Human Corneal Adaptation to Mechanical, Cooling, and Chemical Stimuli

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PURPOSE. To psychophysically investigate adaptation in human corneas using the Belmonte pneumatic esthesiometer.

METHODS. Twenty, 8, and 20 healthy subjects were enrolled in the mechanical, cool, and chemical experiments, respectively. Thresholds were estimated using an ascending method of limits and three intensities (subthreshold, threshold, and suprathreshold, in random order) were each presented 10 or 20 times, and subjects scaled the intensity of the stimuli (0 - 4 [no stimulus to very intense stimulus]). Friedman nonparametric ANOVA was used to analyze the rating data.

RESULTS. There was measurable adaptation with both mechanical and cool stimuli. For both suprathreshold mechanical and cool stimuli, the earlier stimuli were rated more intensely than subsequent stimuli (both P < 0.05). However, this was not the case for subthreshold and threshold mechanical and cool stimuli (all P > 0.05). Paradoxically, for the chemical stimuli, there was adaptation to threshold stimuli (P = 0.05) but no adaptation for subthreshold and suprathreshold stimuli (P = 0.19 and 0.11, respectively).

CONCLUSIONS. Both mechanical (mechanosensory or polymodal) and cold receptors on human corneas show adaptation to repeated suprathreshold stimuli with a reduction in perceived intensity after multiple exposures to the same physical stimulus. This is in accord with the results found in electrophysiological and psychophysical experiments of somatosensation elsewhere in the body (and in other animals). The response to chemical stimuli was different, and this might reflect proximal and distal neural or stimulus-specific effects.

Subjects and Methods

Subjects

Subjects were selected according to the following criteria: None had a history of eye disease, systemic disease, or dry eye symptoms. Contact lens wear was not an exclusion criterion, but lens wearers had to be asymptomatic, have noninvasive tear breakup time of at least 10 seconds, and use only silicon hydrogel lenses. In mechanical, chemical, and cooling stimulus phases, we enrolled 20, 20, and 8 healthy subjects (age range, 21 - 46 years) respectively. There were four contact lens wearers in the mechanical, four in the chemical, and two in the cooling phases respectively. Most subjects were the same in the three stimulus phases except that eight subjects in the chemical experiment did not participate in the mechanical or cooling experiments. When subjects participated in multiple phases, intervals between participation lasted at least 1 week, and the order of participation was randomized. This study adhered to the Declaration of Helsinki for research involving human subjects and received clearance from the University of Waterloo Office of Research Ethics. Informed consent was signed before enrollment.

Instruments

Stimuli were delivered using a computer-controlled Belmonte pneumatic esthesiometer, with temperature, flow, and CO2 proportion monitored and automatically regulated. In addition, the computer collected subject responses and calculated the stimuli based on these.
detectability is a statistical concept, subthreshold stimuli, perceived "1" being the modal response to threshold stimulation. Given that, this scale produced a spread of ratings, with subjects reporting responses by using a computer button box. Pilot experiments showed that this scale was unambiguously detect the chemical stimulus at detection threshold, threshold stimulation when the ISI was 20 seconds (Friedman nonparametric ANOVA; \( P = 0.42 \)).

Psychophysical Methods

Thresholds to mechanical, chemical, and thermal (cooling) stimuli were measured using an ascending method of limits. The final threshold was the average of 6 'yes' responses. In order for subjects to unambiguously detect the chemical stimulus at detection threshold, chemical thresholds were measured using a flow rate of half the chemical experiment.28,29 Subjects were asked to use a 5-point intensity rating scale after each stimulus: 0, no stimulus; 1, very mild stimulus; 2, mild stimulus; 3, moderately strong stimulus; and 4, strong stimulus. Sub-scale after each stimulus: 0, no stimulus; 1, very mild stimulus; 2, mild stimulus; 3, moderately strong stimulus; and 4, strong stimulus. Subjects reported responses by using a computer button box. Pilot experimentation showed that this scale produced a spread of ratings, with '1' being the modal response to threshold stimulation. Given that detectability is a statistical concept, subthreshold stimuli, perceived occasionally, could still be rated lower than 1, if necessary, minimizing biased results because of a floor effect. The experimental stimulation and response sequence are illustrated in Figure 1.

