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Human Tear Serotonin Levels Correlate with Symptoms and Signs of Dry Eye

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Purpose: Serotonin, a neurotransmitter known to be involved in nociceptor sensitization, is present in human tears. The purpose of this study was to correlate tear serotonin levels, as a marker of nociceptor sensitization, to facets of dry eye (DE), including symptoms and signs.

Design: Cross-sectional study.

Participants: A total of 62 patients with normal eyelid and corneal anatomy were prospectively recruited from a Veterans Administration Ophthalmology Clinic over 11 months.

Methods: Dry eye symptoms (Ocular Surface Disease Index [OSDI]), signs (tear break-up time [TBUT], corneal staining, and Schirmer's score), and clinical descriptors of neuropathic ocular pain (NOP) (sensitivity to light or sensitivity to wind) were assessed. For tear analysis, each patient's tears were collected after instilling 50 μl of sterile saline to the lower cul-de-sac of each eye and using capillary action microcaps to collect the ocular wash. Tear serotonin levels were measured using enzyme-linked immunosorbent assay.

Main Outcome Measures: Correlations between tear serotonin concentrations and DE symptoms and signs.

Results: The mean age of the population was 61±14 years, and 84% (n = 52) of the patients were male. Serotonin concentrations negatively correlated with Schirmer's scores ($r = -0.28$; $P = 0.02$) but did not correlate with other DE parameters, such as OSDI scores, sensitivity to light or wind, TBUT, and staining. According to our hypothesis, we divided patients into groups based on both DE symptoms and aqueous tear production; serotonin concentrations were significantly higher in DE group 1 (OSDI ≥6 and Schirmer's <8) compared with both DE group 2 (OSDI ≥6 and Schirmer's ≥8) and controls (OSDI <6 and Schirmer's ≥8). Patients in DE group 2 more frequently reported sensitivity to light (64%) and wind (67%) compared with DE group 1 (40% and 60%, respectively) and controls (8% and 17%, respectively).

Conclusions: Patients with DE symptoms and aqueous tear deficiency had higher tear serotonin levels compared with those with DE symptoms but normal tear production and those without DE symptoms. *Ophthalmology 2015;122:1675-1680 © 2015 by the American Academy of Ophthalmology.*

Dry eye (DE), a multifactorial disease of the ocular surface and tear film, affects millions of men and women in the United States.[1] It is characterized by decreased quantity and altered quality of tears, abnormal ocular surface, and bothersome symptoms. These symptoms include pain, blurry vision, and tearing, all of which adversely affect quality of life.[2,3] Despite their morbidity, the pathophysiology underlying DE symptoms, especially ocular pain, is not well understood.[4] For example, DE symptoms (including ocular pain) do not correlate well with tear film parameters.[5,6] In a prospective study of 263 veteran men, less than 10% of the variability in symptoms was explained by measured tear film parameters.[5] This suggests that factors beyond tear film health are likely important in driving symptoms in certain individuals. Therefore, it is not surprising that some patients have persistent symptoms while on available DE therapies,[7] most of which target the ocular surface and tear film deficiency.

A potential factor that may underlie DE-associated chronic pain is dysfunction of the ocular somatosensory nerves. There is a growing understanding that many patients diagnosed with DE describe features of neuropathic pain,[8] defined as pain resulting from dysfunction of nerves. For example, neuropathic pain and DE share many common descriptors, including spontaneous pain in the absence of any apparent noxious stimulation, dysesthesias, hyperalgesia, and allodynia.[9–11] Neuropathic pain can result from damage or sensitization of peripheral and central somatosensory nerves. With respect to ocular anatomy, this would represent changes in primary afferents[9,12,13] (peripheral sensitization) or in the connecting second- and third-order neurons in the spinal trigeminal nuclei and subcortical and cortical neuronal processes (central sensitization).[9,12]

