Effects of Tear Film Instability on Sensory Responses to Corneal Cold, Mechanical, and Chemical Stimuli

Ping Situ,[1] Carolyn G. Begley,[1] and Trefford L. Simpson[2]

[1]School of Optometry, Indiana University, Bloomington, Indiana, United States
[2]School of Optometry and Vision Science, University of Waterloo, Waterloo, Ontario, Canada

Correspondence: Ping Situ, School of Optometry, Indiana University, 800 East Atwater Avenue, Bloomington, IN 47401, USA; pingsitu@indiana.edu.

Submitted: April 9, 2019
Accepted: June 10, 2019

Citation: Situ P, Begley CG, Simpson TL. Effects of tear film instability on sensory responses to corneal cold, mechanical, and chemical stimuli. *Invest Ophthalmol Vis Sci*. 2019;60:2935–2941. https://doi.org/10.1167/iovs.19-27298

Purpose. To investigate the effects of tear film instability (TFI) induced by sustained tear exposure (STARE) on sensory responses to corneal cold, mechanical, and chemical stimuli.

Methods. Fifteen normal subjects were enrolled. TFI was induced during 10 repeated trials of STARE. Pneumatic cold, mechanical, and chemical stimuli were delivered using a computer-controlled Belmonte esthesiometer on three separate visits. The magnitude of the sensory responses to threshold and suprathreshold (1.25 and 1.50 times threshold levels) stimuli were assessed for intensity, coolness or warmness, irritation and pain, using a 0 (none) to 100 (very strong) scale, before and after STARE trials. Symptoms of ocular discomfort were evaluated using the Current Symptom Questionnaire (CSQ). Repeated measures ANOVA was used for data analysis.

Results. Following STARE trials, the intensity and coolness ratings to cooling stimuli decreased ($P = 0.043$ and 0.044 for intensity and coolness, respectively), while rated irritation to mechanical stimuli was increased ($P = 0.024$). The CSQ scores also increased regardless of visits (all $P < 0.001$). Intensity ratings, coolness to room temperature stimuli and irritation to mechanical and chemical stimuli increased for all suprathreshold stimuli with increasing stimulus levels ($P \leq 0.005$).

Conclusions. Repeated TFI induced by STARE affects neurosensory function of the ocular surface. The decrease in reports of cooling and increase in irritation after repeated TFI suggest a complex interaction of neural mechanisms (particularly nonnociceptive cold and nociceptive mechanical) giving rise to ocular surface sensation in humans.

Keywords: tear film instability, sensory processing, sensory responses to corneal threshold and suprathreshold stimuli, dry eye symptoms

Both tear film instability (TFI) and neurosensory abnormalities play etiological roles in development of the dry eye condition, according to the DEWS II report.[1] Previous studies have shown that repeated TFI induced by a sustained tear exposure (STARE) led to symptoms of discomfort and dryness that mimic those reported in patients with dry eye,[2–4] but the connection between TFI and symptoms remains unclear.[5] While neural control plays an important part in maintaining the integrity of the tear film and the health of the ocular surface,[6,7] the effects of repeated TFI on corneal sensory function has not been systematically studied. It is unclear whether repeated TFI affects corneal sensory responses to different stimulus modalities and how these effects relate to the subjective experience of discomfort.

Ocular discomfort suggests activation of sensory neurons and neural processing pathways that are involved in nociception at the ocular surface. Ocular surface stimuli are detected and encoded by receptors at the terminals of primary afferent neurons of the trigeminal nerve.[8] The decoded signals are carried centrally to two spatially discrete regions of the trigeminal brainstem complex, the interpolaris/caudalis transition region (Vi/Vc) and the caudalis/upper cervical cord junction (Vc/C1), and projected to multiple brain centers that mediate ocular sensations and reflexes.[8] The primary afferents innervating the cornea have been classified as (1) polymodal, with nociceptor terminals activated by noxious mechanical, thermal and chemical stimuli, (2) mechanical, with nociceptors excited only by injurious mechanical forces, and (3) cold-sensitive, with receptors that detect small changes in surface cooling and osmolarity during tear evaporation (high background low-threshold) and respond to cold temperature and hyperosmolarity (low background high-threshold).[5,9,10] Corneal neurons express a range of membrane channels[11–13] such as the transient receptor potential (TRP) family that are thought to transduce environmental and endogenous stimuli to electrophysiological signals.[14,15] Recently, cold receptors have come under increased scrutiny due to their role in regulating tear secretion and tear film hyperosmolarity,[12,16] which is thought to play an etiological role in the development of dry eye.[1]

While the physiology of corneal sensory neurons has been detailed largely in experimental animals,[17] there is an emerging body of evidence from psychophysical studies that link neural physiology to human sensory processing. In particular, Belmonte pneumatic esthesiometry[18] facilitates psychophysical probing of sensory processes by delivering systematically controlled mechanical, chemical, and thermal stimulation to the eye. This allows direct study of human sensory function in health and disease, including dry eye and other ocular surface conditions, surgery and contact lens wear.[5,8] Sensory thresholds and responses to supra-threshold stimuli can be measured,

as well as basic mechanisms, adaptation effects, lacrimation, and blinking responses.[19–23]

Despite the hypotheses that neurosensory abnormalities play an important etiological part in, and both TFI and tear hyperosmolarity are significant entry points contributing to, the pathogenesis and development of a "vicious circle" in dry eye,[1,24] the effects of repeated TFI on suprathreshold sensory processing have not been studied. We hypothesized that repeated TFI will alter corneal sensory function. In order to examine this hypothesis, we measured thresholds and suprathreshold sensory responses following repeated TFI induced by STARE.

