Relationship between electrophysiological signature and defined sensory modality of trigeminal ganglion neurons in vivo

M. Danilo Boada
Department of Anesthesiology, Wake Forest School of Medicine, Winston-Salem, North Carolina; and Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, Alicante, Spain

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Boada MD. Relationship between electrophysiological signature and defined sensory modality of trigeminal ganglion neurons in vivo. J Neurophysiol 109: 749–757, 2013. First published November 14, 2012; doi:10.1152/jn.00693.2012. The trigeminal ganglia (TG) innervate a heterogeneous set of highly sensitive and exposed tissues. Weak, innocuous stimuli can evoke pain as a normal response in some areas such as the cornea. This observation implies, however, the capability of low-threshold mechanoreceptors, inducing pain in the normal condition. To clarify this matter, the present study correlates the electrical signature (both fiber conduction velocity and somatic electrical properties) with receptor field, mechanical threshold, and temperature responsiveness of sensory afferents innervating tissues with dissimilar sensitivity (skin vs. cornea) in the trigeminal domain. Intracellular recordings were obtained in vivo from 148 neurons of the left TG of 62 mice. In 111 of these neurons, the peripheral receptor field was successfully localized: 96 of them innervated the hairy skin, while the remaining 15 innervated the cornea. The electrical signature was defined and peripheral responses correlated with tissue target. No high threshold neurons were found in the cornea. Moreover, the electrical signature of corneal afferent resembles nociceptive neurons in the skin. TG skin afferents showed similar membrane electrical signature and sensory modality as skin afferents from dorsal root ganglion, although TG afferents exhibited a shorter duration of afterhyperpolarization than those previously described in dorsal root ganglion. These data suggest that new or different ways to classify and study TG sensory neurons may be required.

trigeminal ganglia; in vivo; electrophysiology; sensory neurons; nociceptors

NEURONS OF THE DORSAL ROOT (DRG) and trigeminal ganglia (TG) in adult mammals constitute a heterogeneous population in terms of transduction properties of their sensory endings and the quality of sensations evoked by their activation. In TG in particular, the division of a nociceptive afferent to describe primary sensory neurons capable of encoding damaging or potentially damaging stimuli and nonnociceptive afferent to describe those responding to innocuous stimuli (Lewis 1942; Sherrington 1906) is overly simplistic (Bove and Light 1995). This is useful only in the context of the tissue innervated by the afferent and not in absolute terms (Cervero 1994). In vitro strategies have been useful since classically nociceptive and nonnociceptive afferents exhibit different electrical properties based upon expression of various types of functionally distinct ion channels in the cell membrane (Fedtsova et al. 2003; López de Armentia et al. 2000; Lynn and Carpenter 1982; Madrid et al. 2006). Furthermore, these specific electrical properties of the cell soma seem to be directly related to the transducing properties and encoding capacities of the peripheral sensory endings (Belmonte et al. 1991; Hoyes and Barber 1976). The present study combines both the cellular receptive field (RF) characteristics and the cellular electrical signature to differentiate low-threshold mechanosensory neurons from nociceptive mechanosensitive neurons, innervating tissues with dissimilar mechanical sensitivity in the TG domain.

Despite some inconsistencies related to the fast-conducting nociceptive afferents (A-high-threshold mechanoreceptors: AHTMR) and low-threshold mechanosensitive afferents with innocuous cold sensitivity (LTMR-MC), at normothermic temperatures, there is a relationship between cellular electrical signature and RF characteristics (Beuerman and Tanelian 1979; Boada et al. 2010, 2011). However, this has never been tested in the TG system, where different subtypes of primary sensory neurons are innervating tissues with dissimilar exposure to the environment and even with a different embryological origin than the DRG domain (Chen et al. 1995). For instance, in the TG region, peripheral axons of mechanosensory and thermosensory neurons reach not only the skin of the face, but also the eyes, teeth, and meningés. Whether cells of similar sensory modality have differences in their electrical signature [conduction velocity (CV) and somatic electrical properties], and how this signature is related with its targeted tissue in the TG remains unknown. The cornea of the eye, for example, is a tissue devoid of blood vessels and composed of cornified epithelium and is innervated by mechano-polymodal and thermosensory receptors from TG neurons (Beckers et al. 1992), as is the case for the vascularized and cornified skin (Belmonte and Giraldez 1981). Still, low-intensity mechanical, chemical, and thermal stimulation of the cornea evokes mainly sensations of irritation and pain (Acosta et al. 2001; Bessou and Perl 1969; Cabanes et al. 2003; Gallego 1983), while analogous stimuli applied to the skin of the face elicit innocuous mechanical or thermal sensations. Whether this reflects differences in the filtering properties of the supporting tissues, in the central connection of primary sensory neurons or in membrane properties of the neurons that innervate the different tissues, is still undefined.

