

# Selective Changes in Human Corneal Sensation Associated with Herpes Simplex Virus Keratitis

Juana Gallar,<sup>1</sup> Timo M. T. Tervo,<sup>2</sup> Waldir Neira,<sup>2</sup> Juba M. Holopainen,<sup>2</sup> Maria E. Lamberg,<sup>2</sup> Fernando Miñana,<sup>1</sup> M. Carmen Acosta,<sup>1</sup> and Carlos Belmonte<sup>1</sup>

**PURPOSE.** To determine corneal sensitivity to selective mechanical, chemical, and thermal (heat and cold) stimulation in patients with a history of herpes simplex virus (HSV) keratitis.

**METHODS.** Corneal sensitivity to different modalities of stimulus was determined in both eyes of 16 patients with unilateral HSV keratitis diagnosed 1 to 12 months before the study. On slit lamp examination, 13 HSV-affected eyes showed corneal scarring or opacities, and three had no signs of previous keratitis. Corneal sensitivity was determined with the Belmonte gas esthesiometer. Mechanical, chemical, heat, and cold stimuli were applied on the central cornea. Eyes from 10 healthy subjects served as controls.

**RESULTS.** In all control and contralateral eyes, selective mechanical, chemical, heat, and cold stimulation evoked sensations of subjective intensity proportional to the magnitude of the applied stimulus. In one HSV patient, the affected cornea was unresponsive to all types of stimuli, four lost only corneal sensitivity to mechanical stimulation, and three lost only sensitivity to heat. Mechanical ( $P < 0.005$ ) and heat ( $P < 0.05$ ) thresholds were raised in HSV eyes, whereas thresholds for CO<sub>2</sub> were not modified. Also, HSV subjects identified poorly the intensity of mechanical, chemical, and heat stimuli, whereas sensitivity to cold stimulation was unaffected.

**CONCLUSIONS.** In eyes that had had HSV keratitis, corneal sensitivity to mechanical forces and heat was significantly impaired, suggesting that axonal damage and/or altered expression of membrane ion channels involved in transduction and membrane excitability affects primarily the mechano- and polymodal nociceptor terminals. Corneal cold-sensitive terminals remain largely unaffected. (*Invest Ophthalmol Vis Sci.* 2010; 51:4516–4522) DOI:10.1167/iovs.10-5225

Corneal infection by herpes simplex virus (HSV) is a common condition that usually develops as an acute or chronic corneal inflammation.<sup>1</sup> The disease is most often due to reactivation of a latent infection of trigeminal sensory neurons innervating the cornea and possibly also of corneal epithelial cells by the neurotropic HSV (HSV1, HSV2, or both).<sup>2,3</sup>

As a result, the patient develops an epithelial keratitis. This condition is in many cases recurrent, mainly after HSV-1 infection,<sup>4</sup> and is somehow associated with an inadequate local immune response modulated by hormonal status, the presence of exogenous stimuli, use of immunosuppressant drugs or exposure to certain HSV strains.<sup>2,5–7</sup> HSV infection often affects also the corneal stroma, inducing a herpes stromal keratitis (HSK). Occasionally, HSV reaches the corneal endothelium, causing endothelial cell loss and permanent corneal swelling. Recurrent episodes may eventually lead to corneal scarring, opacities, and irregular astigmatism.<sup>2</sup>

Herpes simplex infection is a common cause of corneal sensory loss,<sup>8</sup> although less severe than in keratitis caused by reactivation of varicella-zoster virus.<sup>9</sup> The decrease in corneal sensitivity to mechanical stimulation in HSV keratitis patients is proportional to the number of recurrent episodes.<sup>8</sup> In addition, some patients with active HSV keratitis report sensations of spontaneous pain, discomfort, and dryness. These symptoms are more prominent in patients with stromal inflammation.<sup>8</sup> Corneal hypoesthesia in HSV keratitis is associated with a slightly reduced number of corneal subepithelial nerves.<sup>10</sup>

Sensations evoked at the ocular surface result from the activation of several functional classes of primary sensory neurons located in the trigeminal ganglion (TG), the peripheral axons of which innervate the anterior segment of the eye.<sup>11–13</sup> Polymodal nociceptors, the most abundant receptor type in the cornea, respond to noxious or near-noxious mechanical and thermal stimuli, to exogenous irritants, and to inflammatory agents, predominantly mediating burning pain. Mechanonociceptors are activated only by noxious mechanical forces and possibly elicit mainly pricking pain, whereas cold thermoreceptors respond to small temperature reductions of the corneal surface and evoke cooling and perhaps dryness sensations referred to the eye.<sup>14–16</sup>

Unpleasant and painful ocular sensations arising in HSV keratitis patients may be due to an altered neural activity in infected TG corneal sensory neurons.<sup>17</sup> However, there is no information about the functional subtypes of TG corneal neurons involved in corneal sensitivity disturbances. Mechanical, chemical, heat, and cold stimulation of the cornea with the Belmonte esthesiometer<sup>18</sup> has been used to selectively activate the various functional populations of TG corneal sensory neurons.<sup>19–21</sup> In the present work, corneal gas esthesiometry was performed in patients with a history of HSV keratitis, with the purpose of identifying which types of corneal sensory neurons contribute to their sensory deficits and altered ocular sensations. Preliminary results have been reported in (Gallar J, et al. *IOVS* 2002;43:ARVO E-Abstract 2559).

## METHODS

### Patients

Sixteen patients (nine women and seven men; age  $40.4 \pm 3.7$  years, range 16–66) with a history of unilateral HSV keratitis during the year

From the <sup>1</sup>Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain; and the <sup>2</sup>Department of Ophthalmology, University of Helsinki, Helsinki, Finland.

Supported by Grants SAF2008-00529, BFU2008-04425, and CSD2007-00023 from the Ministerio de Ciencia e Innovación, and Fundación Marcelino Botín, Spain.

Submitted for publication January 18, 2010; revised February 22 and March 17, 2010; accepted March 19, 2010.