Methods

This study was conducted in accord with the Declaration of Helsinki and approved by the Institutional Review Board of Indiana University. Informed consent was obtained from all participants after the nature of the study had been fully explained. Each subject signed the consent form prior to enrollment.

Subjects

Fifteen noncontact lens wearing and healthy subjects (7 females and 8 males) with mean (± SD) age 26.6 ± 2.6 years were enrolled in the study. They had no history of ocular disease or surgeries or any systemic condition or medication that could have affected corneal sensory function.

Apparatus

A computer-controlled pneumatic esthesiometer designed and built at Indiana University was used in the study.[25] The esthesiometer is a dual chamber mechanical, chemical (CO_2), and thermal pneumatic design with computer control of flow, percent CO_2 and temperature, and collection of subject responses to the stimuli. A steady stimulus temperature independent of air-flow and ambient temperature was maintained through feedback provided by a temperature sensing circuit. The distance between and orthogonal alignment of the tip of the esthesiometer and the ocular surface was continuously monitored by a calibrated video camera.

Experimentally Induced TFI (Repeated TFI)

Previously, we and others have used the technique of extended eye opening, termed sustained eye exposure (STARE) to induce TFI.[2–4,26,27] In the current study, subjects kept one eye open as long as possible to induce TFI, which included tear film thinning or tear break-up (TBU). This procedure was repeated for up to 10 trials with approximately 2 seconds between trials. A slit-lamp video camera monitored the tear film during the initial trials of sustained eye exposure to confirm the development of TFI.

Study Procedures

Responses to cool, mechanical, and chemical stimuli were measured on three separate days at approximately the same time of the day (within 0–3.5 hours). The order of the mechanical and chemical sessions was random while the cool session was performed first due to the length of time required to cool down the esthesiometer. All measurements were taken at least 3 hours after subjects awoke[28] on the central cornea of the left eye throughout the study. The left eye was chosen because the esthesiometer setup for this study allowed only left eye testing.

Pneumatic cool, mechanical, and chemical stimuli were delivered using our computerized Belmonte pneumatic esthesiometer. The pneumatic cool and mechanical stimuli consisted of a series of air pulses with flow rates varying from 0 to 200 mL/min. The cool stimulus temperature was set at 20°C (room temperature), and the mechanical and chemical stimulus was approximately 32°C at the ocular surface. Chemical stimulation was induced by increasing the CO_2 concentration (ranged from 0%–80%) in the stimulus air column with a flow rate that was fixed at 70% of the initially estimated mechanical threshold. The stimulus duration was 2 seconds. Subjects were instructed to look at a fixation target during stimulus presentation and blink freely or look down between stimuli. They could interrupt the trials at any time.

An ascending method of limits was used to determine thresholds. A randomly selected initial level of stimulus was used. The threshold was the average of three measurements at each stimulus level.

Following threshold testing, stimuli at 1.00, 1.25, and 1.50 times the threshold were presented in random order. Each stimulus intensity was presented three times. Subjects were asked to assign a number that directly reflected their subjective impression of the sensory attributes of each stimulus measurement. They rated the stimulus intensity, thermal sensation (coolness or warmth), and irritation and painfulness, using a scale ranging from 0 (nonexistent) to 100 (very strong). This testing was repeated after 10 STARE trials using the same methods as before, except that stimulus levels were presented five times to monitor any changes for a longer period of time (up to 10 minutes).

All subjects completed the Dry Eye Questionnaire-5 (DEQ-5)[29] before testing to measure habitual symptoms of ocular irritation. The Current Symptom Questionnaire (CSQ), which queries symptoms at the time of testing was filled out before and after STARE to measure changes in symptoms with STARE. The maximum blink interval (MBI), which is the longest amount of time that subjects could hold their eye open during each STARE trial, was recorded for each trial.

Data Analysis

Data were analyzed using repeated measures ANOVA in SPSS 23 (IBM SPSS) with the statistically significant level set at $P \leq 0.05$. Data were checked for normality using the quantile-quantile plots (Q-Q plots), and the plot for each variable was the appropriate straight line. Estimated magnitudes for intensity, coolness/warmness, irritation, and painfulness for each stimulus type were outcome variables, and time (before and after STARE) and levels (1.00×, 1.25×, and 1.50× threshold) were predictors. In addition, the differences in CSQ scores before and after testing and MBI at different stimulus levels and modalities were compared. Huynh-Feldt corrected P values were calculated to minimize the effects of violating assumptions about data sphericity for repeated-measures ANOVA. Pairwise t-tests with a post hoc Bonferroni correction were used when applicable.

Results

Psychophysical Measures

The mean (± standard error or SEM) of the detection thresholds are 59.2 ± 4.7 mL/min, 72.7 ± 6.0 mL/min, and 26.2% ± 1.8% added CO_2, for room-temperature pneumatic cool, and for mechanical and chemical stimuli (both set at eye temperature), respectively. The thresholds in this study are similar to published results from other studies.[30–32]

Investigative Ophthalmology & Visual Science

Table 1. Sensory Ratings for Cooling Stimulation at Threshold (1.00×) and 1.25 and 1.5 Times Threshold (1.25× and 1.50×) Stratified by Time Before (Pre-) and Following (Post) STARE (Mean ± SEM)