Each subject received up to three training sessions, but 80% received only one or two sessions because during the training, their responses to the threshold stimuli were repeatable. If subjects were unable to give stable results after three training sessions, they were discontinued from the experiment and replaced. One subject from the mechanical and three subjects from the chemical experiments were excluded; no subjects were excluded during the cooling experiments. There was a 5-minute break between sessions. Half the subjects in the mechanical experiment also received an additional suprathreshold (mechanical) session with a 20-second ISI.

Statistical Analysis

Sample sizes were chosen using data derived during the first part of each experiment that served as a pilot phase. Power calculations were based on the effect size of the difference between the “unadapted” and “adapted” intervals (and assuming \( \alpha = 0.05 \) and \( \beta = 0.2 \) [power = 80%]). In two experiments (mechanical and cooling), this occurred during suprathreshold stimulation. For the third (chemical) experiment, this occurred with threshold stimulation.

Five sequential stimulus ratings were averaged; hence, there were four periods in each session. Because these data were derived from an ordinal rating scale, Friedman nonparametric ANOVA was used to evaluate the statistical significance of time after each experimental session began (set at \( P \leq 0.05 \)).

RESULTS

Mechanical Stimulation

Figure 2a shows the average ratings during the four time periods in each session that served as a pilot phase. Power calculations were based on the effect size of the difference between the “unadapted” and “adapted” intervals (and assuming \( \alpha = 0.05 \) and \( \beta = 0.2 \) [power = 80%]). In two experiments (mechanical and cooling), this occurred during suprathreshold stimulation. For the third (chemical) experiment, this occurred with threshold stimulation.

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Chemical Stimulation

Figure 4a shows no change in the average intensity ratings during repeated subthreshold chemical stimulation (ISI 30 seconds, Friedman nonparametric ANOVA \( P = 0.19 \)). Figure 4b shows that there was a significant decrease in intensity ratings with repeated threshold stimulation (Friedman nonparametric ANOVA; \( P = 0.03 \)). Figure 4c shows that the average ratings in the sequential periods of repeated suprathreshold stimulation did not change (Friedman nonparametric ANOVA; \( P = 0.11 \)).

DISCUSSION

To our knowledge, this is the first report of human corneal surface adaptation to mechanical, cold, and chemical stimuli, delivered with a modified Belmonte aesthesiometer.

The result demonstrated that subjective ratings of intensity changed over time, with more intense stimuli (suprathreshold) often producing significant reductions in perceived intensity; this appears to be adaptation in the classic sense.1,3,5 However, the ratings of suprathreshold mechanical, chemical, and thermally cold stimulation were not consistent. The response to chemical stimuli appeared to differ from the other two modes of stimulation. As hypothesized, there was adaptation to the suprathreshold mechanical and cooling stimuli or in the threshold session in the chemical experiment. This difference might reflect a differ-

FIGURE 1. The experimental sequence. Interstimulus intervals were 10 seconds for cooling and mechanical stimulation and 30 seconds for chemical stimulation.
ence in the pneumatic stimuli and how they interact with the tear film and ocular surface, differences between receptor types and underlying sensory channels, functional differences in the central nervous system, or combinations of these.

In the cornea, two types of neurons—mechanosensory and polymodal—are sensitive to mechanical stimulation, both show adaptation to repeated mechanical stimulation. The mechanosensory neurons have slightly higher mechanical thresholds with more apparent adaptation to repeated stimulation than polymodal neurons. In our study, suprathreshold mechanical stimuli would have been expected to activate both of these nociceptive fiber types and perhaps caused adaptation. However, in the subthreshold and threshold stimulus intensity sessions, it is likely that only the more sensitive polymodal neurons would have been activated (if at all), inducing no adaptation.

