Patients with moderate to severe dry eye disease (DE) often report persistent ocular surface pain (POSP).\(^1\)–\(^3\) Conservative therapies are sufficient to manage symptoms in mild DE;\(^4\),\(^5\) however, management in more severe cases has proved difficult.\(^6\)–\(^8\) An alternative approach would be to directly target receptor molecules on corneal nociceptors. The properties and expression of transducer receptor channels on corneal nociceptors are well described;\(^6\),\(^10\) however, the effects of chronic inflammation on corneal polymodal and mechano-nociceptors are less certain. Although factors known to excite corneal nociceptors are elevated in tears of DE patients,\(^11\)–\(^13\) the relationship between nociceptor activity and ocular pain is limited due to a lack of reliable measures of adverse behavior in animals. The present study uses a model for tear deficient DE\(^14\),\(^16\) and recording of muscle activity via orbicularis oculi muscle electromyography (Ooemg)\(^17\) to assess the roles of transient receptor potential vanilloid 1 (TRPV1) and melastatin 8 (TRPM8) ion channels on hypertonic saline (HS)-evoked corneal reflexes in a model for aqueous tear deficient DE.

**METHODS.** Eye wipe behavior and orbicularis oculi muscle activity (Ooemg) were measured after ocular instillation of HS, capsaicin, or menthol 14 days after exorbital gland removal. Total RNA and protein were measured from anterior eye segment and trigeminal ganglion samples from DE rats, whereas TRPM8 levels were not affected.

**RESULTS.** Eye wipe behavior was enhanced in DE rats after HS and capsacin instillation, but not after menthol when compared to sham rats. DE rats displayed greater Ooemg activity after HS and capsacin, but not after menthol, compared to sham rats. HS-evoked Ooemg activity was reduced by selective TRPV1 antagonists and by coapplication of capsain plus QX-314, a charged lidocaine derivative. Menthol did not affect Ooemg activity; however, selective antagonism of TRPM8 reduced HS-evoked Ooemg activity. TRPV1 protein levels were increased in anterior eye segment and trigeminal ganglion samples from DE rats, whereas TRPM8 levels were not affected.

**CONCLUSIONS.** These results suggest that TRPV1 plays a significant role in mediating enhanced nocifensive behavior in DE, while TRPM8 may play a lesser role. Strategies to target specific transducer molecules on corneal nerves may prove beneficial as adjunct therapies in managing ocular pain in moderate to severe cases of DE.

Keywords: dry eye, electromyography, behavior test, transient receptor potential vanilloid 1, transient receptor potential melastatin 8

---

**P**atients with moderate to severe dry eye disease (DE) often report persistent ocular surface pain (POSP).\(^1\)–\(^3\) Conservative therapies are sufficient to manage symptoms in mild DE;\(^4\),\(^5\) however, management in more severe cases has proved difficult.\(^6\)–\(^8\) An alternative approach would be to directly target receptor molecules on corneal nociceptors. The properties and expression of transducer receptor channels on corneal nociceptors are well described;\(^6\),\(^10\) however, the effects of chronic inflammation on corneal polymodal and mechano-nociceptors are less certain. Although factors known to excite corneal nociceptors are elevated in tears of DE patients,\(^11\)–\(^13\) the relationship between nociceptor activity and ocular pain is limited due to a lack of reliable measures of adverse behavior in animals. The present study uses a model for tear deficient DE\(^14\),\(^16\) and recording of muscle activity via orbicularis oculi muscle electromyography (Ooemg)\(^17\) to assess the roles of transient receptor potential vanilloid 1 (TRPV1) and melastatin 8 (TRPM8) ion channels on hypertonic saline (HS)-evoked corneal behavior. HS is well correlated with disease severity and is a risk factor for ocular irritation in DE.\(^18\) The present study sought to determine if a pattern of evoked Ooemg activity existed in anesthetized animals that was consistent with eye wiping in conscious animals, a recognized adverse behavior.\(^19\)–\(^21\) The results indicate that TRPV1 is critical for HS-evoked Ooemg activity at osmotic concentrations of HS necessary to cause pain sensation in humans;\(^22\) while TRPM8 plays only a minor role.

