Cornea

Interaction of Corneal Nociceptive Stimulation and Lacrimal Secretion

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PURPOSE. To investigate the interaction between corneal stimuli at different positions and tear secretion and to establish relationships between nociceptive stimuli detection thresholds and stimulated tearing.

METHODS. Using a computerized Belmonte-esthesiometer, mechanical and chemical stimuli, from 0% to 200% of the threshold in 50% steps, were delivered (in random order) to the central and peripheral (approximately 2-mm inside the limbus) cornea during four separate sessions to 15 subjects. Immediately after each stimulus, tear meniscus height (TMH) was measured using optical coherence tomography to quantify the amount of lacrimal secretion, and subjects reported whether they felt tears starting to accumulate in their eyes. Thresholds (50% detection) for detection of tearing were estimated.

RESULTS. TMH increased with increasing stimulus intensity (P < 0.05), and the overall increase was higher with central stimulation than with peripheral stimulation (P < 0.05). The changes in TMH with threshold-scaled stimulus intensity depended on test location (P < 0.05) and stimulus modality (P < 0.05). The maximum intensity of mechanical stimulation of the central cornea induced the greatest TMH (all P < 0.05). For chemical stimulation, the stimulus intensity required to induce detectable tearing was higher than that required to detect a stimulus and higher in the periphery than at the center (all P < 0.05).

CONCLUSIONS. Noxious mechanical and chemical stimuli evoked measurable tear secretion, with central corneal mechanical stimulation evoking the strongest lacrimation reflex. Central mechanical corneal stimulation is the most effective stimulus-position pairing and appears to be the major sensory driving force for reflex tear secretion by the lacrimal functional unit. (Invest Ophthalmol Vis Sci. 2010;51:5640–5645) DOI:10.1167/iovs.10-5502

Tear secretion is controlled by the integrated lacrimal functional unit that comprises the ocular surface (cornea, conjunctiva, and meibomian glands), the lacrimal glands, and the sensory afferent and autonomic efferent nerves that connect them.¹–⁵ The functional unit regulates the major components of the tear film¹–⁴ and responds to environmental, endocrine, and central neural influences,²,³,⁵,⁶ with overall function to maintain the quality of the principal optical component of the eye.⁷ Sensory signals arising from the ocular surface are an important aspect of the lacrimal functional unit.⁷ It has been considered that wakening tear production is driven by a reflex circuit consisting of the sensory afferents coming mainly from the ocular surface, the synaptic integration at the spinal trigeminal nucleus (Vsp) and a relay to the superior salivatory nucleus located in the brain stem, and the efferent fibers to parasympathetic and sympathetic ganglia and then to the lacrimal glands and other ocular surface tissue.²,⁶,⁸,⁹ During the steady state, without injury or pathology, the sensory nerves of the ocular surface provide low-level input to the functional unit and operate with the efferent parasympathetic and sympathetic nerves to modulate resting tear flow.⁵,⁷ Stimulation of sensory nerves near or above injurious levels results in tear secretion and other reflexes to protect the eye from potential damage.¹⁰

The cornea is richly innervated by sensory nerves that serve important sensory and reflex functions.¹¹–¹² Corneal sensory nerves predominantly originate from neurons located in the ipsilateral trigeminal ganglion and can be classified as thin myelinated (Aδ type) and unmyelinated (C type) fibers.¹⁰ The peripheral axons of the neurons terminate throughout the corneal epithelium as so-called free nerve endings.¹²–¹⁵ Though encapsulated neural terminals have been observed in the limbus and perilimbal bulbar conjunctiva.¹⁶ Despite the lack of morphologic specialization at the endings, different functional sensory fibers, including polymodal nociceptors, mechanonociceptors, and cold receptors, have been identified based on their electrophysiological properties.¹⁷,¹⁸ In addition, a small number of low-threshold mechanical receptors have been found in the limbus.¹⁷ Recently, a study has proposed that the afferent pathways of basal and reflex tearing might involve a different subclass of sensory receptors.¹⁹

Although the peripheral and central neural pathways for the lacrimal reflex induced by corneal stimulation have been partially elucidated, the relationship between the intensity of the sensory input and the outflow of the efferent autonomic nervous system in the lacrimal functional unit has not been defined. It is unclear whether tear secretion induced by stimulation at the central and peripheral cornea is similar, though it has been suggested that the corneal distribution of a subclass of functional types of receptors might be similar.²⁰

In the present study we investigated the interaction between stimulation at various corneal positions and the efferent output, as determined by tear secretion, and defined the psychophysiological function as the relationship between stimulus intensity and stimulated tearing. Systematically varied intensities of mechanical and chemical stimuli were delivered to different positions of the cornea using a Belmonte pneumatic esthesiometer. Tear volume expressed by tear meniscus height (TMH) was quantified using optical coherence tomography (OCT),²¹ and perceived lacrimation was quantified after each stimulus.