On the basis of anatomy, it is biologically plausible that phenotypic alterations or peripheral sensitization of ocular somatosensory nerves could occur as a component of DE in some patients. Free nerve endings interdigitate between superficial epithelial cells and are therefore vulnerable to repeated damage from environmental exposures,[11,14,15] including contact with sensitizing molecules, such as inflammatory mediators. For example, T cells, interleukins, tumor necrosis factor, and matrix metalloproteinase have all been found on the ocular surface and in tears of humans and animals with DE.[16–19]

The presence of inflammation has been shown to affect both directly and indirectly, the function of peripheral nerves

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in animal models through increased expression of serotonin its receptors, and other sensitizing neuropeptides.[20–24] In one study, rat hind paws injected with formalin-induced peripheral inflammation increased serotonin release from mast cells and sensory sensitization.[25] In another model, subcutaneous serotonin injections themselves led to pain (detected by intense flinching and licking behaviors in rats), providing evidence that serotonin is an important peripheral pain mediator.[26] In the trigeminal system, peripheral 5-HT_3 and 5-HT_{2A} receptors were found to mediate pain after formalin injections into masseter muscles.[27,28] The subsequent occurrence of central sensitization has been well described in the setting of ongoing afferent nociceptive traffic seen with peripheral sensitization.[29,30]

Sufficient evidence supports the notion that peripheral serotonin, acting via overexpressed serotonin receptors in primary afferents, contributes to peripheral sensitization and amplification of pain signaling.[20] Because serotonin has been detected in human tears,[31] we hypothesized that tear serotonin levels may act as a surrogate marker for corneal nociceptor sensitization, and therefore could be associated with specific DE subtypes. In this study, we tested our hypothesis that tear serotonin levels would be higher in those with abnormal versus healthy tear parameters.

Methods

Study Population

After approval by the Miami Veterans Administration Institutional Review Board/Ethics Committee, patients were prospectively recruited from the Miami Veterans Administration Eye Clinic between March 2013 and February 2014. Each patient underwent a complete ocular surface examination, and patients with normal eyelid and corneal anatomy were included (n = 62). Exclusion criteria included contact lens wear, ocular medications (with the exception of artificial tears), active external ocular process, history of refractive or retinal surgery, history of glaucoma, and cataract surgery within the past 6 months. Patients with human immunodeficiency virus, sarcoidosis, graft-versus-host disease, collagen vascular disease, or other inflammatory conditions were excluded. On the basis of these inclusion and exclusion criteria, patients with a wide range of DE symptoms (none to severe) and signs (none to severe) were eligible, including those with evidence of aqueous and evaporative tear deficiency.

Data Collection

All patients were examined by the same optometrist (A.L.M.). Demographic and health information was collected for each patient, including age, gender, race, ethnicity, health status, and psychiatric history, including diagnosed depression or anxiety disorder and use of antidepressant and antianxiety medications. Symptoms and severity of DE were assessed via the Ocular Surface Disease Index (OSDI).[32] Clinical features of neuropathic ocular pain (NOP) were assessed by using subscores on the OSDI, specifically question 1 (Have you experienced eyes that are sensitive to light during the last week?) and question 10 (Have your eyes felt uncomfortable in windy conditions during the last week?). In addition, ocular surface examination included (1) tear break-up time (TBUT) (5 μl fluorescein placed in the eye; 3 measurements taken in each eye and averaged); (2) corneal staining (National Eye Institute scale; 5 areas of cornea assessed, with a score of 0–3 in each); and (3) Schirmer's strips with anesthesia.

Tear Collection

Fifty microliters of sterile saline was instilled by a pipette to the lower cul-de-sac of each eye. Tears were immediately collected by capillary action using 1-μl microcaps (Drummond Scientific Company, Broomall, PA) applied gently to the lower temporal lid margins. A minimum of 50 μl of tears (~6 disposable micropipettes) was collected and pooled from the 2 eyes and released by bulb dispenser into 1.5-ml Nalgene polypropylene cryogenic vials (Sigma-Aldrich Co, St. Louis, MO). These vials were labeled with de-identified subject codes and immediately placed in a −80°C freezer.