**METHODS.** A total of 141 male rats (250–350 g, Sprague-Dawley, Harlan, Indianapolis, IN, USA) were used. Data from four rats were excluded from final Ooemg analyses due to persistent low arterial pressure after anesthesia. Animals were housed in pairs and given free access to food and water in a climate- and light-controlled environment (25°C ± 2°C, 12:12-hour light/dark cycle with light on at 7:00 AM). The animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Minnesota (Minneapolis, MN, USA) according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and The National Institutes of Health guide for the Care and the Use of Laboratory Animals (PHS Law 99-158, revised 2015). All efforts were made to minimize the number of the animals used.

**Chemicals.** The following chemicals were applied to the ocular surface: TRPV1 receptor agonist, capsacin (in 10% ethanol, 10% Tween
80, diluted in normal saline); TRPV1 antagonist, 2E-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-3-[4-1,1-imethylethyl-phenyl]-2-2-propenamide (AMG 9810, in 1% DMSO, diluted in saline); TRPV1 antagonist, capsazepine (in 1% DMSO diluted in saline); charged membrane impermeant lidocaine derivative, N-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium bromide (QX-314, diluted in saline); TRPM8 agonist, L-menthol (in 10% ethanol diluted in saline); and TRPM8 antagonist, N-(3-amino-propyl)-2-[3-methylphenyl-methoxyl]-N-2-thienylmethyl benzamide hydrochloride (AMTB, diluted in saline). Drugs were purchased from Bio-Techne (Minneapolis, MN, USA) or from Sigma-Aldrich Corp. (St. Louis, MO, USA).

**Exorbital Gland Excision**

Rats were anesthetized with isoflurane (3%–5%) and the skin anterior to the ear was shaved. A small skin incision was made over the masseter muscle to expose and remove the left exorbital gland. The wound margins were treated with 2% xylocaine gel and the incision was closed with absorbable suture. The gland was exposed in sham rats but was not removed. Ketoprofen (25 mg/kg, intraperitoneally [IP]) was given as a single dose after surgery. Rats survived for 14 days after surgery before testing.

**Eye Wipe Behavior**

Rats were habituated to a plexiglass testing chamber for 1 hour and eye wipe behavior (i.e., purposeful forelimb swipes toward the eye, was measured over 5 minutes). Eye wipes were evoked by ocular instillation of HS (0.15, 1.0, 2.5 M NaCl, pH 7.2), capsaicin (vehicle, 0.327, 3.27, 32.7 μM), or menthol (vehicle, 20, 100, 200 μM) 1 to 2 days prior to OOemg recording. In a separate series, sham and DE rats received vehicle, QX-314 (2%) alone, 2.5 M NaCl or coapplication of QX-314 plus 2.5 M NaCl. Agents were applied to the left eye from a micropipette (20 μL) and eye wipes were counted without prior knowledge of surgical or drug treatment.

**Tear Volume**

Rats were anesthetized with urethane (1.2-1.5 g/kg, IP) and spontaneous tear volume was measured by the increase in wet length of phenol red thread (ZONE-QUICK; Menicon, Inc., San Mateo, CA, USA) at 14 days after surgery. The thread was gently placed in contact with the cornea/conjunctiva at its infero-lateral edge and tear volume was measured over 2 minutes.
TRPV1 and Nociceptive Behavior in Dry Eye

Data Recording and Analysis

OOemg activity was sampled at 1000 Hz, amplified (×10k), filtered (bandwidth 10–300 Hz), displayed and stored offline for later analysis (ADInstruments, Colorado Spring, CO, USA). OOemg activity was recorded continuously for 6 minutes from 3 minutes before (baseline activity) until 3 minutes after stimulus onset, rectified and stored as 1-second bins for off-line analyses. OOemg activity was calculated initially from the raw signal as total activity defined as the integrated area under the curve (AUC) for the 3-minute epoch (μV-s/3 minutes) sampled after the stimulus minus the 3-minute epoch recorded prior to each HS stimulus (i.e., baseline). OOemg activity was analyzed further as long duration activity (OOemgL), defined as continuous epochs lasting more than 200 ms and short duration activity (OOemgS) that lasted less than 200 ms. OOemg activity was assessed by 2-way ANOVA corrected for repeated measures on one factor. Significant treatment effects were assessed by Newman-Keuls after ANOVA. The data were presented as mean ± SEM, and the significant level set at P < 0.05. Power analyses based on previous studies of OOemg activity17,23 indicated that a sample size of n = 5 per treatment group would provide 80% power at P < 0.05.