Disclosure: J. Gallar, None; T.M.T. Tervo, None; W. Neira, None; J.M. Holopainen, None; M.E. Lamberg, None; F. Miñana, None; M.C. Acosta, None; C. Belmonte, None

Corresponding author: Juana Gallar, Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, Avda. Santiago Ramon y Cajal, s/n, 03550 San Juan de Alicante, Spain; juana.gallar@umh.es.

before the study (the inclusion criterion) participated voluntarily in the study. Exclusion criteria were a previous history of ocular infection (other than unilateral HSV keratitis) or chronic ocular inflammation, contact lens wearing, glaucoma, current use of ocular treatments, and previous intraocular surgery. This same group of patients had participated in a previous study.<sup>10</sup> One to 12 months before the study, all the patients presented with corneal disease that had been diagnosed as HSV keratitis. Two patients also had a history of herpes on their lips. From the time of the diagnosis (1–12 months, average  $4.8 \pm 0.8$  months) 5 of 16 patients presented one to two episodes of recurrent keratitis in the affected eye, while the contralateral eyes did not show clinical signs of HSV infection in any of the patients. Patient examination included in all cases biomicroscopy, confocal microscopy, and corneal esthesiometry.

Differences due to age and sex were avoided by recruiting a group of 10 sex and age-matched subjects (6 women and 4 men; age  $41.4 \pm 4.5$  years, range 18–65) without a history of ocular disease to serve as a control group. All subjects signed their informed consent to a protocol approved by the University's Ethical Review Committee and were free to withdraw from the study at any time. The protocol adhered to the tenets of the Declaration of Helsinki and the legal European Union regulations.

### In Vivo Confocal Microscopy

The central cornea and the corneal quadrant involved in HSV keratitis were examined using a tandem scanning confocal microscope (TSCM, model 165A; Tandem Scanning Corp., Reston, VA), as described previously.<sup>10</sup> Images were detected with a low-light-level video camera (VE-1000 Sit System; Dage-MTI Inc., Michigan City, IN), recorded, and digitalized for off-line analysis by using custom software (University of Texas Southwestern Medical Center at Dallas). The morphology of subbasal nerves visualized at the central corneal and in areas adjacent to fibrotic regions was analyzed.

### Corneal Esthesiometry

The original model of the Belmonte gas esthesiometer that was used in previous studies<sup>18–19</sup> was used to apply to the corneal surface 3-second gas jets separated by 2-minute pauses. Mechanical stimulation consisted in a series of eight pulses of air at variable flow (0–300 mL/min) heated up to reach the cornea at around 35°C, to prevent corneal temperature changes during the pulse. For chemical stimulation, five pulses of a warmed mixture of air and CO<sub>2</sub> at increasing concentrations (0%–80% CO<sub>2</sub>, in 20% steps) were applied. Thermal (heat and cold) stimulation was applied via six pulses of air at different temperatures (–10° to +80°C at the tip of the probe, that induced a temperature change at the corneal surface ranging between –4.7°C and +3.8°C around the basal corneal temperature of 34–35°C). For selective chemical and thermal stimulation, flow rates adjusted below the mechanical threshold previously determined for each subject, were used. Pulses of different magnitude were applied randomly. Stimulation was performed in the center of the cornea and sequentially to each eye, except for two subjects in whom only the HSV eye was explored. The protocol was completed in a single session.

The esthesiometer was placed on a slit lamp table and the tip of the stimulating probe was placed perpendicular to the center of the cornea at a distance of 5 mm of the ocular surface, measured with a transparent ruler. The area affected by gas jets becomes larger with the distance. When applied at 5 mm from the corneal surface, the stimuli affected approximately 60% of the total corneal surface, but remained restricted to the cornea and did not extend to the limbus,<sup>16</sup> thus avoiding excitation of limbal, low-threshold mechanoreceptors and ensuring that the receptive fields of most corneal sensory receptors, sometimes covering a full quadrant of the cornea, are stimulated. In the HSV eye of one subject, the probe was also directed to the temporal conjunctiva, 5 mm away from the limbus. Immediately after each gas pulse, the subject scored in separate, continuous horizontal visual analog scales (VASs) the intensity of the sensation experienced as well

as other sensation parameters: degree of irritation, stinging and burning pain, and warming and cooling components of the sensation. Perceptual thresholds for mechanical, chemical, heat, and cold stimulation were determined with the method of the minimum stimulus, which considers the threshold stimulus to be the lowest stimulus intensity that evoked a response  $\geq 0.5$  VAS units.<sup>19–21</sup>

Data are expressed as the mean  $\pm$  SEM. The relationship between VAS scores and the intensity of the applied stimuli was analyzed with linear regression. Sensitivity thresholds obtained in HSV and contralateral eyes and in the eyes of healthy patients of the control group were compared by using the appropriate statistical tests, as indicated in the Results Section.

## RESULTS

### Biomicroscopy

At the time of the esthesiometry, slit lamp examination revealed that the epithelium was intact in all HSV corneas, three corneas had no observable signs of the previous keratitis, and 13 corneas showed stromal scarring and were separated into groups with faint, small stromal scars ( $n = 6$ ), small stromal scars ( $n = 4$ ), medium size stromal scars ( $n = 2$ ), and large stromal scars ( $n = 1$ ). All contralateral and control corneas appeared normal.

### Confocal Microscopy

No superficial epithelial abnormalities were found in HSV corneas but, in 11 of them, dendritic structures were observed in the basal epithelial layer, in the vicinity of stromal fibrosis areas. Rapid proliferation of dendritic cells has been demonstrated.<sup>22</sup> With confocal examination, subbasal nerves appeared to be completely absent in two HSV patients, less numerous than normal in three subjects, and normal in 11 HSV eyes, as previously reported by our group.<sup>10</sup> The subbasal nerve plexus was normal in all contralateral corneas.

### Corneal Esthesiometry: Incidence of Sensitivity Changes

Mechanical, chemical, heat, and cold stimuli were applied to the corneas of both eyes of HSV patients (16 HSV and 14 contralateral eyes) and in both eyes of the group of age-matched healthy subjects (control eyes, ten subjects). No differences in the incidence of responses to selective stimulation of the cornea were found between control and contralateral eyes, which in all cases showed a positive response to mechanical, chemical, heat, and cold stimulation, except for one contralateral eye that did not respond to heat stimulation (Table 1).

