

## Aging and Dry Eye: Age-related Changes in the Function of the Ocular Sensory Apparatus Likely Underlie Dry Eye Symptoms

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### Abstract

Dry eye is a multifactorial disease impacting the ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability associated with potential damage to the ocular sensory apparatus. Dry eye is diagnosed in approximately 3.2 million U.S. women and 1.7 million U.S. men older than 50 years of age. While the natural history of dry eye remains to be determined, dry eye rates increase with age, reaching the highest among women 75-79 years of age and men 80-84 years of age. Many patients with dry eye describe features of neuropathic pain, including spontaneous pain, hyperalgesia and allodynia, suggesting dysfunction of the ocular sensory apparatus, which likely impacts the lacrimal functional unit altering basal and reflex tear secretion. Comorbid conditions such as depression and post-traumatic stress disorder commonly associated with other chronic pain conditions provide further evidence that dry eye may be associated with central processing abnormalities. In fact, recent data show dry eye represents a chronic overlapping pain condition that is significantly impacted by environment and shared genetic factors. In the following sections we review how aging may impact the anatomical structures associated with dry eye. Among other factors, sensitization of the ocular sensory apparatus occurs which occurs with aging is likely influenced by multiple environmental and genetic factors common to other chronic pain conditions and an important driver of ocular pain and dry eye symptoms with aging.

**Keywords:** Aging; Dry eye; Neuropathic pain; Hyperalgesia; Allodynia; Chronic overlapping pain condition

### Introduction

Based on the largest studies of dry eye to date including the Women's Health Study and Physicians Health Study, dry eye is diagnosed in approximately 3.2 million U.S. women and 1.7 million U.S. men older than 50 years of age [1-4]. Moreover, the prevalence of dry eye may be greater in Hispanic and Asian women compared to Caucasian women [2]. While the natural history of dry eye remains to be determined, including prognostic factors, it appears that there is a high likelihood that this disorder progresses with age [3]. In a large managed care population, dry eye was diagnosed or treated in 0.65% of women vs. 0.26% of men ( $P < 0.001$ ), and dry eye rates increased with age, reaching the highest among women 75-79 years of age and men 80-84 years of age [3]. Therefore, in this review our goal is to look at the current understanding of dry eye as a chronic pain condition and how aging may impact this common disorder.

### What is dry eye?

Per the Definition and Classification Subcommittee of the International Dry Eye WorkShop (DEWS), dry eye is "a multifactorial disease of the tears and ocular surface that results in symptoms of

discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface" [5]. Furthermore, the disorder also involves the Lacrimal Functional Unit (LFU) [6], which consists of the ocular surface, the main lacrimal gland and the interconnecting innervation. Just based on these definitions, it is easy to see that dry eye is a complex disorder that manifests with various symptoms and signs.

Symptoms of dry eye are varied and include various descriptors of ocular pain (burning, aching, tender, sharp, shooting, dryness), visual dysfunction (unstable vision, glare), and tearing. Signs are likewise varied and can include evidence of aqueous tear deficiency and/or evaporative dry eye. Several tests can be used to evaluate for these different entities. Schirmer's test with or without anesthesia can be used to assess aqueous production as can tear meniscus height (assessed by inspection or with a high-resolution anterior segment optical coherence tomography machine). Tear break up time, interferometry, and evaluation of meibomian gland parameters can evaluate the anatomy and function of the lipid layer. Other tests, such as tear osmolarity and corneal and conjunctival staining give a global assessment of ocular surface health, abnormalities in which can be seen both with aqueous tear deficiency and evaporative dry eye. A conundrum in dry eye, however, is that none of these objective tests

align well with dry eye symptoms, a finding that has been consistently repeated in several studies [7,8].

A missing link in the puzzle may be that patients with dry eye may have various levels of ocular sensory apparatus dysfunction and that this dysfunction may explain the discrepancy between symptoms and signs of disease. Data supporting this idea include the fact that many patients diagnosed with dry eye describe features of neuropathic pain, including spontaneous pain, dysesthesias (unpleasant, abnormal sensation such as characterizing ocular pain as hot-burning in nature), hyperalgesia (exaggerated pain response to suprathreshold noxious stimuli such as hypersensitivity to wind), and allodynia (pain in response to normally non-noxious stimuli such as sensitivity to light) [9-11]. These pain symptoms are likely due to alterations in function of the ocular sensory apparatus, which may loop back and affect the function of the LFU, with subsequent changes in basal and reflex tear secretion.

