headache may be particularly sensitive to mechanical force transients (see Introduction). Consistent with this, the highest response rates evoked in the dural afferent population occurred during and immediately after stimulus onset in neurons that had an adapting response component. Sustained (nonadapting) responses, which were generally of lower discharge rate, were also observed, both in neurons with and without an adapting response component. Such nonadapting responses might contribute to the headaches that can accompany sustained increases in intracranial pressure or maintained traction or displacement of meningeal tissues.

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