RNA and Protein Measurements

Total RNA was extracted from anterior eye segment samples (cornea plus conjunctiva) and trigeminal ganglia (TG) of sham and DE rats using an RNA kit (Absolutely RNA; Agilent Technologies, La Jolla, CA, USA). cDNA was synthesized from 300 ng of each sample using a cDNA synthesis kit (iScript; Bio-Rad Laboratories, Hercules, CA, USA). qPCR was performed in triplicate on 2 μL cDNA with a DNA engine (Chromo4; Bio-Rad Laboratories) using iQ SYBR Green Supermix (Bio-Rad Laboratories). PCR conditions were as follows: an initial denaturation at 95°C for 3 minutes, followed by 40 cycles of 95°C for 10 seconds, 58.5°C for 20 seconds, and 72°C for 30 seconds. Data were analyzed using the delta Ct method against two reference genes (GAPDH and UBC). Primer sets were as follows: GAPDH: F: 5′-agacagccgcatctttgt-3′, R: 5′-ctgctcctggacgttgcccg-3′. TRPV1: F: 5′-ctgcctctaggacttggcaga-3′, R: 5′-tcgctcctggacgttgcccg-3′. TRPM8: F: 5′-aggactttggccagacagt-3′, R: 5′-aggaaattctggacgcaccgc-3′. A melting curve was employed to ensure amplicon fidelity.

Samples were homogenized in 0.5 mL cold lysis buffer (1% Triton X-100, 10 mM EGTA, 10 mM EDTA, TBS pH 7.4, protease inhibitor cocktail [complete mini; Roche Diagnostic Operations, Indianapolis, IN, USA]). Homogenates were centrifuged at 4°C for 10 minutes at 12,000 g, and the supernatant retained. Protein concentration was determined by BCA assay (Pierce Biotechnology, Inc., Rockford, IL, USA), and 25 μg protein was separated on 7.5% polyacrylamide gels and transferred to a 0.45 μm nitrocellulose membrane (Bio-Rad Laboratories). Membranes were blocked and incubated at 4°C overnight in TRPV1 receptor antibody (1:1000, lot# ACC029AN0550; Millipore, Billerica, MA, USA) or TRPM8 antibody (1:1000, lot# SA2318285; Invitrogen, Waltham, MA, USA) followed by goat anti-rabbit IRDye 680 (LI-COR, Lincoln, NE, USA). Proteins were visualized using an infrared scanner (Odyssey; LI-COR) and arbitrary optical density was determined. Normalizing controls were utilized by simultaneous staining with beta-tubulin antibody (Santa Cruz Biotechnology, Dallas, TX, USA) followed by goat anti-mouse IRDye 800 secondary antibody (LI-COR).

Immunoprecipitation was performed to confirm and compare results from western blots using a commercial kit (Pierce Classic IP Kit; Thermo Fisher Scientific, Rockford, IL, USA). Briefly, anterior eye and TG samples (150 mg) from sham

Experimental Design

At least 1 hour elapsed after placement of the OOemg electrodes before the recording session began. Two experimental designs were used (design one, Fig. 1A). Dose-effect responses were determined for ocular application of: HS (2.5 M), capsaicin (vehicle, 0.327, 3.27, 32.7 μM) or menthol (vehicle, 20, 100, 200 μM). Test solutions remained on the eye for 3 to 4 minutes and then washed out with artificial tears. Each stimulus period was separated by 30 minutes (design two, Fig. 1B). HS (2.5 M) was applied before and 30 minutes after ocular instillation of the following drugs: selective TRPV1 receptor antagonist (AMG9810, 10 μM), nonselective TRPV1 antagonist (capsazepine, 100 μM), selective nociceptor silencing by coapplication of capsaicin (0.327 or 3.27 μM) plus QX-314 (2%), and selective TRPM8 receptor antagonist (AMTB [10, 20, or 50 μM]). Drugs were prepared fresh each day and applied in a total volume of 20 μL. The doses of AMG 9810, capsazepine, and AMTB were similar to those reported to be effective in previous studies.24–26

![Figure 3](https://arvojournals.org/ on 09/30/2018)
and DE rats were homogenized and incubated with 2 μg of antibody for TRPV1 and TRPM8 as noted above. Protein-antibody complexes were separated from homogenate with protein A/G agarose, dissociated with loading buffer and separated on an 8% to 20% precast gel (Protean TGX; Bio-Rad Laboratories). The gel was removed and stained with a commercial stain (GelCode Blue Safe Protein Stain; Thermo Fisher Scientific) before imaging.

