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Topical Tetrodotoxin Attenuates Photophobia Induced by Corneal Injury in the Rat

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Abstract: Corneal injury can produce photophobia, an aversive sensitivity to light. Using topical application of lidocaine, a local anesthetic, and tetrodotoxin (TTX), a selective voltage-sensitive sodium channel blocker, we assessed whether enhanced aversiveness to light induced by corneal injury in rats was caused by enhanced activity in corneal afferents. Eye closure induced by 30 seconds of exposure to bright light (460–485 nm) was increased 24 hours after corneal injury induced by de-epithelialization. Although the topical application of lidocaine did not affect the baseline eye closure response to bright light in control rats, it eliminated the enhancement of the response to the light stimulus after corneal injury (photophobia). Similarly, topical application of TTX had no effect on the eye closure response to bright light in rats with intact corneas, but it markedly attenuated photophobia in rats with corneal injury. Given the well-established corneal toxicity of local anesthetics, we suggest TTX as a therapeutic option to treat photophobia and possibly other symptoms that occur in clinical diseases that involve corneal nociceptor sensitization.

***Perspective:** We show that lidocaine and TTX attenuate photophobia induced by corneal injury. Although corneal toxicity limits use of local anesthetics, TTX may be a safer therapeutic option to reduce the symptom of photophobia associated with corneal injury.*

***Key words:** Eye pain, photophobia, tetrodotoxin, lidocaine, corneal nociceptor.*

Photophobia, an aversive sensation induced by bright visual stimuli,[27] is markedly enhanced in many patients who experience corneal injury, such as occurs after corneal refractive surgery,[4] corneal abrasion,[33] chemical injury,[14] and dry eye syndrome,[34] or in long-term wearers of contact lens.[30] Although even bright light does not activate corneal afferents, it does excite photosensitive melanopsin-containing retinal ganglion cells that activate neurons in the superficial laminae of the trigeminal subnucleus caudalis (Vc/C1) (possibly by increasing ocular blood flow[27]), a site in the central nervous system that is involved in transmitting pain signals. The melanopsin-containing retinal ganglion cells also project to the thalamic relay nuclei, which also receive nociceptive inputs.[20,25] Because the cornea is innervated by nociceptors that encode mechanical, thermal, and chemical stimuli,[1] which also project to Vc/C1[15,28] as well as to the rostral ventrolateral pole of the trigeminal interpolaris/caudalis, the convergence could occur proximally in the trigeminal nuclear complex and/or at higher levels (eg, in thalamic relay nuclei).

Tetrodotoxin (TTX) inhibits a subset of voltage-gated sodium channels that play a key role in inflammatory and neuropathic pain, in particular Na(V) 1.7,[10] which is inhibited by an extremely low subnanomolar concentration of TTX.[17] We have observed that TTX can reverse mechanical hyperalgesia in sensitized muscle nociceptors without eliminating protective nociceptive behaviors (P. Alvarez, unpublished observations, 2015). This is important with respect to treatment of corneal symptoms because elimination of protective nociceptive reflexes (eg, eyelid closure) in response to noxious stimulation of the corneal surface allows continued corneal injury. Chronic use of local anesthetics to treat ocular pain is contraindicated because of major dose- and time-dependent corneal toxicity.[12,29] Because TTX has a lower corneal toxicity, it might be useful for symptoms that are dependent on enhanced input from nociceptive corneal afferents.[37] Therefore, in the present

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study, we evaluated whether topical application of TTX attenuates photophobia in the setting of nociceptor sensitization induced by traumatic injury to the cornea.

Methods

Animals

Experiments were performed on adult male Sprague Dawley rats (200–250 g; Charles River, Hollister, CA). Animals were housed 3 per cage, under a 12-hour light/dark cycle, in a temperature- and humidity-controlled environment. Food and water were available ad libitum. All behavioral testing was performed between 10:00 AM and 4:00 PM. Rats were acclimatized to the testing environment by bringing them to the experimental environment in their home cages, where they were left for 30 to 60 minutes, after which they were placed in cylindrical clear acrylic restrainers for evaluation of their response to stimulation with a bright light.

All experimental protocols were approved by the University of California, San Francisco Committee on Animal Research, and conformed to National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.[9] Efforts were made to minimize the number of animals used and their suffering.