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SUBJECTS, MATERIALS, AND METHODS

Subjects

Fifteen healthy subjects (nine women, six men; age range, 18–47 years; mean ± SD, 31.6 ± 7.9) who did not wear contact lenses and had no history of ocular or systemic diseases or surgery participated in the study.

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and received clearance from the University of Waterloo, Office of Research Ethics (Waterloo, Ontario, Canada). Informed consent was obtained from each subject.

Mechanical and Chemical Stimulation

A computer-controlled Belmonte esthesiometer, described in detail elsewhere,20,22–25 was used to deliver mechanical and chemical stimuli to the central and temporal peripheral cornea (approximately 2 mm from limbus) of the left eye.

Mechanical stimuli consisted of a series of air pulses with flow rate varying from 0 to 200 mL/min. Chemical stimulation was induced by increasing the concentration of CO2 in the air with the flow rate well below the mechanical threshold. The temperature of the stimulus was set at 50°C, which dropped to approximately 35°C at the ocular surface.22–24

At the initial assessment, subjects underwent a training session using stimulation of the central cornea of the right eye. Initial detection thresholds to mechanical and chemical stimuli at both test locations were then estimated using the ascending method of limits (the average of three first reports of stimulus presence) to define the stimulus range. Mechanical and chemical stimuli were set at five levels, ranging from 0% to 200% of the initial detection threshold in 50% steps. Measurements were made during four separate sessions at approximately the same time of day. All measurements were conducted at least 4 hours after subjects awoke to minimize the influence of diurnal effects.25 The order of the test location and the stimulus modality was randomly assigned, and, in each session, the intensity of the stimulus was presented in random order, with a minimum interstimulus interval of 2 minutes; each stimulus was delivered three times.

Tear Meniscus Height Measurement

Before each measurement session and immediately after each stimulus, TMH21 at the mid-lower lid margin was measured with an optical coherence tomographer (model 2010; Zeiss Humphrey System, Dublin, CA) to quantify the amount of lacrimal secretion. Custom software was used to measure TMH of the OCT images.

Estimation of Detectable Tearing Thresholds

Subjects reported (yes or no) the presence of detectable tearing using a button box. Tearing threshold (midpoint of intensity detectable tearing), psychometric function, and slope were estimated using non-linear regression fits of the logistic function: Likelihood of detection = 1 − [1/(1 + (stimulus intensity/threshold)^α)].

Statistical Analysis

Statistical analyses were performed (Statistica 8.0; StatSoft Inc., Tulsa, OK), with P ≤ 0.05 considered statistically significant. Repeated-measures ANOVA and post hoc Tukey HSD tests were used to compare the differences in the TMH measurements between intensity levels, stimulus positions and modalities, and their interactions. The detectable tearing thresholds between locations for each modality and their interactions with the sensory thresholds were also compared.

RESULTS

Effects of Stimulus Intensity, Location, and Modality on TMH

Measurements of TMH at baseline (before each measurement session) and with mechanical and chemical stimulation of differing intensities stratified by modality and location are shown in Figure 1. There was a significant intensity effect regardless of stimulus modality and location, as shown in Figure 2 (ANOVA P < 0.001). TMH increased as a function of increased stimulus intensity. At 200% (2x) threshold, TMH was significantly higher than all stimulus intensities except for 150% (1.5x) threshold (Tukey HSD, all P < 0.05). TMH without stimulation (0% threshold) was significantly lower than any given stimulus intensity (Tukey HSD, all P < 0.05).

There was a significant location effect on TMH independent of stimulus modality (ANOVA, P < 0.001), with higher TMH for central stimulation. The overall increased TMH was similar between mechanical and chemical stimulation regardless of the stimulus locations (ANOVA, P > 0.05).
Interactions between Stimulus Intensity, Location, and Modality

Significant two-way interactions were found between intensity and location, and intensity and modality, as illustrated in Figures 3 and 4 (ANOVA, $P = 0.001$ and $P < 0.001$ for location and modality, respectively). Independent of stimulus modality, intensity at and above the threshold resulted in greater TMH for central stimulation than for peripheral stimulation (Tukey HSD, all $P < 0.05$). On the other hand, TMH induced by different modalities, regardless of location, were similar at most of the intensity levels except for the highest level (Tukey HSD, $P = 0.001$). Although the overall three-way interaction between stimulus intensity, location, and modality was not significant (ANOVA, $P = 0.189$), post hoc pairwise comparison showed that the significant difference between modalities was at the highest suprathreshold level for central stimulation only (Tukey HSD, $P = 0.003$), with the greatest TMH induced by the maximum intensity of mechanical stimulation (Fig. 5).