**RESULTS**

Sham and DE rats displayed normal weight gain after surgery and no overt signs of ocular hyperemia or inflammation. Spontaneous tear volume was measured in 82 rats (sham, n = 59; DE, n = 43). Tear volume was reduced ipsilateral to gland removal (8.63 ± 0.18 mm/2 minutes), compared to the contralateral eye (19.02 ± 0.28 mm/2 minutes) and compared to the left and right eyes of sham rats (19.31 ± 0.3 and 19.26 ± 0.27 mm/2 minutes), respectively. (F$_{1,30}$ = 310, P < 0.001).

**Eye Wipe Behavior**

Ocular application of NaCl evoked dose-related increases in eye wipes in sham and DE rats (F$_{2,20}$ = 13.76, P < 0.001, Fig. 2A). At the highest concentration (2.5 M) eye wipe behavior was significantly greater in DE than sham rats (F$_{1,30}$ = 11.5, P < 0.001). Similarly, capsaicin evoked a dose-related increase in eye wipes in sham and DE rats (F$_{2,20}$ = 16.08, P < 0.001, Fig. 2B). Eye wipes increased after 0.327 μM (0.0001%) and 3.27 μM (0.001%) capsaicin in DE rats, while only the higher dose-evoked eye wipes in sham animals (treatment main effects, F$_{1,12}$ = 9.34, P < 0.01). To determine if HS increased pore dilatation or corneal permeability, sham and DE rats received QX-314 (2%), HS (2.5 M) or coapplication of QX-314 and HS. The results in Figure 2C revealed that QX-314 alone did not affect eye wipe behavior, while HS increased eye wipes in sham (F$_{2,20}$ = 14.1, P < 0.005) and DE rats (F$_{2,20}$ = 25.4, P < 0.001). Coapplication of QX-314 plus HS greatly reduced eye wipes in DE (P < 0.01), but not in sham rats. Menthol (20, 100, and 200 μM) did not affect eye wipe behavior in sham or DE rats compared to vehicle application (F$_{3,42}$ = 1.90, P > 0.1, Fig. 2D). Results after prior application of TRPV1 or TRPM8 receptor antagonists on HS-evoked eye wipe behavior were inconclusive since it was not possible to control drug exposure as the rat began eye wiping after antagonist application. Most rats displayed partial or full eyelid closure after HS or capsaicin; however, this was not quantified. Rats tested for eye wiping behavior also were included in OOemg recording sessions 2 days later.

**OOemg Activity and HS Stimulation**

In an initial series (n = 3), pairs of wire electrodes were positioned in the upper and lower portions of the OO muscle to determine the relative magnitude and timing of HS-evoked OOemg. The pattern and timing of responses were similar; however, the magnitude of OOemg recorded from the lower OO muscle was greater than from the upper OO muscle (F$_{1,16}$ = 9.23, P < 0.001) as shown in Figure 3A. Since OOemg activity increased in upper and lower OO muscles, a single wire electrode was placed in each OO portion in subsequent experiments. As seen in Figure 3B, HS-evoked OOemg activity consisted of an initial sustained period of activity (OOemgl, >200 ms) followed by multiple periods of short duration activity (OOemgS, <200 ms). The dashed vertical lines in Figure 3B indicate time segments of OOemgl and OOemgS that are expanded in Figures 3C and 3D, respectively. Total OOemg activity increased after HS in a concentration-related manner in sham (Fig. 4A, F$_{2,54}$ = 60.8, P < 0.001) and DE animals (Fig. 4B, F$_{2,54}$ = 60.8, P < 0.001). In sham animals, OOemgl represented ~41% of the total OOemg response and increased further with higher concentrations of HS (F$_{2,54}$ = 4.58, P < 0.025). In DE animals, OOemgl represented ~79% of the total OOemg response and displayed a much greater increase with higher HS concentrations (F$_{2,54}$ = 46.1, P < 0.001). HS stimulation did not affect short duration OOemg (OOemgS) in sham DE rats (Fig. 4).