	1.00×		1.25×		1.50×	
Attributes	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Intensity	7.7 ± 2.1	6.2 ± 1.1	13.7 ± 2.2	8.4 ± 1.1	18.2 ± 2.9	12.0 ± 1.5
Coolness	8.4 ± 2.1	6.2 ± 1.4	13.5 ± 2.5	8.5 ± 1.2	15.3 ± 2.4	10.7 ± 1.6
Irritation	0.2 ± 0.2	0.3 ± 0.2	0.6 ± 0.4	0.7 ± 0.5	1.3 ± 0.7	1.2 ± 0.6
Pain	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

The magnitude estimates (mean ± SEM) for the intensity, coolness, irritation, and pain of the pneumatic cool stimulus are listed in Table 1. Both intensity and coolness decreased on average after STARE compared with before (Figs. 1A, 1B). This difference was significant (time main effects; $P = 0.043$ and 0.044 for intensity and coolness, respectively). As expected, magnitude estimates of intensity and coolness increased with increasing stimulus level, regardless of time (stimulus level effect $P < 0.001$ for both intensity and coolness).

Irritation was reported in very few subjects and its magnitude was low. There was no significant difference between the rating of irritation before and after STARE, nor any significant increase with stimulus level ($P = 0.893$ and 0.093 for time and stimulus level, respectively). There were no time and stimulus level interactions for all the sensory attributes (all $P > 0.05$).

The estimated magnitudes of intensity, thermal sensation, irritation, and pain of the mechanical stimulus are shown in Table 2. Irritation increased on average after STARE compared with before (Fig. 1C), and this difference was significant (time main effect; $P = 0.024$). Intensity ratings changed minimally after STARE for the mechanical stimulus and did not show a

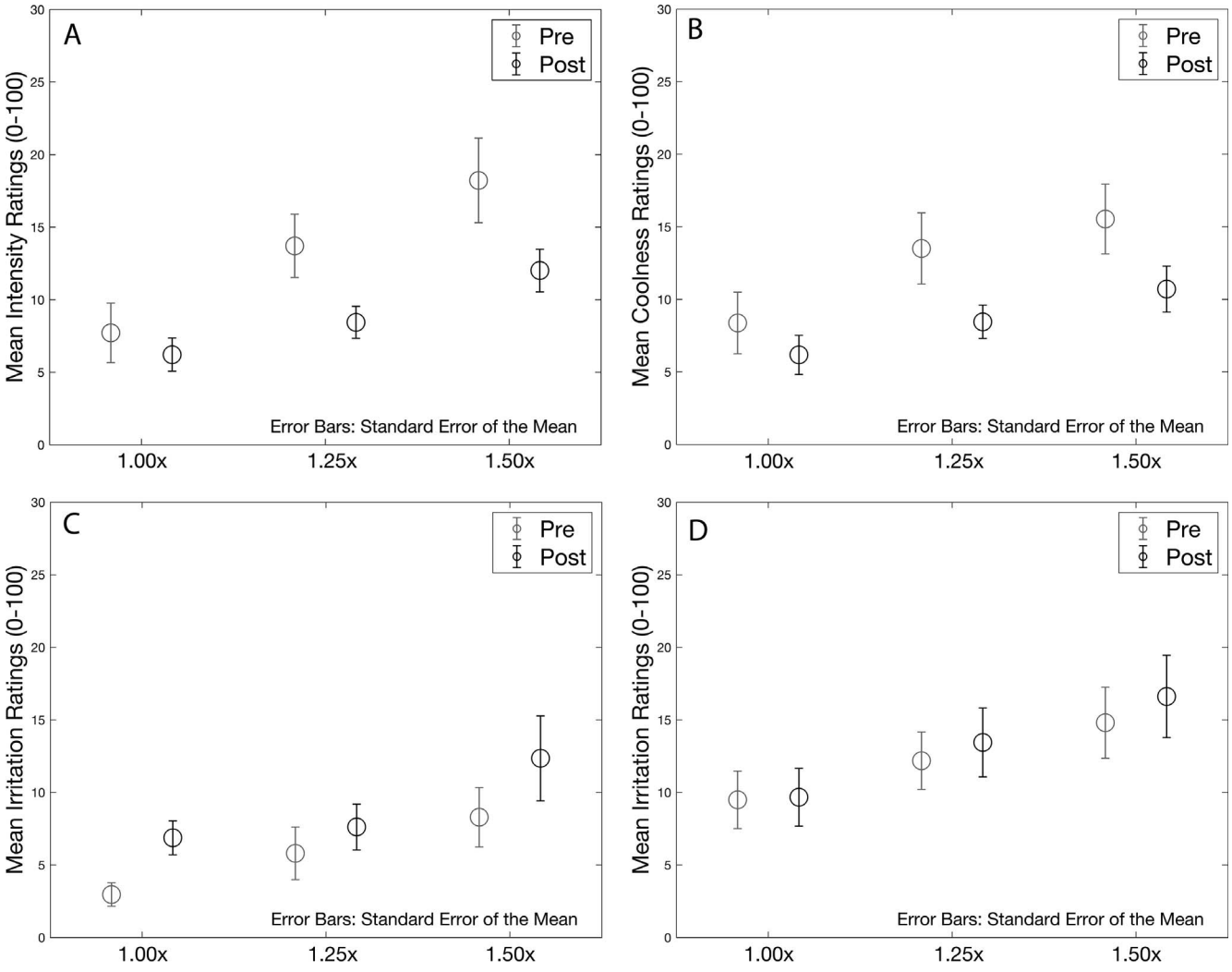

Figure 1. Sensory ratings of intensity, coolness for cooling stimulation, and irritation for mechanical and chemical stimulation at threshold (1.00×) and 1.25 and 1.5 times threshold (1.25× and 1.50×) before (pre-) and following (post) STARE. (**A**) Intensity ratings to cooling stimulus ($P = 0.043$*), (**B**) coolness ratings to cooling stimulus ($P = 0.044$*), (**C**) irritation ratings to mechanical stimulus ($P = 0.024$*), (**D**) irritation ratings to chemical stimulus ($P = 0.257$*). *Repeated measures ANOVA time main effect.