Measuring Photophobia

Bright light stimulates eye closure even in normal control animals.[32,39] We measured this response to bright light in awake rats after they had been acclimated to being in a restrainer (10 min/day for 3 days). Some rats underwent corneal de-epithelialization injury, as previously described.[42] In rats anesthetized with 3% isoflurane, a defect in the central part of the cornea in 1 eye of each rat was made by placing a ~3.5-mm × 3.5-mm square of filter paper (Whatman Grade 40; GE Healthcare, Buckinghamshire, UK), saturated with n-heptanol, on the cornea for 90 seconds. After removing the filter paper, the eye was irrigated with .9% saline, and the n-heptanol–treated epithelial surface removed with a sclerotome (Katena Products Inc, Denville, NJ). Twenty-four hours after de-epithelialization, the magnitude of eye closure in response to 30 seconds exposure to high-intensity light (48 lumens [lm]), wavelength 460 to 485 nm (LXML-PB01-0023 Luxeon Rebel high power light-emitting diode; Quadica Developments Inc, Brantford, Ontario, Canada)[27] was again assessed. The maximal absorbance for melanopsin is ~480 nm,[2] and therefore the wavelength of light we used maximally activates the intrinsically photosensitive melanopsin-containing retinal ganglion cells. Video recordings were made and played back in real time to grade the magnitude of maximal eye closure using the scale illustrated in Fig 1. Bright light–induced eye closure was evaluated before and 10 minutes after topical corneal application of the local anesthetic, lidocaine, or TTX (5 μL volume for each) in awake, lightly restrained rats.

Drugs

TTX was obtained from Abcam (Cambridge, MA), and lidocaine hydrochloride and n-heptanol were obtained from Sigma-Aldrich (St. Louis, MO). TTX and lidocaine were dissolved in .9% saline. The n-heptanol was used undiluted.

Statistical Analyses

Group data are presented as mean ± standard error of the mean. Statistical significance was determined by 1- or 2-way repeated-measures analysis of variance (ANOVA), followed by the Dunnett multiple comparison post hoc test. $P < .05$ was considered statistically significant.

Results

To test the hypothesis that corneal injury enhances aversion to bright light, we compared the eye closure score in response to bright light in control rats and in those that had undergone corneal de-epithelialization injury. Photophobia was significantly increased when measured 24 hours after corneal injury (Fig 2). To explore the possibility that enhanced activity in nociceptors, caused by corneal injury, contributes to the enhanced response to bright light (photophobia), we evaluated the effect of the topical application of a local anesthetic to the cornea on injury-induced photophobia. Compatible with the hypothesis that enhanced activity in

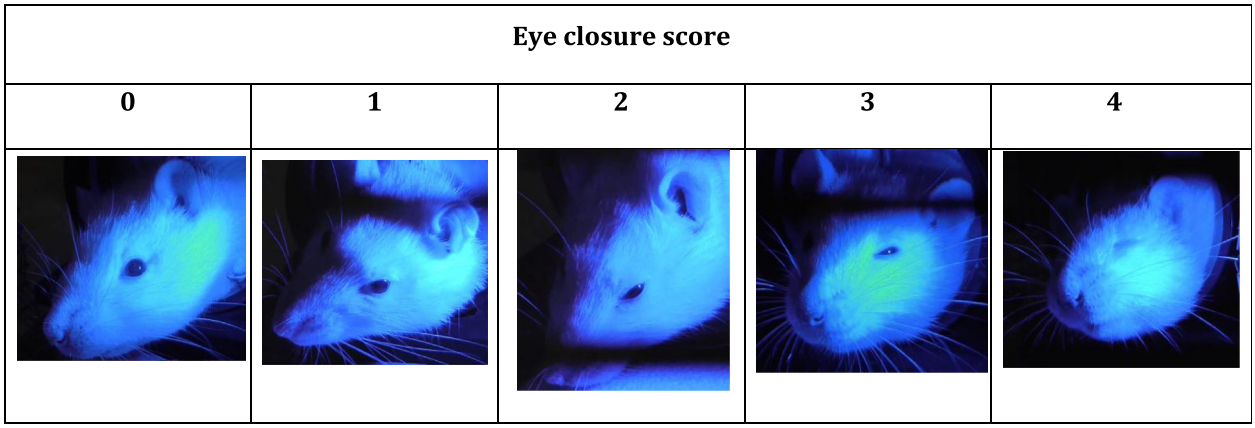

Figure 1. Scale for quantification of eye closure response to bright light in the rat. Eye closure was scored according to its magnitude: no closure = 0, ~25% closure = 1, ~50% closure = 2, ~75% closure = 3, full closure = 4.