Recently, it has been suggested that, in some sensitive tissues in the TG domain (e.g., tooth pulp), pain evoked in response to innocuous mechanical stimulation can be mediated by low-threshold mechanical afferents converging on major nociceptive relays of the trigeminal brain stem complex (Djouhri and Lawson 1999). This novel and attractive idea implies, however, that these cells (algoneurons) should have an
electrical signature similar to a tactile afferent (LTMR). If correct, we should expect these cells to resemble the action potential (AP) characteristics, somatic electrical properties, and CV of other tactile afferents in the TG domain.

In the present work, we explore general aspects of the primary sensory neurons in the TG domain. First, we investigate detectable differences in the electrical signature of these cells with respect to sensory modality in the skin. Secondly, we contrast these skin afferents against their analogs reported in the DRG domain under similar experimental conditions. Finally, we compare the skin TG sensory neuronal electrical properties with those innervating the cornea, to those of the skin.

METHODS

Experiments were conducted on young adult mice (Swiss OF1 strain) of either sex in age from postnatal day (P) 28 to P50 and weighing between 25 and 39 g. All procedures used in the present experiments were approved by Instituto de Neurociencias-Universidad Miguel Hernandez Institutional Animal Care and Use Committee.

Surgical Procedures

Mice were deeply anesthetized with Nembutal (pentobarbital sodium, 50 mg/kg, Euta-Lender, Madrid, Spain), administered intraperitoneally. The trachea was intubated, and animals were ventilated (Minivent, Harvard Apparatus, Holliston, MA) and placed in a custom-designed head fixation support. The hair over the left side of the face was clipped, the scalp cut, and a craniotomy was performed. The brain tissue frontal to the cerebellar tentorium was surgically removed, and the exposed tissues covered with gelatine foam. Heart rate was monitored using an ECG throughout as a guide to the depth of anesthesia. Decerebrated animals were immobilized with d-tubocurarine (420 μg/kg) (Sigma, St. Louis, MO). No additional anesthetics were administered after decerebration.

The skull of the animal was used as a natural perfusion chamber continuously perfused with oxygenated artificial cerebrospinal fluid (in mM: 127.0 NaCl, 1.9 KCl, 2.4 CaCl₂, 1.3 MgSO₄, 1.2 KH₂PO₄, 26 NaHCO₃, and 10.0 D-glucose), at room temperature (22 ± 2°C). Connective tissue at the surface of the internal portion of the ganglia was carefully removed, trying to leave intact the thin, deeper layers with their blood supply.

The preparation was then transferred to a preheated recording chamber (25°C) where the superfusate temperature [measured adjacent to the ganglia by a small thermocouple (IT-23, Physitemp, Clifton, NJ)] was slowly raised to 34°C. Rectal temperature (RET-3, Physitemp) was also monitored and maintained at 28 ± 2°C (Fig. 1A).

Electrophysiology

Neuronal recordings were performed under visual control targeting superficial cells only. The internal-medial portion (all the cells included in this study were recorded from this area, see Fig. 1B) of the left ganglion was illuminated tangentially with a fine (1 mm) fiber-optic light source (F-O-Lite, WPI, Sarasota, FL) that produced a Nomarski-like image of the ganglion surface, viewed through the optics (×20 objective, 0.35 numerical aperture, and 19.9-mm working distance) of an Optiphot-2 upright microscope (Nikon, Tokyo, Japan). The somata were impaled with borosilicate glass microelectrodes filled with 2 M potassium acetate in a 45° angle. The electrode tip was beveled at 44° angle until it reaches a resistance not lower than 80 MΩ (typically 85–125 MΩ) (BV-10 micropipette beveller, Sutter Instruments, Novato, CA). Intracellular recordings with a resting membrane potential of greater than or equal to −40 mV were characterized further. DC output from an Axon clamp 2B (Axon Instruments/Molecular Devices, Sunnyvale, CA) was stored on videotape and/or digitized (sampling rate 28 kHz) with a 1401 A-D converter and analyzed offline using Spike2 (CED, Cambridge, UK). Passive and active membrane properties were determined by passing small-amplitude hyperpolarizing and depolarizing current pulses (150 ms) through the microelectrode. All recordings were performed at 34°C.