Responses of corneas affected by HSV to mechanical, thermal, and chemical stimulation were variably impaired. One of 16 cor-

**TABLE 1.** Proportion of Eyes Showing Sensitivity to Selective Stimulation of the Cornea with the Gas Esthesiometer

Positive Response to	HSV Eyes ( $n = 16$ )	Contralateral Eyes ( $n = 14$ )	Control Eyes ( $n = 10$ )
Mechanical stimulation	10 (63)*	14 (100)	10 (100)
Chemical stimulation	15 (94)	14 (100)	10 (100)
Heat stimulation	12 (75)	13 (92)	10 (100)
Cold stimulation	15 (94)	14 (100)	10 (100)

The number of eyes with positive response to each type of stimulus is shown. Percentage of positive responses in relation to the total number of explored eyes ( $n$ ) is shown in parentheses. The proportion of eyes sensitive to mechanical stimuli was significantly lower in HSV eyes than in contralateral and control eyes.

\*  $P < 0.05$ ,  $z$ -test.

**TABLE 2.** Sensation Threshold for Mechanical, Chemical, Heat and Cold Stimulation of the Cornea with the Gas Esthesiometer

Stimulus Applied	HSV Eyes	Contralateral Eyes	Control Eyes
Mechanical (air flow, mL/min)*	235 ± 21†	191 ± 26	121 ± 21
Chemical (% of CO <sub>2</sub> in air)	24 ± 2	30 ± 5	22 ± 4
Heat (temperature change, °C)‡	+2.2 ± 0.2†	+2.3 ± 0.1§	+1.1 ± 0.3
Cold (temperature change, °C)	-2.6 ± 0.2	-2.9 ± 0.3	-2.0 ± 0.4

Values of the minimum stimulus that evoked a sensation rated with  $\geq 0.5$  VAS units were averaged (mean  $\pm$  SEM;  $n = 16, 14, 10$ ).

\*  $P = 0.005$ , ‡  $P < 0.001$ . One way ANOVA on ranks, showed a significant difference between the three different experimental groups.

†§  $P < 0.05$ . Dunn's test was subsequently used to determine which groups were significantly different from control group.

neas was insensitive to all types of stimuli, in contrast to the conjunctiva of the same eye where detection of all stimuli was normal. Mechanical sensitivity was absent in the affected eye of five other HSV patients, although they still responded to the other stimulus modalities. Finally, in three HSV eyes, only the sensation evoked by heat stimuli was absent. No significant correlation was found between the severity of stromal scarring (graded for statistical purposes from 0, absence of scarring, to 4, large stromal scar) and the absence of corneal sensitivity to any selective stimulus. Clinical observations indicate, however, that, in very severe cases after multiple HSV episodes, the scarred eyes are often clinically less sensitive, but such eyes were not included in this study.

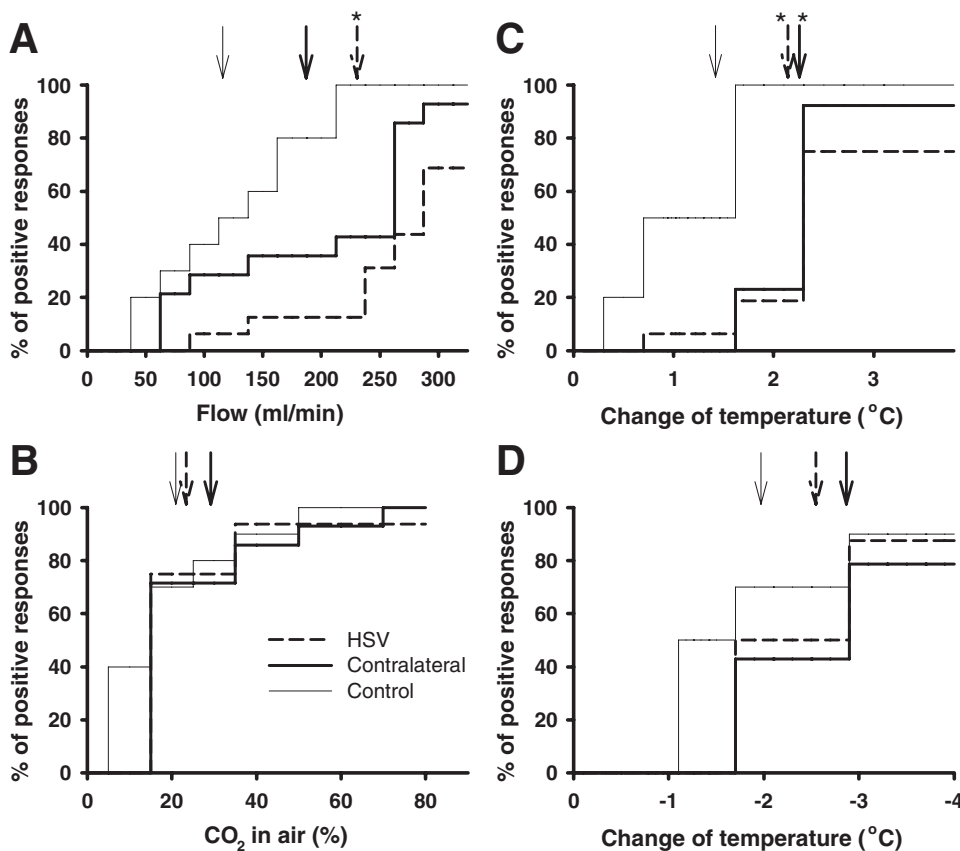
### Sensation Thresholds

Table 2 and Figure 1 present the thresholds for responses to mechanical, thermal, and chemical stimulation, determined in the HSV and contralateral eyes, as well as in the eyes of control subjects. Analysis of variance showed significant differences in