Effect of Stimulus Location on Tearing Thresholds and Relation to Stimulus Detection

Thresholds to mechanical and chemical stimuli and the tearing thresholds at each location in response to different modalities are presented in Table 1. Generally, thresholds to detect mechanical stimulation and detectable tearing were similar across locations, and there was no significant interaction between threshold and location (ANOVA, both $P > 0.05$). A significant difference was found between detectable tearing and detection threshold to the chemical stimulation; tearing thresholds were higher than detection thresholds (ANOVA, $P = 0.043$), and this difference was dependent on the stimulus location (ANOVA, $P = 0.011$). Post hoc pairwise comparison showed that detectable tearing thresholds were higher at the periphery than at the center and higher than the threshold for detecting a stimulus at each corresponding location (Tukey HSD, all $P \leq 0.028$).

DISCUSSION

The primary objective of this study was to investigate the relationship between stimulation of corneal sensory nerves and efferent output of the lacrimal functional unit determined by tear secretion. Tear secretion increased almost linearly with the increase of stimulus intensity.

It has been suggested that the sensory effects and, hence, the lacrimal functional unit operate differently, depending on environmental conditions and pathology. Without stressful stimulation, sensory nerves on the ocular surface provide subthreshold sensory input to the functional unit modulating resting tear flow. When noxious stimuli activate sensory afferents in the functional unit, a series of coordinated reflexes, including reflex tearing, are triggered to protect the eye from potential damage. The present study showed that suprathreshold nociceptive corneal stimuli evoke apparently reflex lacrimation, accompanied by the perception of increasing tear flow in the eye, whereas tear secretion after subthreshold stimuli was the same as unstimulated tear flow (Fig. 1). This physiological evidence supports the hypothesis that two states of a neural control mechanism (i.e., basal-subconscious and augmented-conscious) are involved in regulating tear secretion to protect the ocular surface from injury over a wide range of situations.

Stimuli to the cornea are detected and encoded by the sensory receptors or transducers on the peripheral terminals of
the corneal fibers. Corneal sensory fibers have different functional types of receptors that are preferentially activated by different types of stimulation. In cats, most of the corneal fibers (approximately 70%) are polymodal nociceptors that are equally activated by near-noxious mechanical energy, chemical irritants, heat (higher than 39°C), and noxious cold. Most of the polymodal nociceptor fibers are unmyelinated C type, but some belong to the group of thin myelinated A fibers. Approximately 15% to 20% of the corneal fibers are mechanonociceptors that are fast-conducting, thin myelinated A fibers and are activated exclusively by intense mechanical force. In the present study, mechanical and chemical stimulation generally produced similar effects on reflex tear secretion, consistent with the findings of Acosta et al., although they used different methods to quantify tear volume. Studies in cats have shown that pneumatic mechanical stimuli to the cornea activated mainly the phasic mechanonociceptors and polymodal nociceptors, whereas gas mixtures of increasing CO₂ primarily excited polymodal nociceptors. This similarity between modalities suggests that both polymodal and mechanonociceptors contribute to the afferent pathways of reflex tear secretion. The highest suprathreshold mechanical stimulation in our experiment might have activated not only the polymodal nociceptors but also the high threshold mechanonociceptors, resulting in neural summation and thus producing greater tear reflex than the equivalent intensity of chemical stimulation.