Sensory RF Properties

Once a stable intracellular recording was obtained, the skin was searched, applying gentle pressure with a fine-tipped brush, to locate the peripheral RF. For nonresponding afferents, subsequent searches used increasingly stiffer probes and finally sharp-tipped watchmaker forceps. Neurons with RF located near the nose clamp or the skin incision were excluded because of the potential sensitization on those terminals. Calibrated von Frey filaments (Stoelting, Wood Dale, IL) were used to determine mechanical threshold (applied by hand) and, in some cases, peripheral response properties (e.g., rapid or slow adaptation). Thermal sensitivity was tested using a radiant heat source (cauterizer, >50°C, 3 s) or ice (close but not in contact with the surface), heated or cooled air, and combinations of these stimuli. The gross stimulus temperature value was established by placing an IT-23 thermocouple on the RF at the skin.

Fig. 1. A: schematic diagrams of a lateral view of the preparation and the area where the cellular receptive fields (RFs) were found. V1, ophthalmic nerve; V2, maxillary nerve (red); V3, mandibular nerve (blue). B: schematic and picture of the left trigeminal ganglia (TG) (upper view) showing the general area where the cell bodies were collected (red area). OpN, optic nerve; BS, brain stem.
Cellular Electrical Signature

CV. Because intact TGs give off several peripheral nerves (V1, V2, V3 branches), spike latency was determined by direct stimulation of the tissue’s RF (skin, eye), using a bipolar electrode (0.5 Hz). Electrical stimulation was performed after natural stimulation. All measurements were made with the responses obtained with the minimum stimulus intensity required to excite neurons consistently without jitter, as detailed elsewhere (Beuerman and Tanelian 1979).

Fiber CV was calculated using spike latency (measured from the beginning of the electrical stimulation artifact to the beginning of the AP) and conduction distance from the RF to the ganglia (Boada and Woodbury 2007). Cells were classified according to compound AP velocity values in peripheral nerve recordings of T11-T12 DRG previously reported in mice (Beuerman and Tanelian 1979). Accordingly, cells with CVs over 12 m/s were classified as Aβ; cells between 12 and 1.2 m/s were classified as Aδ, and below 1.2 m/s as C fibers.

Somal electrical properties. Active membrane properties, including the amplitude of the AP (mV), duration of the AP at 50% of the amplitude (D50; ms), maximum and minimum rate of depolarization and repolarization (dmV/s), and duration of the afterhyperpolarization (AHP) at 50% of the amplitude (AHP50), and passive membrane properties, including input resistance (Ri; MΩ) and time constant (τ, ms), were measured [by injecting incremental hyperpolarizing current pulses (≤0.1 nA, 500 ms) through balanced electrodes] and correlated with the sensory modality and the fiber CV (m/s). Only neurons with an evoked AP amplitude ≥40 mV measured from resting membrane potential to the peak were included in the study.

Statistical Analysis

Prior to analysis, parametric assumptions were evaluated for all variables using histograms and descriptive statistics. Some of the measurements were skewed, so all descriptive statistics are reported using medians (range). Standard parametric (Student’s t-test, one-way ANOVA, linear regression) or nonparametric (Mann-Whitney U-test, Kruskall-Wallis) tests were used, depending on normality. Statistical tests were carried out using multiple packages (OriginPro 7.5, Northampton, MA; InStat/Prism, San Diego, CA).