**OOemg Activity and Capsaicin Stimulation**

Capsaicin alone evoked dose-related increases in total OOemg in sham and DE rats (F$_{3,24}$ = 25.9, P < 0.001) that were different between animal groups (treatment main effect, F$_{1,8}$ = 6.85, P < 0.05, Fig. 5).

**OOemg Activity and Menthol Stimulation**

The highest dose of menthol (200 μM) caused small (>2 μV·s/3 minutes), but significant, increases in total OOemg activity in sham (F$_{3,24}$ = 5.12, P < 0.01) and DE rats (F$_{3,24}$ = 4.62, P < 0.025, Fig. 6). However, at this concentration menthol likely is no longer specific for TRPM8 channels.27

**TRPV1 and HS-Evoked OOemg Activity**

Three approaches were used to assess the role of TRPV1 in mediating OOemg responses to HS. In the first series, capsazepine (100 μM), a nonselective TRPV1 antagonist, significantly reduced HS-evoked total OOemg activity in sham
and DE rats (treatment main effect, \(F_{1,9} = 50.9, P < 0.001\), Fig. 7). Capsazepine reduced HS-evoked OOemgL only in DE rats (\(F_{1,9} = 24.9, P < 0.001\); sham, \(F_{1,9} = 1.12, P > 0.1\), Fig. 7B), whereas OOemgS activity was not affected. Second, the highly selective TRPV1 antagonist, AMG9810, reduced HS-evoked OOemgL activity in sham and DE rats (main effect, \(F_{1,8} = 95.9, P < 0.001\), Fig. 8). HS-evoked OOemgL activity was greatly reduced by AMG9810 in DE (\(F_{1,8} = 95.9, P < 0.001\), Fig. 8B) and marginally reduced in sham rats (\(F_{1,8} = 5.73, P < 0.05\), Fig. 8A). Short duration OOemgS activity was not reduced by AMG9810.

Third, we adapted the method of Binshtok et al.\textsuperscript{28} to selectively block voltage-gated sodium channels of TRPV1-expressing corneal nerves by coadministration of QX-314 and low-dose capsaicin (0.327 or 3.27 μM) 30 minutes prior to HS. As seen in Figure 9A, coapplication of 3.27 μM capsaicin with 2% QX-314 caused a significant reduction in HS-evoked total OOemgL activity in sham rats (\(F_{3,21} = 5.29, P < 0.005\)), whereas 0.327 μM capsaicin plus QX-314 had only a minor effect. By contrast, coapplication of 0.327 μM or 3.27 μM capsaicin plus QX-314 significantly reduced HS-evoked OOemgL activity in DE rats (\(F_{3,22} = 9.27, P < 0.001\), Fig. 9B). Note that administration of 3.27 μM capsaicin alone 30 minutes earlier did not affect HS-evoked OOemgL responses in sham or DE rats. HS-evoked OOemgS activity was not affected by capsaicin plus QX-314 in sham or DE rats (data not shown).

**TRPM8 and OOemg Activity**

Prior application of the selective TRPM8 receptor antagonist, AMTB (10–50 μM), significantly reduced the HS-evoked OOemg response in sham (\(F_{1,21} = 6.10, P < 0.005\)) and DE rats (\(F_{1,21} = 5.95, P < 0.005\), Fig. 10). The effect of AMTB on HS-evoked total OOemg activity was greater in DE than sham rats (\(F_{1,7} = 9.15, P < 0.025\)).

**TRPV1 and TRPM8 Expression and Protein Levels**

Anterior eye samples of sham and 14-day DE rats expressed similar levels of TRPV1 (\(F_{1,5} = 6.22, P < 0.1\) and TRPM8 (\(F_{1,5} = 5.25, P < 0.1\)). By contrast, TRPV1 protein levels were...
increased in DE versus sham rats ($F_{1,4} = 10.8$, $P < 0.05$), while TRPM8 protein levels were similar ($F_{1,7} = 0.65$, $P > 0.1$). Trigeminal ganglion (TG) expression of TRPV1 ($F_{1,6} = 0.22$, $P > 0.1$) and TRPM8 ($F_{1,6} = 0.84$, $P > 0.1$) were similar for sham and DE rats. However, TRPV1 protein levels increased and were greater in DE than sham rats ($22.1 \pm 6$ vs. $2.2 \pm 1$ arbitrary units [AU], $n = 4$ per group, $F_{1,6} = 13.7$, $P < 0.025$). Protein levels for TRPM8 were similar for DE and sham rats ($2.5 \pm 1.3$ vs. $0.2 \pm 0.1$ AU, $n = 4$ per group, $F_{1,6} = 2.1$, $P > 0.1$). Immunoprecipitation confirmed the increases in TRPV1 protein from anterior eye samples (sham versus DE = 2.08 and 4.81 relative intensity, respectively) and TG (sham versus DE = 2.29 and 4.09, respectively). Values for TRPM8 protein were low compared to TRPV1 in anterior eye samples (sham versus DE = 1.52 and 0.86 relative intensity, respectively) and TG (sham versus DE = 1.16 and 1.85 relative intensity, respectively, Fig. 11).

