Cold Thermoreceptors, Unexpected Players in Tear Production and Ocular Dryness Sensations

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Physical and chemical agents acting on the ocular surface (extreme environmental temperatures, wind, foreign bodies, and chemicals) elicit conscious sensations and reflex motor and autonomic responses (blinking, lacrimation, conjunctival vasodilation) aimed at protecting the eye from further injury. Sensory nerve terminals of trigeminal ganglion neurons innervating the cornea and conjunctiva are the origin of the peripheral neural information that reaches higher central nervous system areas, evoking ultimately these protective neural responses.\textsuperscript{1–4}

The innervation of the eye surface is provided by functionally distinct types of trigeminal ganglion neurons, whose peripheral nerve endings specialize in the preferential detection of various modalities of physical (mechanical, thermal) and chemical (exogenous irritants and endogenous mediators) stimuli, encoding their spatial and temporal characteristics (intensity and duration) into a discharge of nerve impulses. Most ocular trigeminal ganglion neurons belong to the general group of nociceptor sensory neurons whose peripheral nerve endings are activated by injurious or near injurious stimuli that initiate normal pain sensations.\textsuperscript{5}

CORNEAL PAIN IS SIGNALED BY MECHANO- AND POLYMODAL NOCICEPTORS

Mechanonociceptor neurons are the fastest conducting of the nociceptor neurons that innervate the eye surface. Their peripheral endings are normally recruited only by mechanical forces and respond to sustained mechanical stimulation with a short burst of nerve impulses at intensities that are comparatively low relative to those that cause injury in other territories such as the skin, but become potentially noxious for the fragile, unkeratinized corneal or conjunctival surface epithelium. This population of ocular sensory nerve afferents appears to signal primarily mechanical insults to the eye surface and possibly contributes to the sharp, pricking pain experienced when a foreign body touches the corneal or conjunctival surface.\textsuperscript{6,7}

POLYMODAL NOCICEPTORS

Polymodal nociceptor neurons are the most abundant class of ocular nociceptor neuron. Polymodal nerve endings are activated tonically by mechanical forces, but they also discharge repeatedly in response to heat, external irritant chemicals, and a large variety of endogenous chemical agents released by damaged tissues and immune cells (protons, arachidonic acid metabolites, kinins, cytokines, and growth factors) during injury and inflammation.\textsuperscript{5–8} A prominent feature of polymodal nociceptor neurons is their functional plasticity after injury. Their responsiveness changes drastically a few minutes after tissue damage, that is, they develop sensitization. Sensitizing polymodal nociceptors develop an irregular, low-frequency impulse activity long after stimulation has ceased. Also, the threshold for subsequent stimuli decreases, and their responses to new stimuli increases.\textsuperscript{5,6} Sensitization of the polymodal nociceptors that innervate injured and/or inflamed ocular tissues is the origin of spontaneous pain and of enhanced pain in response to stimuli at the injured area (primary hyperalgesia).\textsuperscript{5} When nociceptor nerve endings are directly damaged, they may exhibit a reduced responsiveness to natural stimuli.\textsuperscript{9,10} Nonetheless, the injured parent axons still display ongoing activity that causes spontaneous pain referred to the wounded area.\textsuperscript{5,10} Sustained stimulation and/or destruction of the terminal part of peripheral nerves additionally trigger long-term molecular and morphologic changes in the surviving segments of polymodal nociceptor neurons. As a consequence, membrane excitability and responsiveness to peripheral stimuli are altered.\textsuperscript{10} Knowledge of the molecular substrates responsible for polymodality and plasticity after inflammation or nerve damage has progressed in recent years, leading to the identification of a large number of transducing and receptor membrane proteins such as TRP channels and receptor molecules for inflammatory agents, as well as to a more detailed definition of the intracellular signaling pathways and gene expression changes associated with injury.\textsuperscript{11}

Postinjury modifications of the impulse firing pattern in polymodal nociceptors and of their responsiveness to new stimuli are determined by the intensity, type, time course, and duration of the tissue insult. This variability explains in mechanistic terms the differences in intensity profile and temporal evolution of ocular discomfort and pain sensations that develop under pathological conditions involving cell destruction, local inflammation, and/or peripheral nerve injury of the eye surface tissues.\textsuperscript{5,9} As expected from their nociceptive nature, experimental, selective stimulation of the population of corneal polymodal nociceptors in humans evokes stinging and burning pain referred to the eye.\textsuperscript{12–15} Moreover, drugs that interfere with inflammation pathways thereby reducing nociceptor sensitization behave as efficacious analgesics. Therefore, it is generally accepted that activity in polymodal nociceptors at the ocular surface is the main basis of the pain that accompanies clinical conditions such as keratitis, corneal ulcers, surgical wounds, contact lens wear, and conjunctivitis. Likewise, the prevalent opinion concerning the origin of the unpleasant sensations that accompany dry eye has been that...
they are primarily due to mechanical and/or chemical excitation of polymodal nociceptors secondary to distortion of nerve endings by ocular surface desiccation, tear hyperosmolality, and/or release of inflammatory mediators into tears.1-3