In addition to adaptation to mechanical stimulation, subjects showed adaptation to room temperature (cooling) pneumatic stimuli; the ratings decreased dramatically after repeated suprathreshold stimuli. A similar phenomenon has also been observed in the skin, and it has been proposed that the unmyelinated C-fibers more readily develop adaptation because of the peculiar membrane processes of the very thin nerve fibers and endings. In the human cornea, all cold and 70% of polymodal neurons are C-fibers. If similar mechanisms operate in the skin as in the cornea, it seems reasonable to conclude that peripheral neural adaptation may play a major role in the adaptation we demonstrated in mechanical and cooling experiments.

Putative central adaptation mechanism could also play a role in our experiment. Subjects’ arousal has been shown to decrease with decreased pain intensity when repeated stimuli caused “bearable” experimental dental pain. Our suprathreshold mechanical stimuli caused bearable sensations (Fig. 2); if a similar central arousal reduction occurred, this might have resulted in the decreased intensity reported by subjects. It has also been reported that cooling stimulation can also activate nociceptive pathways. Therefore, a similar reduction in arousal status during the cooling experiment might also be invoked to account for the cooling results.

The effect of the corneal chemical stimulation is presumably different from mechanical and cooling stimuli. Chemical stimulation arises because CO₂ in the pneumatic stimulus dissolves in the tears to generate local tear film (therefore, presumably corneal) areas that are acidic. In the skin, acidosis causes sensitization. On the other hand, taste receptors adapt to acid. According to a demonstration of adaptation to acidic stimulation in a cat’s corneal polymodal nociceptors, we speculated that subjects would show the same adaptation to suprathreshold stimuli shown with mechanical and cooling stimulation, but this was not found. There was significant reduction in reported intensity with repeated threshold stimulation. There are a number of possible indirect explanations for this lack of adaptation. The first had little directly to do with sensory processing of the actual stimulus. Because subjects responded to suprathreshold chemical stimulation with blinking, blepharospasm, and increased reflex tearing, it was possible that these would minimize the neural effect of protracted stimulation of the nociceptors that, presumably, were partly responsible for the adaptation effects with other stimuli. A second possibility giving rise to an apparent lack of adaptation to suprathreshold stimuli is that sensitization could follow suppression after a recovery period. Hence, in the chemical experiment, the longer ISI might have allowed the C-fibers to operate normally and might have caused less adaptation. Similar results have been found in a human cutaneous heat pain perception study with shorter ISI causing adaptation. Third, a number of possible neurophysiological changes might have occurred. These include the activation of deep (“silent”) additional C-nociceptors after repeated suprathreshold stimulation, the interaction between chemical and mechanical channels (similar to that demonstrated in the skin of rats and psychophysi-
cally in humans, and corneal nociceptive receptive field in the spinal trigeminal complex increasing in size after noxious chemical corneal stimulation. Each of these might effectively result in sensitization that might counteract any reduction in sensitivity because of adaptation. Finally, a central mechanism related to the anticipation of pain could activate the cortical nociceptive system. After experiencing a distinct uncomfortable sensation from chemical stimulation and, perhaps, the peculiarity of the stimulus, a top-down mechanism was triggered to facilitate nociception that could counteract any reduction in the effectiveness of chemical stimuli because of adaptation. Except for the reduction in the effectiveness of the stimuli from tear film alterations, why these mechanisms would occur only for the chemical stimuli and not for the others is not apparent.

This study has provided evidence that the human cornea is similar in its somatosensory processing to other parts of the body, with adaptation to suprathreshold nociceptive mechanical and nonnociceptive cooling stimuli when the inter-stimulus interval is short (10 seconds). Response to chemical stimulation is more complex, perhaps reflecting pneumatic stimulus peculiarities and peripheral neural and cortical factors. Adaptation of the corneal surface to the stimuli, especially mechanical, might have direct implication in the understanding of dry eye symptoms and adaptation to contact lens wear.

In conclusion, we found that the human corneal surface showed adaptation to repeated suprathreshold mechanical and cold stimuli but not to chemical stimuli (CO₂).

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References


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