**Discussion**

A challenge for translational research concerned with mechanisms of chronic pain in humans has been the measurement of animal behavior with predictive value.29 This has been particularly true for studies of ocular pain since there are few options for measuring ocular-evoked aversive behaviors in animals that can be reasonably interpreted as ocular pain in humans. The present study measured forelimb eye wiping behavior in conscious animals and then recorded OOemg activity in anesthetized rats to identical stimuli that are known to cause ocular pain in humans. The results revealed good correspondence between evoked eye wipe behavior and either total or long duration OOemg (OOemgL) activity. Ocular stimuli known to cause pain in humans (e.g., HS and capsaicin) evoked robust concentration-dependent increases in eye wiping in conscious rats and OOemg activity in anesthetized rats and were significantly greater in DE than sham rats.

Eye blink frequency has often been used as a marker for ocular discomfort in DE. Spontaneous blink rates are elevated in patients30–32 and animal models of DE.15,33,34 Although manual counts of HS-evoked eye blinks were enhanced in DE rats,17 blinking also was enhanced after neutral saline35 and...
menthol \(^{56}\) in DE animals suggesting that eye blink frequency is not specific for nociceptive behavior and without videographic confirmation, is difficult to quantify.\(^{57}\) By contrast, squint-like behavior seen as a sustained eyelid closure in response to noxious stimuli has been used to determine the threshold light intensity for photophobia in normal subjects.\(^{58}\) In conscious rats, ocular application of HS at concentrations similar to the present study caused sustained eyelid closure in a concentration-related manner as assessed by videography.\(^{59}\) Collectively, these data supported the notion that sustained OOEmgL activity evoked by HS and capsaicin in anesthetized rats was a valid marker for squint-like behavior in conscious animals.

The tears of DE patients contain several factors able to increase the activity of corneal nociceptors, such as hyperosmolarity and proinflammatory molecules,\(^{13,18}\) and drive ocular pain sensation. A second aim of this study was to determine if TRPV1 and TRPM8 ion channel receptors contributed to the enhanced OOEmgL activity in DE rats. TRPV1 is gated by protons, heat, and hyperosmolar solutions\(^{40}\) and is expressed by 30% to 50% of corneal nerves.\(^{41,42}\) A majority of corneal polymodal nociceptors are gated by protons, heat, and hyperosmolar solutions.\(^{43-45}\) Psychophysical studies report that acidic,\(^{46,47}\) or hyperosmolar solutions\(^ {12,48}\) sufficient to cause burning pain in humans also excite corneal polymodal nociceptors in animals. Three approaches were used to demonstrate that TRPV1 activation contributes to HS-evoked OOEmgL activity: application of a nonselective TRPV1 antagonist (capsazepine), selective TRPV1 antagonist (AMG9810) and coapplication of subthreshold dose of antagonist (capsazepine), selective TRPV1 antagonist, for TRPV1 or TRPM8. The proteins were separated on a gel with molecular weight markers as indicated.

![TRPV1 and TRPM8](Image)

**Figure 11.** Anterior eye segment and TG levels of TRPV1 and TRPM8 in sham and DE rats. Tissues were removed, dissociated, and lysed. The lysate was used in an immunoprecipitation with antibodies for TRPV1 or TRPM8. The proteins were separated on a gel with molecular weight markers as indicated.

evoke pain sensation, TRPV1 alone is sufficient to account for ocular pain.

**Acknowledgments**

Supported by National Institutes of Health Grant EY028143 (DAB).

Disclosure: D. A. Bereiter, None; M. Rahman, None; R. Thompson, None; P. Stephenson, None; H. Saito, None

**References**


42. 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.