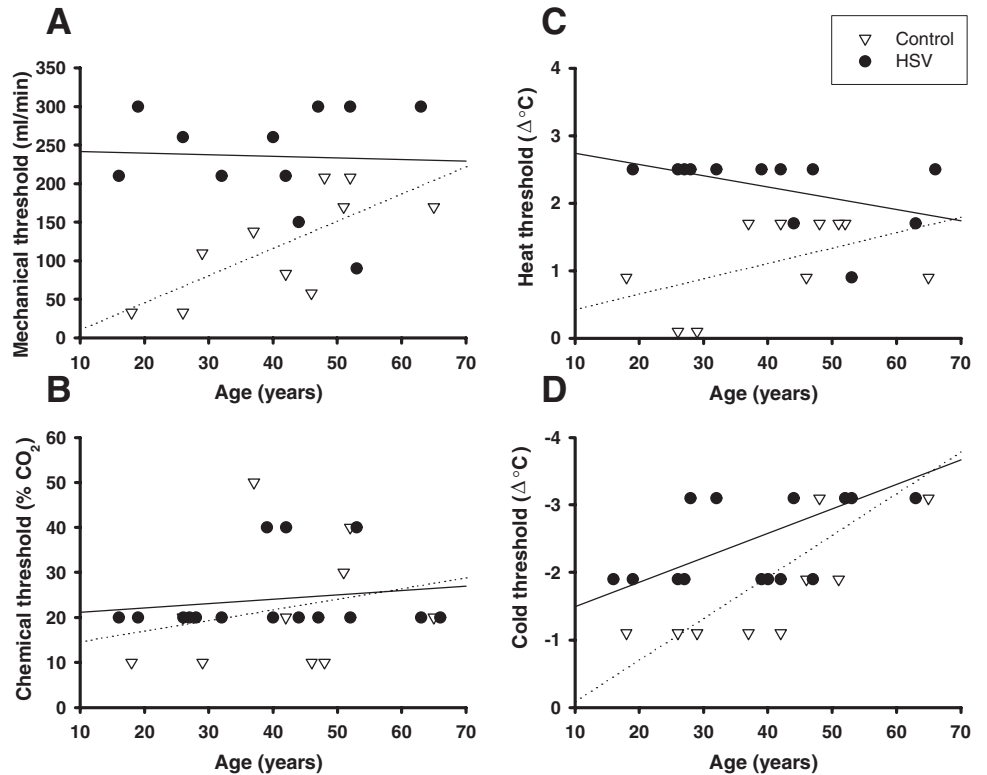
mechanical threshold ( $P = 0.005$ , Kruskal-Wallis test) that were significantly higher in the HSV than in the control eyes ( $P < 0.05$ , Dunn's method) and also slightly higher than in the contralateral eyes, although the difference did not reach the level of significance (Table 2, Fig. 1A). The mechanical thresholds determined in the contralateral and control eyes were not statistically different.

Values of sensory threshold for heat stimulation were also significantly different ( $P < 0.001$ , Kruskal-Wallis test), being higher in HSV and contralateral eyes than in the eyes of the control subjects ( $P < 0.05$ , Dunn's method; Table 2; Fig. 1C). No significant differences were found in sensory threshold for chemical and cold stimulation between HSV, contralateral, and control eyes (Table 2; Figs. 1B, 1D).

As expected from results in previous studies,<sup>23</sup> thresholds increased significantly with age in the control subjects (Pearson correlation coefficients and  $P = 0.733$  and  $0.016$ ,  $0.238$  and  $0.108$ ,  $0.487$  and  $0.154$ , and  $0.701$  and  $0.024$ , for mechani-



**FIGURE 1.** Cumulative distribution of threshold values to (A) air pulses of increasing flow (mechanical stimulation), (B) pulses of increasing CO<sub>2</sub> concentration (chemical stimulation), (C) pulses of air at increasing temperatures (heat thermal stimulation) and (D) pulses of air at decreasing temperatures (cold thermal stimulation), in the HSV ( $n = 16$ ) and their contralateral eyes ( $n = 14$ ) and in the control eyes ( $n = 10$ ). Arrows: corresponding averaged thresholds; \* $P < 0.05$ , different from control group.



**FIGURE 2.** Relationship between age and corneal sensitivity threshold to mechanical (A), chemical (B), heat (C), and cold (D) stimuli in HSV patients ( $n = 16$ ) and in a group of age- and sex-matched control subjects ( $n = 10$ ). Regression lines (solid, HSV; dotted, control) are also plotted (see text for details on  $R$  coefficients and  $P$ -values).

cal, chemical, heat, and cold stimulation, respectively; Fig. 2). In contrast, no significant correlation with age was found in the HSV eyes (Pearson correlation coefficients and  $P = 0.0445$  and  $0.897$ ,  $0.174$  and  $0.535$ ,  $-0.476$  and  $0.117$ , and  $0.313$  and  $0.256$ , for mechanical, chemical, heat, and cold stimulation, respectively), in which the higher mechanical and thermal thresholds (Table 2) appeared to be independent of the patient's age (Fig. 2). In the contralateral eyes, sensitivity thresholds were also independent of the age of the patient (Pearson correlation coefficients and  $P = 0.314$  and  $0.297$ ,  $0.358$  and  $0.208$ ,  $-0.466$  and  $0.126$ , and  $0.235$  and  $0.418$ , for mechanical, chemical, heat, and cold stimulation, respectively).

**Mechanical Stimulation**

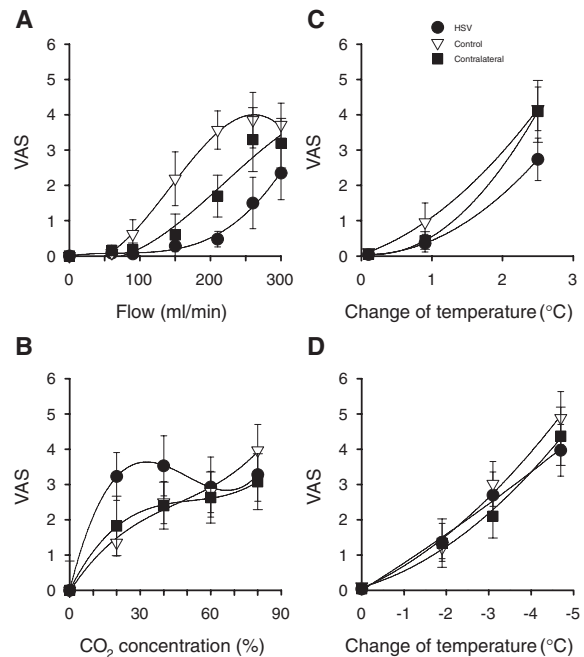
A positive, significant correlation was found between the intensity of the applied stimuli and the reported VAS scores of intensity and irritation in the HSV, contralateral, and control eyes (data not shown). VAS scores for the intensity of mechanical stimuli were significantly lower in the HSV than in the control eyes ( $P = 0.024$ , Bonferroni  $t$ -test; Fig. 3A).