Quantification of Serotonin

Enzyme-linked immunosorbent assay (ELISA) was performed on the tear samples using an ELISA Serotonin Kit (Enzo Life Sciences, Inc, Farmingdale, NY). This kit detects a minimum serotonin concentration of 0.30 ng/ml, and previous research has shown measurable concentrations of serotonin in tears ranging from 0.12 to 50.32 ng/ml, with a mean of 2.74 ng/ml.[31] The negative control was sample without tears (sterile saline; no serotonin present), and the positive control was the serotonin standard (provided by the kit). Equivalent volumes of each sample were tested, and to ensure precision and reproducibility, each sample was run in duplicate and the average of the duplicates was taken. The serotonin concentration was calculated using the average optical density of each sample, and control sterile saline samples were negative for serotonin. After plotting the percent bound versus serotonin concentration for the standards, the serotonin concentration of our samples was interpolated, according to the kit's directions. Samples were prospectively collected over a period of 11 months and thus had different storage times; regression analysis indicated that time in storage did not correlate with serotonin concentrations.

Statistical Analysis

All statistical analyses were performed using SPSS statistical package version 22 (SPSS Inc, Chicago, IL). The strength of association between tear serotonin concentrations and DE signs and symptoms were quantified using Pearson and Spearman coefficients. Analyses also included the Fisher exact test for nominal variables and Student independent t test, Mann–Whitney U test, and analysis of variance (as appropriate) for continuous variables. Multivariable linear regression analyses were performed to evaluate the effect of various factors or serotonin concentrations. All analyses were performed using DE signs from the more severely affected eye. A P value <0.05 was considered statistically significant.

Results

Study Population

The mean age of the population was 61 years, with a standard deviation of 14 years; 84% (n = 52) of the patients were male, 65% (n = 40) self-characterized themselves as white, and 34% (n = 21) self-characterized themselves as Hispanic. Serotonin concentrations were significantly associated with ethnicity but not with other demographic characteristics (age: Pearson $r = 0.14$, $P = 0.26$; Spearman rho = 0.23, $P = 0.07$) (Table 1). Of the 62 patients included in this study, 58 had a psychiatric history available. Sixteen patients (28%) had depression, and of those 10 were

Table 1. Mean Serotonin Concentrations by Patient Demographics and Clinical Features

	Serotonin Concentration (ng/ml), Mean ± SD	P Value
Demographics		
Race		
White	1.58±0.98	0.66
Black	1.73±0.94	
Other	2.01±1.13	
Gender		
Male	1.72±0.97	0.18
Female	1.28±0.91	
Ethnicity		
Hispanic	1.24±0.77	0.02
Non-Hispanic	1.86±0.99	
Comorbidities		
Depression		
Yes	1.74±0.95	0.51
No	1.56±0.88	
Anxiety		
Yes	1.38±0.87	0.48
No	1.64±0.90	
Medication use		
Antidepressants		
Yes	1.74±0.92	0.61
No	1.58±0.90	
Anxiolytics		
Yes	1.60±1.00	0.97
No	1.61±0.89	

SD = standard deviation.

taking antidepressants; 10 (17%) had anxiety, and of those 7 were taking anxiolytics. Mean serotonin concentrations were not different among those with and without a psychiatric history or among those who were or were not taking antidepressant and anxiolytics. There were also no significant differences in OSDI, Schirmer's scores, or DE subgroups among those taking and not taking antidepressants and anxiolytics.

Correlations between Serotonin Concentration and Dry Eye Symptoms and Signs

Serotonin concentrations negatively correlated with Schirmer's scores ($r = -0.28$; $P = 0.01$) but did not correlate with OSDI scores, sensitivity to light or wind, TBUT, or staining (Table 2). A multivariable linear regression analysis considering both ethnicity and Schirmer scores found that both remained significantly associated with serotonin concentrations.

Serotonin Concentration by Dry Eye Subgroups

Overall, patients with aqueous tear deficiency (ATD) (Schirmer's score <8) had higher tear serotonin concentrations compared with those without ATD (2.34±1.16 vs. 1.53±0.89 ng/ml; $P = 0.02$) (Table 3). Serotonin concentrations were significantly higher in the DE group 1 (OSDI ≥6 and Schirmer's <8) compared with both the DE group 2 (OSDI ≥6 and Schirmer's ≥8) and the non-DE control group (OSDI <6 and Schirmer's ≥8) (Table 3). A multivariable linear regression analysis considering both ethnicity and the described 3 groups found that only ethnicity remained significantly associated with serotonin concentrations.