### Epidemiology of dry eye

Population based studies in various United States (US) cities have utilized symptom questionnaires to evaluate the prevalence of dry eye with a prevalence estimate of around 15% [12-16]. Similar studies conducted in several countries around the world have resulted in similar estimates [17-26]. Once thought to be a disease predominantly of women, recent studies out of the Veterans Affairs Medical Center have found that approximately 1 in 5 male veterans carry a diagnosis of dry eye [27,28]. In all the above studies, increasing age has been consistently found to be a risk factor for dry eye.

Twin studies can facilitate our understanding of the genetic and environmental contributions to common disorders. Recently a large twin study was conducted that showed that chronic pain syndromes were impacted significantly by both environment and genetic factors [29]. Vehof et al. showed that four chronic pain syndromes including pelvic pain (PP), chronic widespread pain (CWP), irritable bowel syndrome (IBS), and dry eye were heritable and shared genetic etiologic factors in common biologic pathways [29]. A recent study by Warren et al. found the number of existing antecedent functional somatic syndromes was a significant risk factor for CWP, IBS, and dry eye [30]. We and others have shown that chronic pain syndromes represent a risk factor for dry eye [31,32]. Further support for central processing abnormalities in patients diagnosed with dry eye comes from our work and that of others that studied the relationship between dry eye, depression and post-traumatic stress disorder (PTSD) [33-36]. We found that patients with a diagnosis of depression and PTSD had a 2 fold higher risk of carrying a diagnosis of dry eye [27,28]. Furthermore, these patients complained of more severe dry eye symptoms than patients without PTSD or depression [33].

### Why is dry eye more common in the aging population?

In the following sections we review how aging may impact the anatomical structures associated with dry eye including the lacrimal glands, meibomian glands, goblet cells (e.g., lacrimal functional unit, or LFU) as well as the immune system and ocular sensor apparatus (peripheral and central nervous system components).

The impact of aging on the lacrimal glands, meibomian glands, and goblet cells (LFU): Changes in the lacrimal gland (producer of the aqueous component of tears), meibomian glands (producers of the lipid component) and goblet cells (producers of the mucin component) have all been described with aging. The acini, stroma, and

ducts of aging rat lacrimal glands displayed changes that included acinar degeneration, nuclear abnormalities, lipofuscin-like inclusions; increased collagen, and ductal dilation [37]. Similarly, mice and human aging meibomian glands demonstrated loss of acne and a reduction in cellular proliferation markers (Ki67) [38-41]. Furthermore, decreased goblet cell densities on the ocular surface have been described in older animals, including rats [37] and mice [42] (detected using histology), and in humans (detected using in vivo confocal microscopy) [43]. All of the above changes have been shown to alter the properties and function of the aging tear film. These despite changes, clinically measured dry eye signs (as described above) do not mirror patient reported dry eye symptoms, including ocular pain.

Studies in rodents have also found reduced density of parasympathetic and sympathetic nerves surrounding the acini with aging [44,45]. These differences were also detected via decreased acetylcholine release from lacrimal gland nerves in 24-month-old mice compared to 3 and 12-month-old animals. Functionally, these changes were also associated with decreased tear secretion. Older lacrimal glands also synthesized less protein and secreted less fluid after stimulation with SP, VIP, histamine or 5-HT [46].

The impact of aging on the immune system and ocular inflammation: Inflammation is a well-recognized component of dry eye [47,48]. A classic paper that established this concept in animals was published by Niederkorn et al. in 2006 [49]. In this paper, mice were first subjected to a low humidity environment and were given scopolamine to reduce blinking. These experimental conditions led to the development of T cell-mediated inflammation on the ocular surface with clinical manifestations that resembled dry eye in humans (i.e. corneal staining). The authors were then able to induce a similar disease picture in nude mice by adoptively transferring CD4<sup>+</sup> T cells [49]. Newer data have since demonstrated that older mice spontaneously develop this phenotype, with CD4<sup>+</sup> T cell infiltration into the conjunctiva, expression of inflammatory cytokines (interferon- $\gamma$ , IL-17, matrix metalloproteinase 9), and increased T and B cells in the lacrimal gland. Interestingly, adoptive transfer of CD4<sup>+</sup> T cells isolated from these elderly mice transferred the disease into young immunodeficient recipients [42]. Rats similarly have been found to have increased mast and lymphocytic infiltration in their lacrimal glands with age [37]. Inflammation is a known sensitizer of peripheral nerves [50,51] and it is likely that inflammation on the ocular surface alters the function of the corneal nociceptors, and therefore the function of the ocular sensory apparatus.