Sensations evoked by mechanical stimuli were defined by all subjects as irritating, always with a predominantly stinging component. VAS scores for burning, cooling, and warming components of the sensation were lower than for stinging. Low VAS scores were also assigned to the sensations evoked by mechanical stimulation of HSV eyes (Fig. 4A). In the contralateral eyes, mechanical stimulation evoked sensations rated with VAS scores higher than those from the HSV eyes and lower than the values obtained in control eyes (Fig. 3A).

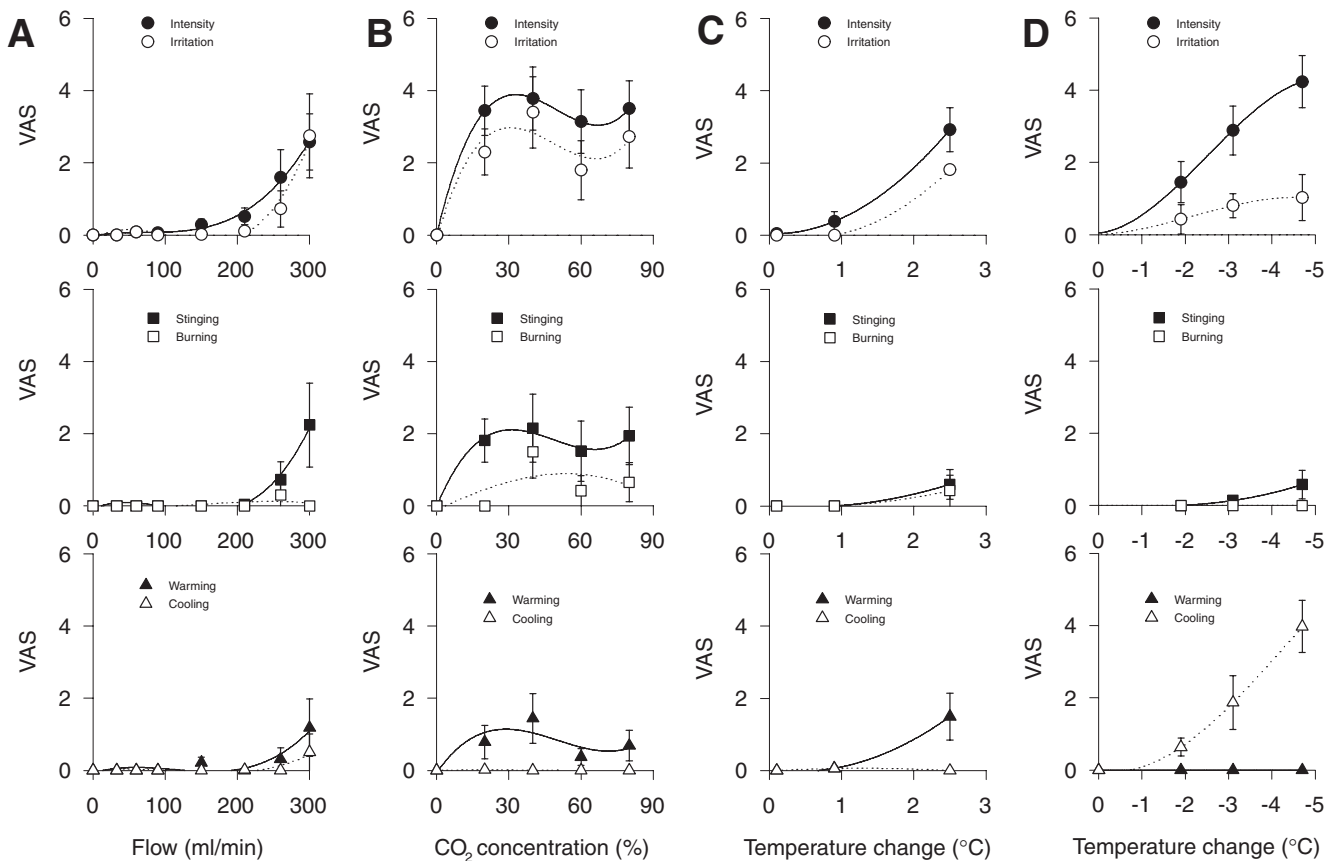
**Chemical Stimulation**

As shown in Figure 3B, the intensity of the sensation evoked in HSV eyes was independent of the concentration of  $CO_2$  in the stimulating gas (Pearson correlation coefficient =  $0.675$ ,  $P = 0.211$ ), whereas VAS scores were proportional to stimulus intensity in control subjects (correlation coefficient =  $0.984$ ,

$P = 0.002$ ) and in contralateral eyes (Fig. 3B; correlation coefficient =  $0.918$ ,  $P = 0.028$ ). In all subjects, chemical stimuli evoked irritation with a predominantly stinging component (Fig. 4B).



**FIGURE 3.** Relationship between VAS scores attributed to the intensity of evoked sensation and the amplitude of mechanical (A), chemical (B), heat (C), and cold (D) stimuli applied to the corneal surface of HSV ( $n = 16$ ), contralateral ( $n = 14$ ), and control ( $n = 10$ ) eyes. Data are the mean  $\pm$  SEM.



**FIGURE 4.** Intensity-response curves obtained in HSV eyes for the different components of the sensation evoked by selective mechanical (A), chemical (B), heat (C), and cold stimulation (D) of the cornea. Subjective intensity and irritation (*top*), stinging and burning components of the irritation (*middle*), and the thermal components of the evoked sensation (*bottom*) are represented. Data are the mean  $\pm$  SEM,  $n = 16$ .

### Heat Stimulation

VAS scores for the intensity of the sensation evoked by jets of air heated over basal corneal temperature were similar in control and contralateral eyes and slightly lower in the HSV eyes (Fig. 3C), increasing with the stimulus intensity. Heat thermal stimulation also evoked a sensation of warming proportional to the stimulus intensity (Fig. 4C).