RESULTS

Intracellular recordings were obtained in 148 neurons of the left TG from 62 animals. In 111 of these neurons, the peripheral RF was successfully localized: 96 of them innervated the hairy skin, while the remaining 15 innervated the ocular surface. The RFs of hairy skin neurons were located in the left side of the face and around the eye, corresponding to the distribution field of the V1/V2 branches of the TG. Using a cutoff value for mechanical threshold of 1.6 mN, neurons were tentatively classified as low (LTMRs, 61/96) and high threshold (HTMRs 35/96). This initial classification was combined with their response to a suprathreshold, sustained mechanical compression of the RF with the stimulating probe, and then further subdivided into rapidly adapting (RA) neurons (highly phasic response firing at the beginning and end of a 2-s mechanical pulse) and slowly adapting (SA) neurons (tonic response firing throughout a 2-s mechanical pulses with regular or irregular pattern of discharge). Corneal sensory neurons were analyzed as an independent group. Representative somal APs and peripheral response properties of analyzed neurons are shown in Fig. 2, A and B, respectively; physiological properties of this sample are presented in Table 1.

Types of Mechanosensory Neurons Innervating the Skin

Afferents were classified by combining the following. LTMRs. Neurons conducting in the A range (>1.2 m/s) and with a mechanical threshold below 1.6 mN were initially classified as low-threshold, myelinated neurons and further grouped according to their adaptation rate to mechanical pulses and its CV.

RA (on-off response to sustained mechanical stimulation), low-threshold mechanosensory neurons (LTMR-RA) included hair follicle afferent neurons with both fast- and slow-conducting fibers (FH: fast hairs; SH: slow hairs) (41/61) that were discerned by their short-lasting response to sustained suprathreshold mechanical activation and dynamic sensitivity to single-hair displacements (Boada and Woodbury 2007; Bove and...
Table 1. Cellular properties of trigeminal sensory neurons recorded in vivo from adult mice

<table>
<thead>
<tr>
<th>Type</th>
<th>Subtype</th>
<th>n</th>
<th>Passive</th>
<th></th>
<th>Active</th>
<th></th>
<th>Somatic Electrical Properties</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Em, mV</td>
<td>Ri, MΩ</td>
<td>τ, ms</td>
<td>Amplitude, mV</td>
<td>D50, ms</td>
</tr>
<tr>
<td>Skin LTMR</td>
<td>SA</td>
<td>8</td>
<td>−61 ± 1.2</td>
<td>190 ± 23</td>
<td>1.4 ± 0.4</td>
<td>69 ± 3.9</td>
<td>0.3 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>FH/SH</td>
<td>41</td>
<td>−61 ± 0.8</td>
<td>250 ± 16</td>
<td>1.9 ± 0.2</td>
<td>71 ± 1.8</td>
<td>0.4 ± 0.03</td>
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<tr>
<td></td>
<td>MC</td>
<td>7</td>
<td>−58 ± 1.7</td>
<td>330 ± 52</td>
<td>1.5 ± 0.2</td>
<td>64 ± 2.5</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Vb</td>
<td>5</td>
<td>−60 ± 1.7</td>
<td>200 ± 64</td>
<td>1.7 ± 0.4</td>
<td>62 ± 3.3</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>HTMR</td>
<td>AHTMR</td>
<td>25</td>
<td>−60 ± 1.2</td>
<td>230 ± 21</td>
<td>2.2 ± 0.2</td>
<td>88 ± 2.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>CHTMR</td>
<td>10</td>
<td>−56 ± 1.5</td>
<td>410 ± 68</td>
<td>4.3 ± 0.7</td>
<td>89 ± 1.5</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Cornea Mech</td>
<td>10</td>
<td>61</td>
<td>−63 ± 1.9</td>
<td>270 ± 59</td>
<td>3.0 ± 0.6</td>
<td>87 ± 3.9</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Poly</td>
<td>5</td>
<td>61</td>
<td>−62 ± 1.3</td>
<td>300 ± 87</td>
<td>3.5 ± 0.4</td>
<td>89 ± 3.5</td>
<td>0.9 ± 0.2</td>
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Receptor Field Properties

| Type | X, Y | Z | | Mechanical threshold, mN |
|------|-----|---|-----------------|
| Skin | LTMR SA | 8 | 17 (11.4–30) | 1.6 (0.98–1.6) |
| FH   | 30   | 11 (6–25) | 0.98 (0.98–9.8) |
| SH   | 11   | 1.8 (1.5–3.1) | 0.98 (0.98–9.8) |
| MC   | 7    | 13 (10–30) | 0.98 (0.1–9.8) |
| Vb   | 5    | 8 (6.3-15) | 0.98 (0.1–0.98) |
| HTMR | AHTMR | 25 | 5 (1–10) | 9.8 (1.6–98) |
| CHTMR | 10 | 0.7 (0.15–1.2) | 71.5 (9.8–98) |
| Cornea Mech | 10 | 5 (1–10) | 0.98 (0.98–2.5) |
| Poly | 5    | 4.5 (1.8–8) | 0.98 (0.98–0.98) |