Table 2. Correlations between Serotonin Concentrations and Dry Eye Symptoms and Signs (More Severely Affected Eye)

Parameter	Pearson r	P Value	Spearman rho	P Value
OSDI total	−0.02	0.88	0.002	0.99
OSDI question 1: light	0.007	0.96	0.008	0.95
OSDI question 10: wind	−0.11	0.37	−0.09	0.49
TBUT	0.04	0.73	0.1	0.42
Corneal staining	0.09	0.48	0.1	0.43
Schirmer's score	−0.28	0.02	−0.22	0.08

OSDI = ocular surface disease index; TBUT = tear break-up time.

Neuropathic Ocular Pain Features by Study Populations

Demographic characteristics did not influence the frequency of NOP features, but patients with a diagnosis of depression or antidepressant use had a higher frequency of sensitivity to wind (depression: 80% [n = 12] vs. 49% [n = 19], $P = 0.04$; antidepressant use 100% [n = 9] vs. 49% [n = 22], $P = 0.01$). Patients in DE group 2 had the highest frequency of sensitivity to wind and light, compared with DE group 1 and controls (Table 3).

Discussion

The results of this study show that patients with DE symptoms and ATD have higher tear serotonin concentrations compared with those with DE symptoms and normal tear production and those without DE symptoms. However, patients with DE symptoms and normal tear production more frequently endorsed wind and light sensitivity than those with DE symptoms and reduced tear production and those without DE symptoms.

We chose to measure tear serotonin concentrations on the ocular surface as a potential marker of corneal nociceptor sensitization because serotonin has been detected in human tears[31] and is a known peripheral nerve sensitizer.[20,33] In addition, serotonin concentrations are known to increase not only with inflammation (a well-described component of DE) but also with peripheral nerve abnormalities.[20,26,34,35] With the use of high-performance liquid chromatography with electrochemical detection, the average serotonin concentration in tears in a previous study of 22 volunteers (64% were female) with an average age of 36.5±9 years was 2.74±1.99 ng/ml.[31] In our older, mostly male population, average serotonin concentrations were slightly lower using an ELISA methodology.

Serotonin produces its sensitizing effect on peripheral nerves through direct and indirect mechanisms.[20] Serotonin binds to G protein-coupled receptors and mobilizes downstream slow modulatory responses through second messenger signaling pathways[36] with activation of ligand-gated ion channels and rapid depolarizing responses in nerves.[37] Serotonin also may directly amplify the conductance of tetrodotoxin-resistant sodium channels in primary afferents, shift the conductance-voltage curve toward a hyperpolarized direction, and enhance the channel kinetics.[38] This subsequently has the capacity to affect the

Table 3. Tear Serotonin Concentrations, Dry Eye Symptoms, and Signs (More Severely Affected Eye) in Dry Eye Subgroups

	Control Group (OSDI <6 and Schirmer's ≥8)	DE Group 1 (OSDI ≥6 and Schirmer's <8)	DE Group 2 (OSDI ≥6 and Schirmer's >8)	*P* Value
Patients (n)	12	5	45	
Serotonin (mean [ng/ml] ± SD)	1.72±1.07	3.03±0.76	1.48±0.84	0.005*
Symptoms				
OSDI Q1: light ≥0 (%, n)	8%, 1	40%, 2	64%, 29	<0.0005
OSDI Q10: wind ≥0 (%, n)	17%, 2	60%, 3	67%, 30	0.001
OSDI Q1: light (mean ± SD)	0.33±1.2	1.0±1.7	1.5±1.5	0.04†
OSDI Q10: wind (mean ± SD)	0.33±0.89	1.0±1.2	1.6±1.6	0.01†
OSDI total (mean ± SD)	1.5±1.4	18.1±11.5	27.7±19.5	<0.0005
Signs				
TBUT (mean [sec] ± SD)	8.4±2.9	6.3±1.7	7.4±2.8	0.47
Corneal staining (mean ± SD)	2.2±2.2	4.2±3.3	3.1±2.9	0.45
Schirmer's score (mean [mm] ± SD)	17.8±5.7	4±2.3	15.5±5.0	<0.0005*