The impact of aging on the ocular sensory apparatus: Changes in peripheral nerves, including a reduction in the number of specialized peripheral receptors and deterioration of myelinated and unmyelinated axons, are known to occur with age [52] and have been related to decreased sensitivity within the auditory, gustatory, and visual systems [53]. Similar changes have been shown within the somatosensory system, including mechanical, thermal, and nociceptive responsiveness. Evidence from this line of research may be useful for understanding changes in mechanical and thermal sensory processing within the ocular sensory apparatus.

In aged animals the proportions of cutaneous mechano- and/or heat-responsive C-nociceptors have been shown to be significantly lower in older compared to younger rats [54]. Similarly, microneurographic studies in humans have reported decreased ratios of mechano-sensitive to mechano-insensitive C-fibers in healthy older

subjects (age range: 41-67 years) compared to younger subjects (21-36 years) when measuring from the peroneal nerve [55].

Electrophysiologically, the mechanical stimulus needed to elicit a response in cutaneous mechano-responsive C-fibers was higher in older compared to younger rats, but responses to heat and chemical stimuli were similar irrespective of age [54]. In contrast, responses recorded from mechano-responsive C-fibers from deep tissue (muscle) in aged rats, showed the opposite effect: lower thresholds and greater responsiveness to mechanical stimuli compared to fibers from young rats [56]. In humans, decreased thresholds for heat stimuli in cutaneous mechano-insensitive C-fibers, and slightly increased thresholds in mechano-responsive fibers, have been found in aged individuals compared to fibers in their younger counterparts [55]. Additionally, microneurographic recordings from single C-fibers in humans have demonstrated that a higher frequency of fibers in older adults had atypical discharge characteristics (13%) compared to fibers recorded from younger individuals, similar to, but less pronounced than, what has been described in patients with neuropathic pain [55].

Behaviorally, it has generally been found in humans that aging caused increases in threshold for pain report, but decreases in pain tolerance [53]. Similarly, animal studies have reported enhanced nociceptive behaviors to supra-threshold noxious levels of cold and heat (but similar responses to mechanical stimuli) between aged and young animals [54]. The interplay of peripheral and central changes due to aging which give rise to these changes in perceptual response involves a complex and minimally understood set of mechanisms.

With regards to the eye, confocal microscopy data studying peripheral corneal nerves have demonstrated mixed results, with one study reporting significant declines in sub-basal nerve fiber density with age (0.9% per year) [57], and others reporting no alterations in number, density, or beadings of nerves with age [58,59].

Central nerve changes are also known to occur with age, with decreased connectivity and network integrity noted in healthy older adults [60]. With regards to the eye, changes in peripheral nerves due to age related environmental stress and inflammation may propagate upwards and affect the function of the central aspect of the ocular sensory apparatus. The anatomy of the ocular sensory apparatus lends biologic plausibility to this idea. The first synapse of the ocular sensory apparatus occurs in the Vi/Vc zone, and specific dry-responsive projection neurons have been identified in this area. A subset of these neurons, receive additional converging input from corneal primary afferents sensitive to protons, heat and chemicals [61]. Therefore, projection neurons in the transition zone integrate innocuous as well as noxious sensory information from the eye, and may constitute the neurophysiological substrate of central sensitization. In fact, the Vi/Vc transition zone is a well-recognized area involved in the generation and maintenance of hyperexcitability and central sensitization [62].

With regards to the overall function of the ocular sensory apparatus, both the Cochet-Bonnet and Belmonte aesthesiometry devices have been used to characterize somatosensory function within the eye in animals and humans. Using the Cochet-Bonnet aesthesiometer, corneal sensitivity was found decrease with age in horses [63] and humans [64]. The Belmonte aesthesiometer, a more robust instrument, has displayed mixed results with some studies reporting lower detection thresholds (higher sensitivity) [65] and others reporting higher thresholds (lower sensitivity) with age [66-68]. Unfortunately, none of the available instruments can directly measure

the function of the peripheral and/or central components of the ocular sensory apparatus in-vivo in humans.

## Conclusion

To summarize, among other factors, sensitization of the ocular sensory apparatus is likely influenced by multiple environmental and genetic factors common to other chronic pain conditions and is likely an important driver of ocular pain and dry eye symptoms in the elderly, but remains an understudied component of dry eye. New technology is needed to functionally assess the status of the ocular sensory apparatus, determine prognostic factors, and understand the impact of aging on dry eye. This information, combined with ocular surface measurements, will likely improve treatment algorithms, with a focus on agents that modulate ocular sensory apparatus function in appropriate individuals.