### Cold Stimulation

VAS scores for intensity of the sensation evoked by cold stimulation were proportional to the stimulus amplitude in all groups (Pearson correlation coefficients =  $-0.980$ ,  $-0.995$ , and  $-0.975$ ,  $P = 0.02$ ,  $0.005$ , and  $0.03$ , for control, HSV, and contralateral eyes, respectively; Fig. 3D). Average VAS scores of the six parameters of the sensation evoked by cold air were similar in the HSV (Fig. 4D), contralateral, and control eyes. In the HSV eyes, cold stimulation was slightly irritating and evoked a cooling sensation proportional to the stimulus intensity (correlation coefficient =  $-0.957$ ,  $P = 0.04$ ; Fig. 4D).

## DISCUSSION

The present work shows that patients who had had one or several episodes of HSV corneal infection experienced a reduction in corneal sensitivity of variable magnitude. Perceptual characteristics of corneal sensation were also disturbed to a variable degree, suggesting that a different level of impairment occurs in the functionally heterogeneous population of sen-

sory neurons innervating the cornea after an HSV corneal infection.

During the first several days after corneal inoculation, HSV replicates in corneal epithelial cells and is then cleared by the host immune response.<sup>2,24</sup> Over this period, HSV enters nerve terminals and is transported retrogradely to the neuronal cell bodies, where it undergoes acute replication. Nonspecific and specific immune effector cells infiltrate along the trigeminal nerve and form foci around individual infected neurons. Although viral replication is not detectable and most infiltrating cells are cleared by 1 month after corneal inoculation, low levels of immune effector cells remain in ganglia harboring latent HSV.<sup>25-27</sup> During the latency period, genes in sensory neurons encoding proteins involved in neurotransmission, voltage-gated ion channels, neuropeptides, and axonal elongation and remodeling, change their expression pattern.<sup>28,29</sup> After reactivation, herpes viruses spread within sensory neurons and transfer across junctions formed between nerve terminals and corneal epithelium cells.<sup>2,30</sup> It has been suggested that small quantities of viral antigens from the HSV-infected epithelial cells spill into the stroma and promote HSK. The long delay between virus spread in the epithelium and development of HSK may reflect the time necessary to overcome control mechanisms that dampen host immunity in the stroma.

Patients included in our study were all in the latent period, and one third of them had experienced reactivation episodes. At the time of esthesiometry, many of the corneas exhibited dendritic structures at the level of the basal epithelial cells and areas of stromal fibrosis but no overt signs of active inflammation. Thus, the low corneal sensitivity observed in most of the

patients could be the consequence of a decrease in the number of sensory axons innervating the cornea and/or of a permanent impairment of the transducing capacities of corneal sensory terminals.

Localization of sensitivity loss after HSV keratitis varies from patient to patient. In a classic study, Norn<sup>8</sup> reported that hypoesthesia could be restricted to the central cornea (12% of patients), to the periphery (14%), or to one half of the cornea (22%), although in a majority of subjects (52%), mechanical sensitivity was uniformly impaired or normal.<sup>8</sup> In the present study, the tip of the esthesiometer was directed to the center of the cornea. However, air puffs applied with this gas esthesiometer and measured by corneal thermography reached up to 60% of the corneal surface.<sup>16</sup> Besides, receptive fields (RFs) of polymodal neurons often cover more than one fourth of the corneal surface and extend several millimeters into the adjacent limbus and conjunctiva; average RF of mechanonociceptive neurons are smaller and cover ~10% of the corneal surface; finally, RFs of corneal cold thermosensitive neurons are dotlike and small and more abundant in the peripheral than in the central cornea.<sup>31</sup> Considering the size, location, and overlapping of the RFs of sensory neurons innervating the cornea and the size of the area stimulated by the gas pulses, it can be assumed that most if not all corneal sensory receptors were stimulated by the applied stimuli. Thus, sensory deficits found in the present work are safely attributable to a decrease in the number of sensory nerves or to an alteration of their function.

In some of the HSV patients, nerve fiber density at the corneal subbasal nerve plexus was found by confocal microscopy to be reduced *in vivo*. This technique does not possess enough resolution to quantify epithelial nerve terminals and may not even reveal all subbasal fibers and thus, the possibility that some of them were permanently damaged after the HSV-induced inflammatory episodes cannot be excluded. However, overall nerve density in the subbasal plexus appears to be largely unaffected in most cases with resolved HSV keratitis,<sup>10</sup> supporting the view that a lower nerve fiber density is not the sole cause of the decreased corneal sensitivity observed after HSV keratitis.

A decreased sensory input from corneal afferent nerves to the brain, leading to reduced sensitivity could also result from an abnormally low excitability of TG neurons due to the altered gene expression reported during the HSV latent state.<sup>28</sup> Experimental infection of cultured primary sensory neurons with HSV *in vitro* causes, within 24 hours, a profound, rapid, and selective loss of voltage-dependent Na<sup>+</sup> currents due to internalization of Na<sup>+</sup> channels from the plasma membrane.<sup>32</sup> The density and functional state of voltage-gated Na<sup>+</sup> channels in the cell membrane determine the excitability of adult sensory neurons.<sup>33</sup> Therefore, long-lasting disturbances of ion channel function—in particular, Na<sup>+</sup> channels of the cell body, peripheral axons, and terminals of TG neurons—may contribute to the changes in sensitivity found in postherpetic patients. The presence of allodynia and hyperalgesia during the latent period in mice infected with HSV-1 for at least 40 days supports this interpretation.<sup>34</sup> Such persistent allodynia and hyperalgesia signs resemble the abnormal sensations, including spontaneous pain and mechanical allodynia, described by postherpetic neuralgia patients long after clinically evident disease episodes.<sup>35</sup> Finally, it has been reported that several Na<sup>+</sup> channel blockers such as gabapentin and amitriptyline reduce the pain sensation that appears during acute herpetic episodes and also during the postherpetic outbreaks,<sup>36</sup> supporting evidence of an involvement of Na<sup>+</sup> channels in the generation of postherpetic sensitivity disturbances.