Investigative Ophthalmology & Visual Science

Table 2. Sensory Ratings for Mechanical Stimulation at Threshold (1.00×) and 1.25 and 1.5 Times Threshold (1.25× and 1.50×) Stratified by Time Before (Pre-) and Following (Post) STARE (Mean ± SEM)

	1.00×		1.25×		1.5×	
Attributes	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Intensity	9.6 ± 1.3	9.3 ± 1.4	13.8 ± 1.8	13.0 ± 1.7	20.0 ± 2.7	20.3 ± 2.9
Thermal	0.4 ± 0.3	0.5 ± 0.5	1.1 ± 0.6	0.4 ± 0.4	0.6 ± 0.5	1.6 ± 1.5
Irritation	3.0 ± 0.8	6.9 ± 1.2	5.8 ± 1.8	7.6 ± 1.6	8.3 ± 2.0	12.3 ± 2.9
Pain	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

statistically significant difference ($P = 0.824$). Regardless of time, ratings of intensity and irritation to mechanical stimuli increased significantly with the level of stimulation ($P < 0.001$ for intensity and $P = 0.005$ for irritation, respectively). No significant time and stimulus level interactions were found for any of the sensory attributes (all $P > 0.05$).

Table 3 contains the estimated magnitudes of intensity, thermal (coolness/warmness), irritation, and pain to chemical stimuli. On average, the magnitude estimates for intensity and irritation tended to increase after STARE (Fig. 1D), but the changes were not statistically significant (time main effects, $P =$ 0.233 and 0.257, respectively). Intensity and irritation ratings to chemical stimuli increased with stimulus level ($P < 0.001$ for both intensity and irritation), regardless of time. There were no time and stimulus level interactions for chemical sensory attribute ratings (all $P > 0.05$).

Perhaps because participants were nondry eye healthy subjects and the intensity of suprathreshold stimulus was not very high, no pain was recorded by any of the subjects for any stimulus modality. Thus, statistical analysis was not performed for estimated magnitude of pain. Similarly, statistical analysis was not performed for estimated magnitude of thermal ratings for chemical and mechanical stimuli since a low magnitude of thermal (warm) sensation was reported by only four subjects during one session and by one subject in another session.

Symptoms of Ocular Discomfort Measured by the CSQ

Symptoms assessed using the CSQ before and immediately after STARE for cool, mechanical, and chemical stimuli are presented in Figure 2. Regardless of stimulus level, CSQ scores were higher after STARE for each of the cool, mechanical, and chemical stimulus sessions (all $P < 0.001$). There were no significant differences between stimulus levels nor interactions between stimulus level and time for all three modalities (all $P >$ 0.05).

Maximum Blink Interval (MBI)

The average MBI during repeated trials of holding the eye open for each stimulus level, stratified by stimulus modality, are listed in Table 4. There were no statistical differences between stimulus levels and modality sessions ($P = 0.712$ and 0.131 for level and modality, respectively).

Discussion

The primary objective of the study was to investigate the effects of experimental TFI on corneal responses to cool, mechanical, and chemical stimuli. The present study psychophysically demonstrates in humans that TFI induced by repeated trials of STARE produced bidirectional effects on corneal sensory processing, decreasing the responses to suprathreshold cold stimulation and enhancing irritation with mechanical and chemical stimuli. These novel results confirmed our working hypothesis that continuous ocular stimulation resulting from prolonged eye-opening (STARE) and associated TFI and ocular surface stress alters sensory processing/sensations. Although the STARE technique does not represent normal blinking as shown by the MBI results (Table 4), the induced TFI may provide a model for understanding the neurosensory abnormalities that play an etiological role in dry eye.[5]

Corneal sensations are mediated by different functional types of corneal sensory neurons through activation of modality-specific receptors.[33–35] The irritation/discomfort induced by mechanical stimuli increased (Fig. 1C), with a similar trend for chemical stimuli (Fig. 1D) in the study, suggesting that repeated STARE and TFI may result in promoting nociception of noxious stimuli. A few possible changes occurred in the ocular surface during STARE that may contribute to the altered neurosensory processing although the events during TFI remain a subject for speculation and are not well understood.[36] As the tear film thins and breaks up, increased evaporation should act to elevate tear film osmolarity, possibly as high as 800 mOsm/kg or higher.[37–39] It has been suggested that corneal polymodal neurons are excited when tear osmolarity is greater than 600 mOsm,[40] which has been postulated to occur during tear breakup.[38,39] In addition, it is possible that cell shrinkage may induced by hyperosmotic exposure to underlying corneal epithelial cells[41] during TBU. Deformation of surface cells secondary to drying, as suggested increased surface scatter with wavefront measurement,[42] may also stimulate corneal nociceptors. As corneal polymodal and mechanical neurons (and perhaps chemo-nociceptors[9,43])

Table 3. Sensory Ratings for Chemical Stimulation at Threshold (1.00×) and 1.25 and 1.5 Times Threshold (1.25× and 1.50×) Stratified by Time Before (Pre-) and Following (Post) STARE (Mean ± SEM)