Values are means ± SE or medians (with ranges in parentheses); n, no. of mice. Em, resting membrane potential; Ri, input resistance; τ, time constant; AHP, afterhyperpolarization; D50, duration of the action potential at 50% of the amplitude; AHP50, AHP at 50% of the amplitude; LTMR, low-threshold mechanoreceptors; HTMR, high-threshold mechanoreceptors; SA, slowly adapting; FH, fast hairs; SH, slow hairs; MC, mechano-cold; Vb, vibrissae; AHTMR, A-high-threshold mechanoreceptors; CHTMR, C-high-threshold mechanoreceptors; Mech, mechanical; Poly, polymodal. Temperatures: ganglia 34 ± 1°C; skin 28°C ± 2°C.

Light 1995). They were insensitive to thermal stimuli. Another type of RA neuron was activated by light movements of the vibrissae (LTMR-Vb) (whiskers) (5/61), and exhibited a certain level of directionality in their response to displacement (Marfurt and Del Toro 1987).

SA, low-threshold mechanosensory neurons (LTMR-SA) (8/61) were characterized by their tonic regular activity that persisted along the duration of the mechanical compression of the skin without poststimuli discharge and fast CV (>10 m/s). Both SA type I and type II were grouped under this category (Gallar et al. 1993; Hensel et al. 1974; Lewin et al. 1992).

High-threshold mechanoreceptors. These neurons exhibited a broad but relative high mechanical threshold (median 9.8 mN; range: 1.6–98 mN). Within them, the group with mechanical threshold over 1.6 mN (25/35) with fast-conducting fibers (median: 5 m/s; range: 3–10 m/s) was segregated (AHTMR). They displayed a regular discharge (fairly steady AP-AP interval at static-phase firing) in response to sustained skin pressure and in a few cases (6/25) gave a vigorous response to heat stimulation (more than 10 AP/stimuli). None of them was activated by cold. Their RFS were typically spotlike, with one or two points of maximal sensitivity, and did not show postdischarge or ongoing activity after the stimulus was interrupted (Fig. 2).

A separate group of TG neurons (10/35), also with a high mechanical threshold (median: 71.5 mN; range: 9.8–98 mN), exhibited a CV within the C range (median: 0.6 m/s; range: 0.2–1.2 m/s) with spotlike RFS (CHTMR). Some were activated (5/10) also by noxious heat, often developing ongoing activity afterwards. This is consistent with previous reports of high-threshold mechanosensory neurons being classified as pure mechano-nociceptive (Chen et al. 1995) or polymodal-nociceptive neurons (Belmonte and Giraldez 1981; Kenshalo 1960) (Fig. 2).

LTMRs conduction velocity was in general faster than (mean CV: 11.3 ± 0.9 m/s) HTMRs neurons (mean CV: 4.5 ± 0.5 m/s) (P < 0.0001). Nevertheless, as shown in Fig. 3, A and B, a marked variability in CV among neurons within each of these two categories was observed. Within LTMRs, hairs (FH) and LTMR-Vb had a CV (median: 10.5 m/s) slower than LTMR-SA afferents (median: 17 m/s) (P < 0.001). This is also illustrated in Fig. 3A, where the APs of the different functional types of neurons have been represented according to their apparent latency in response to electrical stimulation of the peripheral RF.

Mechano-cold-sensitive neurons (LTMR-MC). A small fraction (7/61) of neurons with CV within the myelinated range (median: 15 m/s) showed an ongoing discharge (mean frequency: 8 ± 3 Hz) at rest and exhibited a vigorous response to surface cold stimulation (Fig. 2), characterized by a marked increase in firing frequency (21.3 ± 7.7 Hz) with decreases of

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Electrical Signature of Mechanosensory Neurons

AP characteristics. In LTMRs, the amplitude of the AP (mean: 66.8 ± 1.9 mV) was significantly smaller than in HTMRs (mean: 87.3 ± 1.7 mV) (P < 0.002). No difference in AP amplitude was found among the different subtypes within each of these main groups of sensory neurons.