Our results not only show a general decrease in corneal sensitivity after HSV keratitis but also provide evidence that impairment of the different corneal sensation modalities was

not homogeneous. The final quality of corneal sensations depends on the degree of recruitment of the functionally different subpopulations of corneal sensory neurons (mechanonociceptor, polymodal nociceptor, and cold receptor TG neurons) whose preferential activation by their specific stimulating energy evokes conscious sensations with different perceptual components.<sup>11–13,16,19</sup> The specificity of the different functional classes of sensory neurons to physical and chemical stimuli depends on the selective expression of characteristic transduction molecules. Detection of mechanical forces involves stretch-activated channels, whereas TRPV1 and TRPA1 channels are the main but not exclusive transducers of heat, acid, and a large number of endogenous and exogenous chemical agents. Transduction of cold stimuli is primarily mediated by TRPM8 channels.<sup>37,38</sup>

In HSV keratitis patients, decreases in the detection sensitivity for mechanical forces were pronounced, as was also the case for sensitivity to heat stimulation and, to a lesser degree, to CO<sub>2</sub>, whereas responsiveness to cold was unaffected. Both mechanosensory and polymodal neurons respond to mechanical stimuli, whereas heat and acidic stimuli are detected only by polymodal neurons. Differences in the impairment of the various sensory parameters after HSV infection may reflect variable effects on polymodal, mechanosensory, and cold sensitive TG neurons and on their transduction mechanisms for the different types of stimuli. The significant decrease in mechanical sensitivity after HSV infection suggests a reduction of the neural input to the brain that originates at mechanonociceptor and polymodal nociceptor terminals, where transduction of mechanical forces takes place. Detection of heat and of the reductions in pH produced by CO<sub>2</sub> occurs only in polymodal neurons. Impaired function of these neurons could explain the selective deficit in sensitivity to these stimuli, whereas preservation of cold sensitivity suggests that the function of cold-specific neurons remained virtually intact. There is convincing experimental evidence showing that different neuronal subtypes have different degrees of susceptibility to productive and latent infection with HSV-1. The virus preferentially establishes a latent infection in IB4 negative neurons, which are TRPV1 positive and immunoreactive for neuropeptides and the high-affinity nerve growth factor receptor.<sup>7</sup> At the best of our knowledge, there is no experimental information on whether expression of TRP or other transducing channels is selectively compromised in HSV-1 infected TG neurons. It could be speculated that in addition to Na<sup>+</sup> channels, expression of mechanosensory transduction channels and of TRPV1 and TRPA1 channels decreases in mechanosensory and polymodal neurons after HSV keratitis, whereas the density of TRPM8 channels in cold-sensitive neurons does not appear to be significantly modified.

A slight decrease in corneal sensitivity was also detected in contralateral eyes. This response could be caused by a subclinical HSV extension to the contralateral side, a possibility that has been repeatedly suggested.<sup>39,40</sup> Alternatively, some sensory nerve endings of the contralateral cornea may become damaged if the bilateral alteration of tear composition and volume that has been reported in HSV patients<sup>40</sup> persists for prolonged times. A similar mechanism has been proposed to explain corneal hyposensitivity of dry eye patients.<sup>41,42</sup> Nevertheless, in postherpetic patients, normal tear secretion can still be evoked by stimulation with alcohol vapor of the nose mucosa, which is innervated by the maxillary division of the trigeminal nerve, suggesting that in these patients decreased tearing is probably the consequence and not the cause of the reduced corneal sensory input observed after HSV keratitis.

In summary, the present results suggest that sensory deficits and altered ocular sensations experienced after HSV keratitis are primarily associated with an impairment of polymodal and

mechanonociceptive trigeminal sensory neurons, possibly resulting from an altered expression in HSV-infected neurons of ion channels involved in sensory transduction and/or the termination of neuronal excitability.

### Acknowledgments

The authors thank Alfonso Pérez-Vegara and Manuel Bayonas for technical assistance, Jukka Moilanen and Minna Vesaluoma for their collaboration in the initial experiments, and Elspeth M. McLachlan for critical reading of the manuscript.