The neural activities encoded by sensory receptors are carried centripetally by trigeminal ganglion neurons to higher levels in the central nervous system. The ocular surface is represented mainly in two spatially distinct regions of the spinal trigeminal nucleus in the lower brain stem: the trigeminal nucleus interpolaris-caudalis (Vi/Vc) transition and the subnucleus caudalis-upper cervical spinal cord (Vc/C1) junction. Because of this unique dual representation, it has been proposed that neurons at Vi/Vc and Vc/C1 transition regions mediate different aspects of corneal nociception and that their efferent projection to supraspinal areas might also be different. The corneal neurons located at the Vi/Vc transition region that project to the superior salivatory nucleus serve ocular-specific functions such as blink and tear reflexes, whereas those located within the superficial laminae (I-II) of the Vc/C1 transition that project to the posterior thalamic nucleus may play a prominent role in the sensory-discriminative aspects of corneal nociception. The present study showed that at and above threshold, stimulation of the central cornea produced greater reflex tearing than the equivalent stimulus to the periphery, suggesting that reflex lacrimation responses vary depending on the stimulus location. Given that suprathreshold stimulation may damage the cornea and that the ultimate goal of corneal reflexes is to protect the ocular surface and, therefore, the eye itself, it is plausible that the circuitry involved in reflex responses to noxious stimulation of the two locations may be different (e.g., efferent projection to the superior salivatory nucleus vs. the posterior thalamic nucleus) because the visual axis is situated within the central cornea.

**TABLE 1.** Thresholds of Detectable Tearing and Stimulus Detection

<table>
<thead>
<tr>
<th>Threshold</th>
<th>Mechanical (mL/min)</th>
<th>Chemical (% CO₂ added)</th>
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<tr>
<td></td>
<td>Central</td>
<td>Peripheral</td>
</tr>
<tr>
<td>Tearing</td>
<td>60.12 ± 11.09</td>
<td>65.25 ± 11.94</td>
</tr>
<tr>
<td>Stimulus detection</td>
<td>54.36 ± 5.34</td>
<td>51.60 ± 6.01</td>
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Values are mean ± SEM.
It has been reported that a small number of thick, fast-conducting nerve fibers innervate the perilimbal episcera.\textsuperscript{17} These fibers possibly terminate at the limbal region with Krause-like corpuscular endings, as described by Lawrenson and Ruskell.\textsuperscript{16} and respond to gentle mechanical stimulation of the ocular surface (they are mechanoreceptors).\textsuperscript{10} Activation of this type of receptor has been reported to be less effective than activation by polymodal nociceptors in evoking the tear reflex.\textsuperscript{19} This may also contribute to the location differences in the study.

In addition, although the density of corneal innervation in humans seems to be more uniform\textsuperscript{19} than previously believed based on evidence from animal histology,\textsuperscript{12} it is possible that there is a reduction in density in the periphery that is not clearly revealed by the current methods (e.g., if the periphery is not fully explored from limbus to limbus). The result in Figure 3 might simply reflect this higher sensory fiber packing in the central cornea that could lead to stronger activation of neurons with nociceptive central stimulation compared with the periphery. On the other hand, this explanation is untenable in light of the interaction between position and stimulus type (Fig. 4). This is more complex than a simple distinction between central and peripheral processing (or density differences) because the difference between center and periphery also depends on stimulus type.

As expected, the intensity required to trigger detectable tearing was generally higher than that required to detect a noxious stimulus, though for mechanical stimulation the differences between the two thresholds did not reach statistical significance. Additionally, as in previous studies,\textsuperscript{20,24–37} we did not find a strong positional effect on thresholds to detect mechanical and chemical stimulation. However, for supratreshold chemical stimulation, the intensity required to induce subjectively detectable tearing was higher for the peripheral cornea than for the central cornea, consistent with the difference in amount of tear secretion between the two locations. It appears that chemical stimulation of the central and peripheral cornea is similar for mediating corneal sensation at the threshold level, but it is different for detecting stimulated tearing. This suggests that chemosensory information from the central cornea and the peripheral cornea may be processed differently at the spinal trigeminal nucleus, depending on the sensory-discriminative or tear reflex aspect.

In conclusion, the present study demonstrates that a systematic increase in tear volume as determined by TMH is monotonically related to the intensity of the sensory input from the cornea in a dose-response manner. This provides physiological evidence that sensory innervation of the cornea (thus, the ocular surface) is the major neural driving force for lacrimal gland secretion. Acting through areas of the central nervous system, the sensory nerves and efferent parasympathetic and sympathetic nerves of the lacrimal functional unit modulate tear secretion to ensure a healthy ocular surface and to protect the eye under normal as well as environmentally challenging conditions (such as during this experiment).

The components of the lacrimal functional unit are linked in a homeostatic loop by complex and precise sensory, parasympathetic, and sympathetic neural control.\textsuperscript{3} Establishing the relation between activation of the sensory nerves from the ocular surface and the graded output of the lacrimal gland secretion may enable further understanding of the neural mechanisms contributing to the development of dry eye and ultimately to the development of effective treatments for ocular surface diseases in which the functioning of the innervation of the lacrimal unit may be compromised.

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**References**