LTMRs also consistently showed a narrow (D50) and fast AP amplitude was found among the different subtypes within each of these main groups of sensory neurons.

The membrane τ (ms) was significantly different between low- and high-threshold neuron groups. As appears in Fig. 4A, faster conducting fibers corresponded to somas with shorter APs (r² = −0.322, P < 0.001). Likewise, the duration of the AHP (AHP50) was correlated with the CV, and in general cells with faster CVs have shorter AHPs (r² = −0.258 (P < 0.001)) (Fig. 4B).

The membrane τ (ms) was significantly different between low- and high-threshold neuron groups (P < 0.001). Low-threshold neurons had smaller τ values (mean: 1.8 ± 0.1 ms) than high-threshold neurons (mean: 3 ± 0.3 ms); τ values were also correlated with CV, r² = −0.25 (P < 0.05). Likewise, cells with faster CV exhibited lower Ri than slower neurons, with a correlation value of r² = −0.25 (P < 0.05) for the entire population. However, this relationship appears to be independent of the cellular function; SH and AHTMR nociceptors shows the same τ value.
Corneal Sensory Neurons

Two subtypes of sensory neurons were identified which responded to stimulation of the corneal surface. The first one (10/15) was exclusively responsive to mechanical stimulation, whereas the second type also responded to radiant heat stimulation when applied in close proximity to the RF (5/15) (cold was not tested). Responses to mechanical stimulation did not differ between these types. Both subtypes had exquisitely low mechanical thresholds ($<0.98$ mN) (Fig. 3C) and exhibited a tonic discharge when sustained mechanical stimuli were applied (Fig. 2). CV was within the A$\beta$ range with a mean value of $5.1\pm0.9$ m/s (Fig. 3B), and only one mechanically sensitive cell showed a CV below the CV range typically ascribed to A$\beta$ fibers. Interestingly this cell was also the only one with a mechanical threshold in the range considered to be high threshold (2.5 mN). Active and passive somatic electrical properties of corneal neurons (Table 1) were indistinguishable from the AHTMRs of skin TG neurons, with similar CV and RF properties, except for the markedly lower mechanical threshold (Fig. 4, C and D).

DISCUSSION

In the present study, long-duration recordings of TG neuron somata allowed determination of electrical activity, permitting the correlation of transducing characteristics and soma electrical properties of primary sensory neurons innervating the TG territory. The principal observations and conclusions are as follows: 1) there is a correlation between the somatic electrical signature and modality (LTMR vs. HTMR); 2) LTMR fast-conducting nociceptors exist in the TG domain and show a dual modality (responding to moderate cold in addition to mechanical stimuli); 3) corneal afferents show extremely high mechanical sensitivity, despite the fact that their somatic electrical properties are identical to the AHTMR afferents recorded from the skin of the face. This is the first time that intracellular recordings were achieved in the TG of a living animal. As a result, the data presented in this study are unique in nature and provide novel insight to peripheral sensory processing in TG in vivo.

Technical Considerations

The preparation developed (TG in vivo mouse) to perform these records required mild hypothermia. In the present study, background discharges were observed only in the LTMR-MC subtype. Moderate increases in the mechanical thresholds of some tactile afferents (SH) were also observed (see below). Despite the temperature limitation, no spontaneous activity was present in any nociceptor [some cold-induced sensitization has been observed in AHTMR after long exposure (personal observation)]. This can be interpreted as a lack of sensitization, and therefore their mechanical thresholds appear to reflect a nonsensitized baseline. However, whether mechanical thresholds of both LTMR and HTMR populations would have been different at normothermia is unknown. Another limitation on this study is the consistent thermal stimulation of the cornea. It is possible to consistently apply both thermal (heat and cold) and mechanical stimulation to the skin. However, in the cornea, this is technically very challenging. Due to animal size and the short distance between cellular RF and the ganglia, any small movement during ice or cold water application results in the immediate loss of neuronal recording. Therefore, a more
controlled method will be needed to combine multimodal stimulation of this area.