	1.00×		1.25×		1.5×	
Attributes	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Intensity	9.9 ± 2.0	10.4 ± 2.1	12.7 ± 1.9	14.0 ± 2.4	15.0 ± 2.4	17.1 ± 2.8
Thermal	0.4 ± 0.3	0.4 ± 0.3	0.6 ± 0.4	0.3 ± 0.3	0.3 ± 0.3	0.5 ± 0.4
Irritation	9.5 ± 2.0	9.7 ± 2.0	12.2 ± 2.0	13.4 ± 2.4	14.8 ± 2.5	16.6 ± 2.8
Pain	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Investigative Ophthalmology & Visual Science

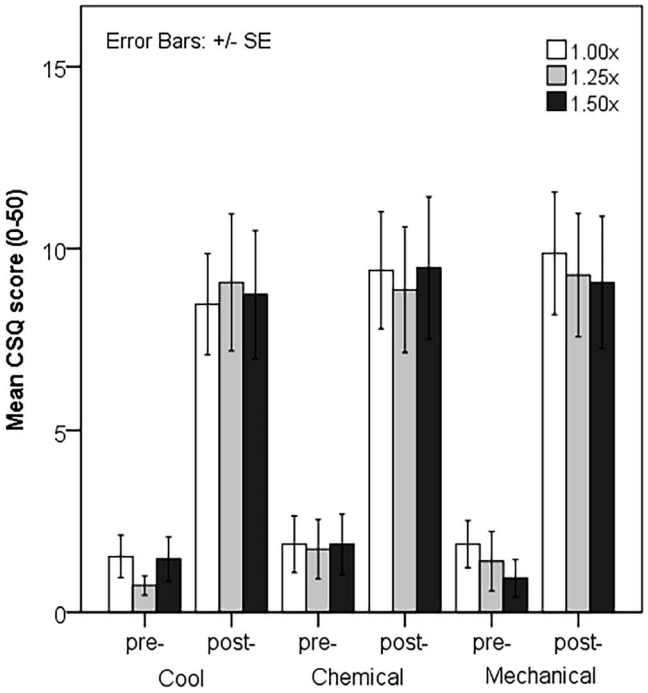

Figure 2. CSQ scores before (pre-) and after (post) STARE for the cooling (cool), mechanical (mechanical) and chemical (chemical) sessions, stratified by stimulation at threshold (1.00×), 1.25 and 1.5 times threshold (1.25× and 1.5×). All $P < 0.001$.

connect centrally to the second- and higher-order neurons that are responsible for nociception,[10,17,44] activation of these neurons is likely to increase sensory inputs to the nociceptive pathway evoking pain (including irritation).[5]

Additionally, the hyperosmolarity that was likely to occur during STARE secondary to tear film evaporation during TFI[45] could have altered the activity of cold receptors as reported in animal studies.[16,40,46–48] These abnormal activities have been thought to underlie dry eye symptoms[49] and are perhaps involved in activation of high threshold cold-sensitive neurons and the connecting nociceptive pathway.[50,51] In the present study, besides the increased irritation with threshold and suprathreshold stimuli discussed above, symptoms of ocular discomfort increased after repeated STARE, as demonstrated by the shift in the CSQ scores to worse symptoms (Fig. 2), similar to previous reports.[3,4] The abnormal sensory inputs from the ocular surface and the activation of the nociceptive pathway may contribute to this increased ocular irritation following STARE, supporting the hypothesis that TFI may induce neurosensory abnormalities,[1] which play an important role in dry eye development.[5]

Presumably, activation of low threshold cold-sensitive neurons is in response to a small surface temperature reduction during normal blink cycle and elicits an innocuous cooling sensation,[5] while corneal high threshold cold receptors are activated by stronger stimulation evoking irritation.[52,53] The nonnoxious room-temperature pneumatic stimuli in the study elicited a cooling sensation similar to previous reports,[32,52,54] but the magnitude of coolness to pneumatic cool stimuli reduced after repeated STARE (Figs. 1A, 1B), suggesting adaptation, inhibition, and/or masking of the neural mechanisms responsible for innocuous cold perception. The detection of cold was most likely through the opening of TRPM8 transducing channels of the corneal primary cold-sensitive neurons.[15,55] The sensory signals detected are transmitted by second-order neurons that respond to cooling

Table 4. MBI During Cooling, Mechanical, and Chemical Sessions Stratified by Stimulation at Threshold (1.00×) and 1.25 (1.25×) and 1.5 (1.5×) Times Threshold (Mean ± SEM)

Sessions	1.0×	1.25×	1.5×
Cooling	27.9 ± 6.2	28.3 ± 5.8	31.3 ± 7.1
Mechanical	34.9 ± 8.2	31.4 ± 6.2	28.9 ± 5.8
Chemical	20.8 ± 2.4	20.5 ± 3.0	20.1 ± 3.4

and hyperosmolarity at the Vi/Vc transition region.[34,47] These second-order neurons appear to have distinct functional properties, with one group exclusively responding to innocuous cooling and another group having lower response to cooling and menthol but also responding to acid and noxious heat.[34] They likely receive input from different types of corneal primary afferent neurons, suggesting distinct pathways ("labeled-lines") for processing sensory signals arising from the ocular surface involved in signaling cooling and drying[34] to regulate ocular homeostasis.[5] While the abnormal sensory inputs induced by TFI and associated ocular surface changes during STARE might lead to activation of the nociceptive pathway as discussed earlier, the reduction in coolness perception in the present study provides evidence that the neuronal pathway processing innocuous cold could also be affected, perhaps at the afferent and/or higher levels.