### References

- Liesegang J. Herpes simplex virus epidemiology and ocular importance. *Cornea*. 2001;20:1-13.
- Kaye S, Choudhary A. Herpes simplex keratitis. *Prog Retin Eye Res*. 2006;25:355-380.
- Kaye SB, Baker K, Bonshek R, et al. Human herpes viruses in the cornea. *Br J Ophthalmol*. 2000;84:563-571.
- Toma HS, Murina AT, Areaux RG Jr, et al. Ocular HSV-1 latency, reactivation and recurrent disease. *Semin Ophthalmol*. 2008;23:249-273.
- Wilhelmus KR, Dawson CR, Barron BA, et al. Risk factors for herpes simplex virus epithelial keratitis recurring during treatment of stromal keratitis or iridocyclitis. Herpetic Eye Disease Study Group. *Br J Ophthalmol*. 1996;80:969-972.
- Herpetic Eye Disease Study Group. Predictors of recurrent herpes simplex virus keratitis. *Cornea*. 2001;20:123-128.
- Margolis TP, Elfman FL, Leib D, et al. Spontaneous reactivation of herpes simplex virus type 1 in latently infected murine sensory ganglia. *J Virol*. 2007;81:11069-11074.
- Norn MS. Dendritic (herpetic) keratitis IV: follow-up examination of corneal sensitivity. *Acta Ophthalmol*. 1970;48:383-395.
- Kaufman HE. Treatment of viral diseases of the cornea and external eye. *Prog Retin Eye Res*. 2000;19:69-85.
- Rosenberg ME, Tervo TM, Müller LJ, Moilanen JA, Vesaluoma MH. In vivo confocal microscopy after herpes keratitis. *Cornea*. 2002;21:265-269.
- Belmonte C, Acosta MC, Gallar J. Neural basis of sensation in intact and injured corneas. *Exp Eye Res*. 2004;78:513-525.
- Belmonte C, Aracil A, Acosta MC, Luna C, Gallar J. Nerves and sensations from the eye surface. *Ocul Surf*. 2004;2:29-34.
- Belmonte C, Tervo T. Pain in and around the eye. In: McMahon S, Koltzenburg M, eds. *Wall and Melzack's Textbook of Pain*. 5th ed. London: Elsevier Science; 2005:887-901.
- Belmonte C, Gallar J, Pozo MA, Rebollo I. Excitation by irritant chemical substances of sensory afferent units in the cat's cornea. *J Physiol*. 1991;437:709-725.
- Gallar J, Pozo MA, Tuckett RP, Belmonte C. Response of sensory units with unmyelinated fibres to mechanical, thermal and chemical stimulation of the cat's cornea. *J Physiol*. 1993;468:609-622.
- Acosta MC, Belmonte C, Gallar J. Sensory experiences in humans and single-unit activity in cats evoked by polymodal stimulation of the cornea. *J Physiol*. 2001;534:511-525.
- Belmonte C. Eye dryness sensations after refractive surgery: impaired tear secretion or "phantom" cornea? *J Refract Surg*. 2007;23:598-602.
- Belmonte C, Acosta MC, Schmelz M, Gallar J. Measurement of corneal sensitivity to mechanical and chemical stimulation with a CO<sub>2</sub> esthesiometer. *Invest Ophthalmol Vis Sci*. 1999;40:513-519.
- Acosta MC, Tan ME, Belmonte C, Gallar J. Sensations evoked by selective mechanical, chemical, and thermal stimulation of the conjunctiva and cornea. *Invest Ophthalmol Vis Sci*. 2001;42:2063-2067.
- Gallar J, Acosta MC, Moilanen JA, Holopainen JM, Belmonte C, Tervo TM. Recovery of corneal sensitivity to mechanical and chemical stimulation after laser in situ keratomileusis (LASIK). *J Corneal Refract Surg*. 2004;20:229-235.
- Gallar J, Morales C, Freire V, Acosta MC, Belmonte C, Duran JA. Corneal sensitivity and tear production are decreased in fibromyalgia patients. *Invest Ophthalmol Vis Sci*. 2009;50:4129-4134.
- Hamrah P, Pavan-Langston D, Dana R. Herpes simplex keratitis and dendritic cells at the crossroads: lessons from the past and a view into the future. *Int Ophthalmol Clin*. 2009;49:53-62.
- Acosta MC, Alfaro ML, Borrás F, Belmonte C, Gallar J. Influence of age, gender and iris color on mechanical and chemical sensitivity of the cornea and conjunctiva. *Exp Eye Res*. 2006;83:932-938.
- Kaye SB, Lynas C, Patterson A, Risk JM, McCarthy K, Hart CA. Evidence for herpes simplex viral latency in the human cornea. *Br J Ophthalmol*. 1991;75:195-200.
- Simmons A, Tschärke D, Speck P. The role of immune mechanisms in control of herpes simplex virus infection of the peripheral nervous system. *Curr Top Microbiol Immunol*. 1992;179:31-56.
- Shimeld C, Whiteland JL, Nicholls SM, Grinfeld E, Easty DL, Gao H, Hill TJ. Immune cell infiltration and persistence in the mouse trigeminal ganglion after infection of the cornea with herpes simplex virus type 1. *J Neuroimmunol*. 1995;61:7-16.
- Liu T, Tang Q, Hendricks RL. Inflammatory infiltration of the trigeminal ganglion after herpes simplex virus type 1 corneal infection. *J Virol*. 1996;70:264-271.
- Kramer MF, James Cook W, et al. Latent herpes simplex virus infection of sensory neurons alters neuronal gene expression. *J Virol*. 2003;77:9533-9541.
- Hamza MA, Higgins DM, Ruyechan WT. Two alphaherpesvirus latency-associated gene products influence calcitonin gene-related peptide levels in rat trigeminal neurons. *Neurobiol Dis*. 2007;25:553-560.
- Polcivova K, Biswas PS, Banerjee K, Wisner TW, Rouse BT, Johnson DC. Herpes keratitis in the absence of anterograde transport of virus from sensory ganglia to the cornea. *Proc Natl Acad Sci USA*. 2005;102:11462-11467.
- Belmonte C, García-Hirschfeld J, Gallar J. Neurobiology of ocular pain. *Prog Retin Eye Res*. 1997;16:117-156.
- Storey N, Latchman D, Bevan S. Selective internalization of sodium channels in rat dorsal root ganglion neurons infected with herpes simplex virus-1. *J Cell Biol*. 2002;158:1251-1262.
- Cummins TR, Sheets PL, Waxman SG. The roles of sodium channels in nociception: implications for mechanisms of pain. *Pain*. 2007;131:243-257.
- Takasaki I, Sasaki A, Andoh T, Nojima H, Shiraki K, Kuraishi Y. Effects of analgesics on delayed postherpetic pain in mice. *Anesthesiology*. 2002;96:1168-1174.
- Oaklander AL. Mechanisms of pain and itch caused by herpes zoster (shingles). *J Pain*. 2008;9(1 suppl):S10-S18.
- Kuraishi Y, Takasaki I, Nojima H, Shiraki K, Takahata H. Effects of the suppression of acute herpetic pain by gabapentin and amitriptyline on the incidence of delayed postherpetic pain in mice. *Life Sci*. 2004;74:2619-2626.
- Belmonte C, Viana F. Molecular and cellular limits to somatosensory specificity. *Mol Pain*. 2008;4:4-14.
- Nilius B, Owsianik G, Voets T, Peters JA. Transient receptor potential cation channels in disease. *Physiol Rev*. 2007;87:165-217.
- Shimeld C, Tullo AB, Hill TJ, Blyth WA, Easty DL. Spread of herpes simplex virus and distribution of latent infection after intraocular infection of the mouse. *Arch Virol*. 1985;85:175-187.
- Keijser S, van Best JA, Van der Lelij A, Jager MJ. Reflex and steady state tears in patients with latent stromal herpetic keratitis. *Invest Ophthalmol Vis Sci*. 2002;43:87-91.
- Bourcier T, Acosta MC, Borderie V, et al. Decreased corneal sensitivity in patients with dry eye. *Invest Ophthalmol Vis Sci*. 2005;46:2341-2345.
- Benítez-Del-Castillo JM, Acosta MC, Wassfi MA, et al. Relation between corneal innervation with confocal microscopy and corneal sensitivity with noncontact esthesiometry in patients with dry eye. *Invest Ophthalmol Vis Sci*. 2007;48:173-181.