Relationship Between Somal Electrical Properties and Sensory Modality

For DRG sensory neurons supplying the thoracolumbar dermatomes, a relationship between the cell’s mechanical threshold and AP shape (duration and amplitude) was previously described in cats (Hoyes and Barber 1976; Parra et al. 2010), rats (López de Armentia et al. 2000), guinea pig (Cervero 1994), and mice (Cain et al. 2001). Our results extend the relationship between mechanical threshold and AP shape and amplitude to the mechanosensory neurons innervating the trigeminal territory of mice. LTMR neurons have narrower APs, shorter duration AHPs, and smaller AHP amplitude than HTMR neurons (presumably nociceptor). Although absolute values of passive (Ri, τ) and active membrane properties (D50, AP amplitude) of TG neurons and those of DRG neurons reported by other authors are quite different, differences are likely attributable to the relatively low body temperature at which animals were maintained in our experiments (see technical limitations). Temperature dramatically alters some passive electrical properties (such as Ri), and also modifies the appearance of the AP (Beuerman and Tanelian 1979). For example, APs of HTM neurons of the DRG recorded at 34°C had a duration that was approximately one-half of that reported for APs at room temperature (Cervero 1994).

LTMR neurons (some types of hairs and SA receptors) usually show CVs in the Aβ range (Boada and Woodbury 2007; Kumazawa and Mizumura 1980), whereas D-hairs and AHMTM neurons have CVs in the Aδ range in rats (Knibestöl 1975; Koerber et al. 1988; Koerber and Woodbury 2002; Kumazawa and Mizumura 1980), monkeys (Lewin and McMahon 1991), and humans (Adriaensen et al. 1983; Parra et al. 2010). More recently, Koltzenburg et al. (1997) described the relation between CV and sensory modality in an in vitro nerve skin preparation of mice and reported some AHTMRs with CVs > 10 m/s. They also found unmyelinated mechanosensitive afferent fibers with CV below 1 m/s (thermal sensitive or not). Our results support these findings and extend them to the trigeminal system, particularly in regard to the relationship between the sensory modality and CV.

AHP Duration (AHP50) at TG A-Fiber Nociceptors

The AHP duration has been described as one of the most consistent parameters to distinguish LTMR from AHMTM neurons conducting at similar CVs. Similar studies (Boada and Woodbury 2007; Koerber et al. 1988) show that AHMTM afferents innervating hairy skin have almost twofold longer AHP duration than those in LTMR nociceptors. This unique property of the AHMTM afferents appears to be diminished in the TG (for direct comparison with this study, see Boada and Woodbury 2007, Fig. 4, A–C). In the TG, both skin and corneal afferents show considerably shorter AHP duration than those in the DRGs innervating hairy skin. It is also important to note that low temperatures increase AP and AHP durations, which in the context of this study implies that the observed AHP duration values could be even shorter at normothermic temperatures. The significance of the differences between the DRG and TG AHMTM afferents is difficult to evaluate. It has been proposed that long AHP associated with broad APs might be of importance to control discharge frequency in the soma, thereby influencing the cellular metabolism via this activity (Koerber et al. 1988). It is therefore conceivable that these cells (TG AHMTM) may be able to reach higher discharge frequencies to the same stimulus intensity compared with the same response in DRGs. However, the physiological significance of the use of the similar stimulation to evaluate these electrical differences will greatly depend on the tissue target of this afferents (see Corneal Sensory Neurons and Conclusions).

Low-Threshold Mechano-Cold Neurons (LTMR-MC)

Intracellular recordings of identified cold neurons in vivo are scarce. Their spontaneous firing and impulse response to cooling are similar to those described in the hind paw of rat (Leem et al. 1993). Also, cold-sensitive neurons were found to show high Ri and AP shape similar to those described in cultured cold-sensitive neurons of the mouse TG (Madrid et al. 2006). Somas of presumed cold-sensitive neurons recorded in vitro (Burgess and Perl 1973) and in dissociated cells (Kumazawa and Perl 1977; Perl and Burgess 1973) do not usually display ongoing activity. This suggests that ongoing impulse activity seen in the soma in vivo originates at the peripheral terminals. The presence of mechanical sensitivity in cold neurons was somewhat surprising because responsiveness to mechanical forces is usually absent in cold receptor fibers of the cat or guinea pig (Djouhri and Lawson 2001; Fried et al. 2001). However, combined mechanical and cold sensitivity have been reported in sensory fibers of rodents (Burgess and Perl 1967; Keller et al. 1991). The proportion of cold neurons was similar to that found in in vitro studies (Perl and Burgess 1973). A similar population of mechno-cold neurons has been described in the thoracic ganglia (Beuerman and Tanelian 1979).