While there are many putative mechanisms that might account for these two seemingly paradoxical mechanical and cooling effects on corneal sensation following STARE, one possible explanation is related to the suppression of TRPM8-mediated responses to innocuous cooling in corneal cold receptors and sensitization of the nociceptors by inflammatory mediators as reported in animal models of allergic conjunctivitis and UV keratitis.[56,57] There are reports that inflammatory mediators inhibit the activation of TRPM8 channels[58,59] by the G protein subunit $G\alpha_q$.[59] In the present study, repeated STARE and TFI could potentially produce ocular surface stress resulting in local release of inflammatory mediators that may reduce the activity of cold receptors and enhance the activity of nociceptors. These effects on peripheral nerve activity may contribute to the changes in the magnitude of sensations evoked by cooling and mechanical stimulation.

Another possibility may be "labeled line" crosstalk or inhibitory and excitatory effects between corneal cooling and nociceptive pathways. Peripheral sensory neurons are presumably connected to specific neuronal pathways or labeled lines, evoking particular modality of sensation such as touch, itch, pain, and temperature sensitivity,[60–63] and the crosstalk among these labeled lines generates and shapes somatosensory perception, as has been reported in somatic sensation and pain reserch.[61,63] Human and animal studies have shown that innocuous cold could suppress nociception or pain and vice versa.[52,53,64,65] Using a spinal cord slice preparation, Zheng et al.[66] have shown that while two distinct populations of inhibitory interneurons in the superficial dorsal horn received specific inputs from TRPM8 (cold) and TRPV1 (heat-pain) expressing afferents, these interneurons converged and were reciprocally inhibitory, allowing interactions between specific afferent messages.[65,66] Like the spinal dorsal horn, the neurons within the trigeminal brainstem complex are extensively interconnected.[35] It is plausible, therefore, that differential engagement of modality specific primary corneal neurons using inhibitory and excitatory circuitries in the trigeminal brainstem complex and/or higher central processing pathway may underlie the crosstalk between coolness and irritation in the present study.

In this study, the sample size was relatively small, and it could be argued that STARE does not represent normal blinking conditions. However, although the induced TFI effects were short-lived and reversible, the present study reveals the complexity of sensory inputs arising from the ocular surface affecting human sensory processing and supports the notion that psychophysical channels[52] do not act independently. These results are important for a number of reasons. First, the experiment points to the utility of pneumatic stimuli for examining hypotheses that are more complex than simple sensitivity issues. We were only able to determine different cooling and discomfort effects because pneumatic esthesiometers provide us with the means to examine mechanical, chemical, and thermal effects. Secondly, the results highlight the usefulness of studying suprathreshold processing (that in the current context is much more experimentally tractable than threshold sensory processes). Finally, the dissociation of the effects on cold and pain sensing pathways reinforces the separability of these paths.

In conclusion, repeated STARE and TFI results in a reduction of innocuous cooling sensations and promotes irritation to noxious stimuli. While further study is needed to understand STARE induced TFI as a sensory stimulus, the present study provides physiological evidence for the first time that prolonged repeated periods of ocular surface stimulation by TFI lead to significant differences in suprathreshold scaling and appears to differently affect mechanical and cooling pathways. The bidirectional responses suggest complex interactions of neural mechanisms underlying the ocular surface sensations in normal and disease conditions such as dry eye.

Acknowledgments

Supported by Grant Number R01EY021794 (CGB) from the National Eye Institute. The authors alone are responsible for the content and writing of the paper.