**The Cornea Is Also Innervated by Cold Thermoreceptors**

Cold thermoreceptors represent a separate class of corneal conjunctival sensory neurons with unique, specific properties. These include spontaneous firing of nerve impulses, often in bursts, at the normal background temperature of the cornea or conjunctiva (34–35°C) and immediate increase of their impulse activity when the corneal temperature drops, which thereafter stabilizes gradually at a higher impulse frequency level, proportional to the new value of corneal temperature.16 This, thus, cold thermoreceptors encode in their firing frequency the speed and magnitude of temperature reductions at the ocular surface as well as the final static temperature. In the normal range of corneal temperatures (36–29°C), cold thermoreceptors are able to discriminate transient temperature variations of 0.5°C or less.7,17,18 As expected, psychophysical experiments using corneal esthesiometry in human subjects have shown that they are able to discriminate corneal temperature drops of 1°C to 2°C below basal level.14,15

The prominent sensitivity of ocular cold receptors to small temperature reductions of the eye surface is puzzling. There is no evidence that corneal thermal information is used for environmental temperature assessment in animals, including humans. In fact, only under precise experimental conditions are humans capable of clearly ascribing a thermal quality to the mildly irritant sensation evoked by moderate cooling of the ocular surface.14,19,20

**Corneal Cold Receptors Detect Ocular Surface Wetness and Contribute to Basal Tearing**

An alternative and perhaps more relevant role for corneal and conjunctival cold thermoreceptors seems to be providing sensory information about the degree of wetness of the ocular surface.18,21 During the interblink periods, the ocular surface temperature, which is approximately 34°C when the eyes are closed, falls gradually, at a rate of 0.3°C/s,22 because of evaporation. As mentioned above, corneal cold receptor endings exhibit a remarkably high sensitivity for dynamic temperature reductions and are thus able to encode into their background firing frequency such small temperature oscillations.17 Moreover, when eyes remain open for longer times or when corneal evaporation is enhanced, corneal temperature decreases are more pronounced, increasing substantially cold fiber activity. The exquisite thermal sensitivity of corneal trigeminal neurons is attributable to the expression of TRPM8, a membrane ion channel specifically gated by cold.23-25 Cold trigeminal neurons of other bodily territories may also express Kv1 potassium channels, which are responsible for the current I_{Kov} that acts as a “brake” against cold excitation24,25 and counteracts the responsiveness to small temperature drops. However, I_{Kov} is absent in corneal cold thermoreceptors, which explains their particularly high sensitivity. Moreover, genetic deletion of TRPM8 channels in mice renders corneal cold thermoreceptors insensitive to temperature reductions and silences their background activity, proving that TRPM8 channels are essential for cold signaling in corneal cold afferent fibers.18 A surprising consequence of TRPM8 elimination is that basal tearing in Trpm8<sup>−/−</sup> mice is reduced to almost half compared with wild-type animals. This strongly supports the view that under normal circumstances, the continuous impulse firing from cold thermoreceptors represents a tonic stimulus for basal tear fluid secretion, conceivably activating at the superior salivary nucleus the parasympathetic secretory drive to the lacrimal glands and goblet cells (Fig. 1).26,27 Moreover, experimental warming of the ocular surface in mice or human subjects—a maneuver that transiently silences corneal cold thermoreceptors—decreases basal tearing, further confirming in humans the hypothesis that the basal flow of tears is partly dependent on the tonic input from the peripheral cold thermoreceptor endings located at the ocular surface.18

The activity of cold thermoreceptors within the normal range of corneal temperatures in healthy eyes appears to exert a nearly maximal stimulatory effect on tear secretion, because further increases in cold receptor activity obtained by larger corneal cooling stimuli within the innocuous level causes only modest elevations of the basal tearing rate.18 However, it is worth noting that the magnitude of the tonic excitatory input of cold receptors to central parasympathetic neurons possibly depends on the total number of active fibers and their mean firing frequency, as occurs with other secretory and vascular autonomic responses driven by cutaneous thermoreceptor sensory input.28,29 Cold-dependent basal tearing is expected to decay if the overall sensory inflow provided by the whole population of ocular cold afferent fibers decreases, as presumably occurs when cold trigeminal neurons progressively die with aging.30 This mechanism could contribute to the increased incidence of deficient basal tear secretion with age (Fig. 1).