Corneal Sensory Neurons

The functional similarity of corneal neurons with the different subtypes of identified skin nociceptors has been a matter of discussion. Previous data of Lopez de Armentia et al. (2000) showed that TG corneal neurons recorded in vitro exhibited AP durations and other membrane properties that make them similar to nociceptive neurons. Our results show that corneal sensory neurons in vivo share the active and passive membrane characteristics of cutaneous high-threshold mechanosensitive and polymodal nociceptive neurons, with the sole difference of exhibiting a much lower mechanical threshold. This may be attributable to the closeness of corneal sensory endings to the corneal surface and to the absence of keratinized superficial cells which are found in the skin (Beckers et al. 1992; Belmonte and Gallego 1983; Djouhri and Lawson 2001). A large body of data show that afferent innervations of the cornea are either unmyelinated or thinly myelinated (Beckers et al. 1992; Hoyes and Barber 1976; Keller et al. 1991; Marfurt and Del Toro 1987; Rózs and Beuerman 1982; Zander and Weddell 1951). Unexpectedly, in our study, we mostly found neurons with Aδ fibers innervating this organ. These A-fiber nociceptors were mostly mechanically sensitive, even though a large proportion of these afferents has been described as polymodal nociceptors (Belmonte and Giraldez 1981; Belmonte et al. 1991; Pozo and Cervero 1993; Tanelian and Beuerman 1984). The most obvious explanation for this discrepancy is a poten-
tial bias introduced by the electrodes used in this study, which make it more likely to record from cells with larger cellular bodies. However, this does not explain why two-thirds of our cells were sensitive only to mechanical stimulation. This observation suggests that A-fiber nociceptors innervating the cornea may be more abundant in mice than in other species (e.g., rat, guinea pig, cat from cited studies).

Conclusions

Are these corneal cells nociceptors? As argued by Cervero (1994), the nociceptive quality of a given cell capable of encoding potential or actual damaging stimulation “should be defined in terms of the tissue innervated and not in absolute terms” (cited by Bove and Light 1995). This argument appears to be appropriate for the understanding of corneal afferents. Corneal afferents recorded in this study appear to be nociceptors based on electrical signature similarities (somatic electrical properties, CV, thermal sensibility, and response dynamics) with nociceptors innervating the skin.

Strictly speaking, corneal neurons are low-threshold afferents and yet they share no electrical properties with tactile afferents in the face. The justification for defining corneal afferents with mechanical thresholds below 1 mN as nociceptive come solely from the observed pain-related response in psychophysical studies in humans (Beuerman and Tanelian 1979; Kenshalo 1960) and the recording of cellular activity in the spinal trigeminal complex (Pozo and Cervero 1993). Therefore, our data provide the first intracellular electrophysiologically-based evidence that these cells should be rightly considered LTMR nociceptors, nociceptive cells with an extremely low threshold to mechanical stimulation.

One might conclude that more susceptible and exposed tissues may have more sensitive nociceptors. However, it also appears that, when cells with nociceptive electrical profiles have the sensitivity of tactile afferents, it is hard to apply the traditional markers of nociceptive afferents by simply sampling the mechanical thresholds (Kumazawa and Mizumura 1980; Paintal 1960 cited by Bove and Light 1995). Since the susceptibility to damage in corneal tissue from mild stimuli is real, it appears that the sensitivity of mechanical nociceptive afferents needs to be different. Despite the logic of this last statement, it is clear that the definition of mechanical nociceptive afferents based only on a given cell RF mechanical threshold is inappropriate for the TG domain. Due to their low mechanical threshold [equivalent to a tactile afferent (LTMR)] and the complexity of the task to discriminate them from the tactile population, new ways to classify and study these afferents (mechanical, thermal, plus electrical characteristics) are required. In accordance with this, a revision in our current definition of mechano-nociceptors may be needed for cells in the TG domain.

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REFERENCES