DE = dry eye; OSDI = ocular surface disease index; SD = standard deviation; TBUT = tear break-up time; Q = question.
*Significant differences between DE group 1 compared with control and DE group 2 by least significant difference subanalysis.
†Significant difference between DE group 2 and control group by least significant difference subanalysis.

threshold of activation, sensitivity, and nociceptive transmission of peripheral nerves.[21,22] Likewise, 5-HT_3 receptor stimulation enhances perception of pain.[39]

To our knowledge, this study is unique because it correlates serotonin concentrations with DE parameters. However, others have examined the relationship between other peripheral nerve sensitizers, such as nerve growth factor, and DE.[40,41] Serotonin may act synergistically together with several neuropeptides and inflammatory mediators, as a component of an "inflammatory soup,"[20] contributing to neuronal peripheral sensitization and hyperexcitability.[24] This has been shown in humans, in whom serotonin may act synergistically with bradykinin in producing pain and hyperalgesia.[20,42]

Understanding the role of peripheral sensitizers in DE may open up new therapeutic pathways. For example, serotonin receptor antagonists administered systemically or topically, such as ketanserin (5-HT_{2A} antagonist), granisetron (5-HT_3 antagonist), and cromolyn (prevents the degranulation of mast cells), may have therapeutic potential in some patients with DE. Ketanserin and granisetron, for instance, have been shown to have a peripheral antihyperalgesic effects, the latter in trigeminal system pain in humans.[20,43,44]

Study Limitations

As with all studies, our findings must be considered in light of our study limitations. First, our study was conducted at a Veterans Affair Hospital, and therefore our population consists of predominantly older men. However, we argue that this group is an important one to study DE because of the high incidence of central pain disorders and depression in this population. Second, our cross-sectional study design precludes commenting on persistence or change in DE symptoms, signs, serotonin concentration, and NOP features over time. Third, we recognize that serotonin concentrations and patients' self-reported responses on questionnaires are not the same as direct assessments of ocular somatosensory nerve function. However, direct nerve conduction studies of the cornea are not currently available, and therefore measures are needed to provide indirect information on this understudied parameter of DE. Fourth, it is not clear why other aspects of tear dysfunction (corneal staining and TBUT) were not significantly associated with serotonin. Further studies, with increased numbers and in other DE populations, will be needed to replicate our findings and assess which parameters of tear dysfunction, including ones not measured in this study, most closely align to serotonin concentrations. Finally, we did not measure inflammation on the ocular surface or measure other sensitizers previously described in DE, such as nerve growth factor. As such, we cannot evaluate the link among serotonin, inflammation, and other nerve sensitizers.

Despite these limitations, we found that patients with DE symptoms and ATD had higher concentrations of serotonin on the ocular surface. Previous studies have found associations between low tear levels and inflammation,[45,46] thus supporting our theory that ocular surface inflammation can increase local concentrations of neurotransmitters and thus modulate neuronal function. Of note, patients with DE symptoms but normal tear production had serotonin concentrations similar to those of patients without DE symptoms, but they tended to describe a higher frequency of NOP symptoms, suggesting the presence of central abnormalities in this subgroup of patients. The implication of this study supports a disease model in which there is a connection among ocular surface stress, inflammation, and peripheral sensitization of ocular somatosensory nerves in some patients with DE. Moreover, our data suggest that still other patients may have developed central sensitization with neuropathic pain–like symptoms even in the absence of ongoing ocular surface abnormalities. Identifying novel biomarkers and developing new therapeutics to target the underlying pathology in each case to better manage these different patients with DE will likely be an important area of research in the future.