While maintenance of basal tear secretion presumably depends on the tonic activity of cold afferent fibers, sudden increases in tear flow produced by injurious or irritant ocular surface stimuli are mediated by polymodal nociceptors.31 The effect of polymodal nociceptors appears to be independent of the tonic influence on basal tearing exerted by cold thermoreceptors. Irritation-induced, reflex increases in tear flow remain fully operative in Trpm8<sup>−/−</sup> mice, in which cold fibers are silent.18

**Cold Thermoreceptors Contribute to Dryness Sensations**

Experimental selective stimulation of corneal cold receptors in humans by small decreased in temperature (1–3°C) evokes distinct, conscious sensations of cooling that become increasingly unpleasant when larger temperature decreases are applied.14,15 Likewise, in the skin, increasing cooling evokes escalating unpleasant cold sensations that correlate directly with a progressive recruitment of cold receptor fibers.28 Possibly, the neural information provided by ocular cold thermoreceptors about the subtle temperature oscillations occurring in healthy eyes under normal environmental conditions remains subconscious. This is actually the case for thermal information provided by skin thermoreceptors under comfortable external temperatures.26 However, it can be speculated that when a sufficient number of ocular cold sensory fibers firing at higher frequencies are recruited, a conscious sensation of eye dryness of a magnitude proportional to the number and firing rate of cold receptor afferents can be expected (Fig. 1).

According to this hypothesis, sensations of ocular dryness accompanying the augmented tear evaporation would be the ocular counterpart of the sensory experience of unpleasant cold elicited by external temperature reductions of the skin. We propose that they are at least in part, evoked by an augmented activity at corneal cold receptor fibers caused by an enhanced, evaporation-induced ocular surface cooling.22 Ele-
Temperature (ºC)

Fire at higher frequency due to faster evaporation and evoke lower number of cold fibers that remain functional presumably aged individuals in whom basal tear secretion is reduced, the firing and to the ensuing unpleasant sensation of dryness. In thermoreceptors, may further contribute to the augmented vated tear osmolarity levels, which also activate corneal cold thermoreceptors, may further contribute to the augmented firing and to the ensuing unpleasant sensation of dryness. In aged individuals in whom basal tear secretion is reduced, the lower number of cold fibers that remain functional presumably fire at higher frequency due to faster evaporation and evoke dryness sensations even though their summated sensory inflow may be still insufficient to maintain the fraction of the tear flow dependent on cold fiber tonic effects on parasympathetic pathways. This interpretation does not exclude the possibility that age-dependent excitability changes in central nervous system neurons further contribute to the augmented dryness sensitivity in elderly people.

The interpretation that corneal cold thermoreceptors contribute to conscious dryness sensations does not exclude a participation of corneal and conjunctival polymodal nociceptors to the production of unpleasant feelings during ocular surface dryness. In fact, peripheral nociceptor fibers and their higher order neurons of the brain stem trigeminal complex gradually increase their activity when the cornea dries, suggesting that nociceptor pathways also contribute to the sensory inflow that encodes the level of ocular desiccation. It is tempting to speculate that mild sensations of ocular dryness are mainly dependent on cold receptor activation, whereas the contribution of polymodal nociceptors becomes more relevant when ocular drying is intense enough to become potentially damaging for corneal and conjunctival epithelial cells.

Finally, it is worth noting that unpleasant sensations of ocular dryness may also appear after traumatic or inflammatory damage to corneal sensory nerves, in the absence of concomitant alterations in tear film composition or volume. Peripheral injury gives rise to an abnormal spontaneous firing in damaged sensory nerves. This is also the case for corneal sensory nerves after photorefractive surgery, thus suggesting that such aberrant activity is interpreted by the brain as ocular surface dryness. Nevertheless, the relative contribution of injured cold- and nociceptor peripheral fibers to this type of dysesthesia is still ignored.
MULTIPlicity OF ROLES OF OCULAR COLD THERMORECEPTORS

Conscious discrimination of small temperature changes is not the sole role performed by cold thermoreceptors that innervate the skin, mucosae, and viscera. They additionally mediate thermal visceral reflexes, measure fluid flow in the respiratory pathways, and evoke pain. In the eye, there is experimental evidence that choroidal and scleral cold thermoreceptors also respond to changes in uveal blood flow and may participate in its regulation.

Signaling and maintaining ocular surface wetness is a new role for corneal and conjunctival cold thermoreceptors that was hitherto unknown and that cold thermoreceptors innervating other external mucosae (e.g., mouth and vagina) may also share. Finally, the possibility that the sensory message sent by ocular surface cold receptors also contributes to the modulation of automatic blinking is a functional alternative that deserves experimental scrutiny.

The receptors appear to be critical for cold transduction and generation of background impulse activity in ocular thermoreceptors. The dependence of both characteristics on a sole molecular entity opens new and exciting possibilities in the search for new drugs to treat reduced tearing and unpleasant dryness sensations, the most clinically relevant symptoms manifested by the majority of dry eye patients.

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References

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