Disclosure: **P. Situ**, None; **C.G. Begley**, None; **T.L. Simpson**, None

References

1. Craig JP, Nichols KK, Akpek EK, et al. TFOS DEWS II definition and classification report. *Ocul Surf*. 2017;15:276–283.
2. Begley CG, Himebaugh N, Renner D, et al. Tear breakup dynamics: a technique for quantifying tear film instability. *Optom Vis Sci*. 2006;83:15–21.
3. Zhang J, Begley CG, Situ P, Simpson T, Liu H. A link between tear breakup and symptoms of ocular irritation. *Ocul Surf*. 2017;15:696–703.
4. Begley C, Simpson T, Liu H, et al. Quantitative analysis of tear film fluorescence and discomfort during tear film instability and thinning. *Invest Ophthalmol Vis Sci*. 2013;54:2645–2653.
5. Belmonte C, Nichols JJ, Cox SM, et al. TFOS DEWS II pain and sensation report. *Ocul Surf*. 2017;15:404–437.
6. Stern ME, Beuerman RW, Fox RI, Gao J, Mircheff AK, Pflugfelder SC. The pathology of dry eye: the interaction between the ocular surface and lacrimal glands. *Cornea*. 1998;17:584–589.
7. Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC. The role of the lacrimal functional unit in the pathophysiology of dry eye. *Exp Eye Res*. 2004;78:409–416.
8. Stapleton F, Marfurt C, Golebiowski B, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the subcommittee on neurobiology. *Invest Ophthalmol Vis Sci*. 2013;54:TFOS71–TFOS97.
9. MacIver MB, Tanelian DL. Free nerve ending terminal morphology is fiber type specific for A delta and C fibers innervating rabbit corneal epithelium. *J Neurophysiol*. 1993;69:1779–1783.
10. MacIver MB, Tanelian DL. Structural and functional specialization of A delta and C fiber free nerve endings innervating rabbit corneal epithelium. *J Neurosci*. 1993;13:4511–4524.
11. Alamri A, Bron R, Brock JA, Ivanusic JJ. Transient receptor potential cation channel subfamily V member 1 expressing corneal sensory neurons can be subdivided into at least three subpopulations. *Front Neuroanat*. 2015;9:71.
12. Parra A, Madrid R, Echevarria D, et al. Ocular surface wetness is regulated by TRPM8-dependent cold thermoreceptors of the cornea. *Nat Med*. 2010;16:1396–1399.
13. Bron R, Wood RJ, Brock JA, Ivanusic JJ. Piezo2 expression in corneal afferent neurons. *J Comp Neurol*. 2014;522:2967–2979.
14. Stucky CL, Dubin AE, Jeske NA, Malin SA, McKemy DD, Story GM. Roles of transient receptor potential channels in pain. *Brain Res Rev*. 2009;60:2–23.
15. McKemy DD, Neuhausser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature*. 2002;416:52–58.
16. Hirata H, Meng ID. Cold-sensitive corneal afferents respond to a variety of ocular stimuli central to tear production: implications for dry eye disease. *Invest Ophthalmol Vis Sci*. 2010;51:3969–3976.
17. Belmonte C, Acosta MC, Gallar J. Neural basis of sensation in intact and injured corneas. *Exp Eye Res*. 2004;78:513–525.
18. Belmonte C, Acosta MC, Schmelz M, Gallar J. Measurement of corneal sensitivity to mechanical and chemical stimulation with a CO2 esthesiometer. *Invest Ophthalmol Vis Sci*. 1999;40:513–519.
19. Chen J, Feng Y, Simpson TL. Human corneal adaptation to mechanical, cooling, and chemical stimuli. *Invest Ophthalmol Vis Sci*. 2010;51:876–881.
20. Feng Y, Simpson TL. The inhibitory interaction between human corneal and conjunctival sensory channels. *Invest Ophthalmol Vis Sci*. 2005;46:1251–1255.
21. Situ P, Simpson TL. Interaction of corneal nociceptive stimulation and lacrimal secretion. *Invest Ophthalmol Vis Sci*. 2010;51:5640–5645.
22. Acosta MC, Peral A, Luna C, Pintor J, Belmonte C, Gallar J. Tear secretion induced by selective stimulation of corneal and conjunctival sensory nerve fibers. *Invest Ophthalmol Vis Sci*. 2004;45:2333–2336.
23. Wu Z, Begley CG, Situ P, Simpson T. The effects of increasing ocular surface stimulation on blinking and sensation. *Invest Ophthalmol Vis Sci*. 2014;55:1555–1563.
24. Bron AJ, de Paiva CS, Chauhan SK, et al. TFOS DEWS II pathophysiology report. *Ocul Surf*. 2017;15:438–510.
25. Situ P, Simpson T, Begley C. Hypersensitivity to cold stimuli in symptomatic contact lens wearers. *Optom Vis Sci*. 2016;93:909–916.
26. Liu H, Begley CG, Chalmers R, Wilson G, Srinivas SP, Wilkinson JA. Temporal progression and spatial repeatability of tear breakup. *Optom Vis Sci*. 2006;83:723–730.
27. Varikooty J, Simpson TL. The interblink interval I: the relationship between sensation intensity and tear film disruption. *Invest Ophthalmol Vis Sci*. 2009;50:1087–1092.
28. du Toit R, Vega JA, Fonn D, Simpson T. Diurnal variation of corneal sensitivity and thickness. *Cornea*. 2003;22:205–209.
29. Chalmers RL, Begley CG, Caffery B. Validation of the 5-Item Dry Eye Questionnaire (DEQ-5): discrimination across self-assessed severity and aqueous tear deficient dry eye diagnoses. *Cont Lens Anterior Eye*. 2010;33:55–60.