References

1. Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. Am J Ophthalmol 2003;136:318–26.
2. Miljanovic B, Dana R, Sullivan DA, Schaumberg DA. Impact of dry eye syndrome on vision-related quality of life. Am J Ophthalmol 2007;143:409–15.
3. Lemp MA. Epidemiology and classification of dry eye. Adv Exp Med Biol 1998;438:791–803.
4. Nichols KK, Mitchell GL, Zadnik K. Performance and repeatability of the NEI-VFQ-25 in patients with dry eye. Cornea 2002;21:578–83.
5. Galor A, Feuer W, Lee DJ, et al. Ocular surface parameters in older male veterans. Invest Ophthalmol Vis Sci 2013;54: 1426–33.
6. Sullivan BD, Whitmer D, Nichols KK, et al. An objective approach to dry eye disease severity. Invest Ophthalmol Vis Sci 2010;51:6125–30.
7. Rajagopalan K, Abetz L, Mertzanis P, et al. Comparing the discriminative validity of two generic and one disease-specific health-related quality of life measures in a sample of patients with dry eye. Value Health 2005;8:168–74.
8. Galor A, Zlotcavitch L, Walter SD, et al. Dry eye symptom severity and persistence are associated with symptoms of neuropathic pain. Br J Ophthalmol 2015;99:665–8.
9. Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. Annu Rev Neurosci 2009;32:1–32.
10. Gartner R, Jensen MB, Nielsen J, et al. Prevalence of and factors associated with persistent pain following breast cancer surgery. JAMA 2009;302:1985–92.
11. Rosenthal P, Borsook D. The corneal pain system. Part I: the missing piece of the dry eye puzzle. Ocul Surf 2012;10:2–14.
12. McGreevy K, Bottros MM, Raja SN. Preventing chronic pain following acute pain: risk factors, preventive strategies, and their efficacy. Eur J Pain Suppl 2011;5:365–72.
13. Hains LE, Loram LC, Weiseler JL, et al. Pain intensity and duration can be enhanced by prior challenge: initial evidence suggestive of a role of microglial priming. J Pain 2010;11: 1004–14.
14. Muller LJ, Marfurt CF, Kruse F, Tervo TM. Corneal nerves: structure, contents and function. Exp Eye Res 2003;76:521–42.
15. Guthoff RF, Wienss H, Hahnel C, Wree A. Epithelial innervation of human cornea: a three-dimensional study using confocal laser scanning fluorescence microscopy. Cornea 2005;24:608–13.
16. Luo L, Li DQ, Doshi A, et al. Experimental dry eye stimulates production of inflammatory cytokines and MMP-9 and activates MAPK signaling pathways on the ocular surface. Invest Ophthalmol Vis Sci 2004;45:4293–301.
17. Solomon A, Dursun D, Liu Z, et al. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Ophthalmol Vis Sci 2001;42:2283–92.
18. Stern ME, Schaumburg CS, Pflugfelder SC. Dry eye as a mucosal autoimmune disease. Int Rev Immunol 2013;32:19–41.
19. Aragona P, Aguennouz M, Rania L, et al. Matrix metalloproteinase 9 and transglutaminase 2 expression at the ocular surface in patients with different forms of dry eye disease. Ophthalmology 2015;122:62–71.
20. Bardin L. The complex role of serotonin and 5-HT receptors in chronic pain. Behav Pharmacol 2011;22:390–404.
21. Cardenas CG, Mar LP, Vysokanov AV, et al. Serotonergic modulation of hyperpolarization-activated current in acutely isolated rat dorsal root ganglion neurons. J Physiol 1999;518: 507–23.
22. Lang PM, Moalem-Taylor G, Tracey DJ, et al. Activity-dependent modulation of axonal excitability in unmyelinated peripheral rat nerve fibers by the 5-HT(3) serotonin receptor. J Neurophysiol 2006;96:2963–71.
23. Michaelis M, Vogel C, Blenk KH, Janig W. Algesics excite axotomised afferent nerve fibres within the first hours following nerve transection in rats. Pain 1997;72:347–54.
24. Moalem G, Grafe P, Tracey DJ. Chemical mediators enhance the excitability of unmyelinated sensory axons in normal and injured peripheral nerve of the rat. Neuroscience 2005;134: 1399–411.
25. Nakajima K, Obata H, Ito N, et al. The nociceptive mechanism of 5-hydroxytryptamine released into the peripheral tissue in acute inflammatory pain in rats. Eur J Pain 2009;13:441–7.
26. Hong Y, Abbott FV. Behavioural effects of intraplantar injection of inflammatory mediators in the rat. Neuroscience 1994;63:827–36.
27. Okamoto K, Imbe H, Tashiro A, et al. The role of peripheral 5HT2A and 5HT1A receptors on the orofacial formalin test in rats with persistent temporomandibular joint inflammation. Neuroscience 2005;130:465–74.
28. Okamoto K, Imbe H, Tashiro A, et al. Blockade of peripheral 5HT3 receptor attenuates the formalin-induced nocifensive behavior in persistent temporomandibular joint inflammation of rat. Neurosci Lett 2004;367:259–63.
29. Woolf CJ, Chong MS. Preemptive analgesia—treating postoperative pain by preventing the establishment of central sensitization. Anesth Analg 1993;77:362–79.
30. Koltzenburg M, Torebjork HE, Wahren LK. Nociceptor modulated central sensitization causes mechanical hyperalgesia in acute chemogenic and chronic neuropathic pain. Brain 1994;117:579–91.
31. Martin XD, Brennan MC. Serotonin in human tears. Eur J Ophthalmol 1994;4:159–65.
32. Schiffman RM, Christianson MD, Jacobsen G, et al. Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol 2000;118:615–21.
33. Sommer C. Serotonin in pain and analgesia: actions in the periphery. Mol Neurobiol 2004;30:117–25.
34. Anden NE, Olsson Y. 5-hydroxytryptamine in normal and sectioned rat sciatic nerve. Acta Pathol Microbiol Scand 1967;70:537–40.
35. Vogel C, Mossner R, Gerlach M, et al. Absence of thermal hyperalgesia in serotonin transporter-deficient mice. J Neurosci 2003;23:708–15.
36. Bradley PB, Engel G, Feniuk W, et al. Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 1986;25:563–76.
37. Yakel JL, Jackson MB. 5-HT3 receptors mediate rapid responses in cultured hippocampus and a clonal cell line. Neuron 1988;1:615–21.
38. Gold MS, Reichling DB, Shuster MJ, Levine JD. Hyperalgesic agents increase a tetrodotoxin-resistant Na+ current in nociceptors. Proc Natl Acad Sci U S A 1996;93:1108–12.
39. Richardson BP, Engel G. The pharmacology and function of 5-HT receptors. Trends Neurosci 1986;9:424–8.
40. Levi-Montalcini R. The nerve growth factor 35 years later. Science 1987;237:1154–62.
41. Lambiase A, Bonini S, Micera A, et al. Expression of nerve growth factor receptors on the ocular surface in healthy

subjects and during manifestation of inflammatory diseases. Invest Ophthalmol Vis Sci 1998;39:1272–5.
42. Babenko V, Graven-Nielsen T, Svensson P, et al. Experimental human muscle pain and muscular hyperalgesia induced by combinations of serotonin and bradykinin. Pain 1999;82:1–8.
43. Nishiyama T. Effects of a 5-HT2A receptor antagonist, sarpogrelate on thermal or inflammatory pain. Eur J Pharmacol 2005;516:18–22.
44. Ernberg M, Lundeberg T, Kopp S. Pain and allodynia/hyperalgesia induced by intramuscular injection of serotonin in patients with fibromyalgia and healthy individuals. Pain 2000;85:31–9.
45. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 2007;5:75–92.
46. Prabhasawat P, Tseng SC. Frequent association of delayed tear clearance in ocular irritation. Br J Ophthalmol 1998;82:666–75.

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Abbreviations and Acronyms:
ATD = aqueous tear deficiency; **DE** = dry eye; **ELISA** = enzyme-linked immunosorbent assay; **NOP** = neuropathic ocular pain; **OSDI** = Ocular Surface Disease Index; **TBUT** = tear break-up time.

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