30. 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.
31. Feng Y, Simpson TL. Nociceptive sensation and sensitivity evoked from human cornea and conjunctiva stimulated by CO2. *Invest Ophthalmol Vis Sci*. 2003;44:529–532.
32. Situ P, Simpson TL, Fonn D. Eccentric variation of corneal sensitivity to pneumatic stimulation at different temperatures and with CO2. *Exp Eye Res*. 2007;85:400–405.
33. Hirata H, Okamoto K, Tashiro A, Bereiter DA. A novel class of neurons at the trigeminal subnucleus interpolaris/caudalis transition region monitors ocular surface fluid status and modulates tear production. *J Neurosci*. 2004;24:4224–4232.
34. Kurose M, Meng ID. Corneal dry-responsive neurons in the spinal trigeminal nucleus respond to innocuous cooling in the rat. *J Neurophysiol*. 2013;109:2517–2522.
35. Bereiter DA, Hirata H, Hu JW. Trigeminal subnucleus caudalis: beyond homologies with the spinal dorsal horn. *Pain*. 2000; 88:221–224.
36. Willcox MDP, Argueso P, Georgiev GA, et al. TFOS DEWS II tear film report. *Ocul Surf*. 2017;15:366–403.
37. Braun RJ, King-Smith PE, Begley CG, Li L, Gewecke NR. Dynamics and function of the tear film in relation to the blink cycle. *Prog Retin Eye Res*. 2015;45:132–164.
38. Peng CC, Cerretani C, Braun RJ, Radke CJ. Evaporation-driven instability of the precorneal tear film. *Adv Colloid Interface Sci*. 2014;206:250–264.
39. Liu H, Begley C, Chen M, et al. A link between tear instability and hyperosmolarity in dry eye. *Invest Ophthalmol Vis Sci*. 2009;50:3671–3679.
40. Parra A, Gonzalez-Gonzalez O, Gallar J, Belmonte C. Tear fluid hyperosmolality increases nerve impulse activity of cold thermoreceptor endings of the cornea. *Pain*. 2014;155: 1481–1491.
41. Capó-Aponte JE, Wang Z, Bildin VN, Pokorny KS, Reinach PS. Fate of hypertonicity-stressed corneal epithelial cells depends on differential MAPK activation and p38MAPK/Na-K-2Cl cotransporter1 interaction. *Exp Eye Res*. 2007;84:361–372.
42. Himebaugh NL, Nam J, Bradley A, Liu H, Thibos LN, Begley CG. Scale and spatial distribution of aberrations associated with tear breakup. *Optom Vis Sci*. 2012;89:1590–1600.
43. Tanelian DL, MacIver MB. Simultaneous visualization and electrophysiology of corneal A-delta and C fiber afferents. *J Neurosci Methods*. 1990;32:213–222.
44. Belmonte C, Garcia-Hirschfeld J, Gallar J. Neurobiology of ocular pain. *Prog Retin Eye Res*. 1997;16:117–156.
45. Kimball SH, King-Smith PE, Nichols JJ. Evidence for the major contribution of evaporation to tear film thinning between blinks. *Invest Ophthalmol Vis Sci*. 2010;51:6294–6297.
46. Hirata H, Mizerska K, Marfurt CF, Rosenblatt MI. Hyperosmolar tears induce functional and structural alterations of corneal nerves: electrophysiological and anatomical evidence toward neurotoxicity. *Invest Ophthalmol Vis Sci*. 2015;56: 8125–8140.
47. Hirata H, Rosenblatt MI. Hyperosmolar tears enhance cooling sensitivity of the corneal nerves in rats: possible neural basis for cold-induced dry eye pain. *Invest Ophthalmol Vis Sci*. 2014;55:5821–5833.
48. Kovács I, Luna C, Quirce S, et al. Abnormal activity of corneal cold thermoreceptors underlies the unpleasant sensations in dry eye disease. *Pain*. 2016;157:399–417.
49. Belmonte C, Gallar J. Cold thermoreceptors, unexpected players in tear production and ocular dryness sensations. *Invest Ophthalmol Vis Sci*. 2011;52:3888–3892.
50. Campero M, Baumann TK, Bostock H, Ochoa JL. Human cutaneous C fibres activated by cooling, heating and menthol. *J Physiol*. 2009;587:5633–5652.
51. Madrid R, de la Pena E, Donovan-Rodriguez T, Belmonte C, Viana F. Variable threshold of trigeminal cold-thermosensitive neurons is determined by a balance between TRPM8 and Kv1 potassium channels. *J Neurosci*. 2009;29:3120–3131.
52. Feng Y, Simpson TL. Characteristics of human corneal psychophysical channels. *Invest Ophthalmol Vis Sci*. 2004; 45:3005–3010.
53. 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.
54. Murphy PJ, Patel S, Morgan PB, Marshall J. The minimum stimulus energy required to produce a cooling sensation in the human cornea. *Ophthalmic Physiol Opt*. 2001;21:407–410.
55. McCoy DD, Knowlton WM, McKemy DD. Scraping through the ice: uncovering the role of TRPM8 in cold transduction. *Am J Physiol Regul Integr Comp Physiol*. 2011;300:R1278–R1287.
56. Acosta MC, Luna C, Quirce S, Belmonte C, Gallar J. Changes in sensory activity of ocular surface sensory nerves during allergic keratoconjunctivitis. *Pain*. 2013;154:2353–2362.
57. Acosta MC, Luna C, Quirce S, Belmonte C, Gallar J. Corneal sensory nerve activity in an experimental model of UV keratitis. *Invest Ophthalmol Vis Sci*. 2014;55:3403–3412.
58. Linte RM, Ciobanu C, Reid G, Babes A. Desensitization of cold- and menthol-sensitive rat dorsal root ganglion neurones by inflammatory mediators. *Exp Brain Res*. 2007;178:89–98.
59. Zhang X, Mak S, Li L, et al. Direct inhibition of the cold-activated TRPM8 ion channel by Gαq. *Nat Cell Biol*. 2012;14: 851–858.
60. Handwerker HO. Sixty years of C-fiber recordings from animal and human skin nerves: historical notes. *Prog Brain Res*. 1996;113:39–51.
61. Craig AD. Pain mechanisms: labeled lines versus convergence in central processing. *Annu Rev Neurosci*. 2003;26:1–30.
62. Belmonte C, Brock JA, Viana F. Converting cold into pain. *Exp Brain Res*. 2009;196:13–30.
63. Green BG. Temperature perception and nociception. *J Neurobiol*. 2004;61:13–29.
64. Bini G, Cruccu G, Hagbarth K-E, Schady W, Torebjörk E. Analgesic effect of vibration and cooling on pain induced by intraneural electrical stimulation. *Pain*. 1984;18:239–248.
65. McCoy ES, Taylor-Blake B, Street SE, Pribisko AL, Zheng J, Zylka MJ. Peptidergic CGRPα primary sensory neurons encode heat and itch and tonically suppress sensitivity to cold. *Neuron*. 2013;78:138–151.
66. Zheng J, Lu Y, Perl ER. Inhibitory neurones of the spinal substantia gelatinosa mediate interaction of signals from primary afferents. *J Physiol*. 2010;588:2065–2075.

Investigative Ophthalmology & Visual Science