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## References

1. Schaumberg DA, Dana R, Buring JE, Sullivan DA (2009) Prevalence of dry eye disease among US men: estimates from the Physicians' Health Studies. *Arch Ophthalmol* 127: 763-768.
2. Schaumberg DA, Sullivan DA, Buring JE, Dana MR (2003) Prevalence of dry eye syndrome among US women. *Am J Ophthalmol* 136: 318-326.
3. [No authors listed] (2007) The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye Workshop. *Ocul Surf* 5: 93-107.
4. Schein OD, Hochberg MC, Muñoz B, Tielsch JM, Bandeen-Roche K, et al. (1999) Dry eye and dry mouth in the elderly: a population-based assessment. *Arch Intern Med* 159: 1359-1363.
5. [No authors listed] (2007) The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye Workshop. *Ocul Surf* 5: 75-92.
6. Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC (2004) The role of the lacrimal functional unit in the pathophysiology of dry eye. *Exp Eye Res* 78: 409-416.
7. Galor A, Feuer W, Lee DJ, Florez H, Venincasa VD, et al. (2013) Ocular surface parameters in older male veterans. *Invest Ophthalmol Vis Sci* 54: 1426-1433.
8. Schein OD, Tielsch JM, Muñoz B, Bandeen-Roche K, West S (1997) Relation between signs and symptoms of dry eye in the elderly. A population-based perspective. *Ophthalmology* 104: 1395-1401.
9. Costigan M, Scholz J, Woolf CJ (2009) Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu Rev Neurosci* 32: 1-32.
10. Gärtner R, Jensen MB, Nielsen J, Ewertz M, Kroman N, et al. (2009) Prevalence of and factors associated with persistent pain following breast cancer surgery. *JAMA* 302: 1985-1992.
11. Rosenthal P, Borsook D (2012) The corneal pain system. Part I: the missing piece of the dry eye puzzle. *Ocul Surf* 10: 2-14.
12. Begley CG, Chalmers RL, Mitchell GL, Nichols KK, Caffery B, et al. (2001) Characterization of ocular surface symptoms from optometric practices in North America. *Cornea* 20: 610-618.

13. Schein OD, Muñoz B, Tielsch JM, Bandeen-Roche K, West S (1997) Prevalence of dry eye among the elderly. *Am J Ophthalmol* 124: 723-728.
14. Moss SE, Klein R, Klein BE (2000) Prevalence of and risk factors for dry eye syndrome. *Arch Ophthalmol* 118: 1264-1268.
15. Bandeen-Roche K, Muñoz B, Tielsch JM, West SK, Schein OD (1997) Self-reported assessment of dry eye in a population-based setting. *Invest Ophthalmol Vis Sci* 38: 2469-2475.
16. Muñoz B, West SK, Rubin GS, Schein OD, Quigley HA, et al. (2000) Causes of blindness and visual impairment in a population of older Americans: The Salisbury Eye Evaluation Study. *Arch Ophthalmol* 118: 819-825.
17. Brewitt H, Sistani F (2001) Dry eye disease: the scale of the problem. *Surv Ophthalmol* 45: 199-202.
18. McCarty CA, Bansal AK, Livingston PM, Stanislavsky YL, Taylor HR (1998) The epidemiology of dry eye in Melbourne, Australia. *Ophthalmology* 105: 1114-1119.
19. Chia EM, Mitchell P, Rochtchina E, Lee AJ, Maroun R, et al. (2003) Prevalence and associations of dry eye syndrome in an older population: the Blue Mountains Eye Study. *Clin Experiment Ophthalmol* 31: 229-232.
20. Hikichi T, Yoshida A, Fukui Y (1995) Prevalence of dry eye in Japanese eye centers. Graefes archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv für klinische und experimentelle Ophthalmologie 233:555-558.
21. Uchino M, Schaumberg DA, Dogru M, Uchino Y, Fukagawa K, et al. (2008) Prevalence of dry eye disease among Japanese visual display terminal users. *Ophthalmology* 115: 1982-1988.
22. Uchino M, Dogru M, Uchino Y, Fukagawa K, Shimmura S, et al. (2008) Japan Ministry of Health study on prevalence of dry eye disease among Japanese high school students. *Am J Ophthalmol* 146: 925-929.
23. Shimmura S, Shimazaki J, Tsubota K (1999) Results of a population-based questionnaire on the symptoms and lifestyles associated with dry eye. *Cornea* 18: 408-411.
24. Lekhanont K, Rojanaporn D, Chuck RS, Vongthongsri A (2006) Prevalence of dry eye in Bangkok, Thailand. *Cornea* 25: 1162-1167.
25. Sahai A, Malik P (2005) Dry eye: prevalence and attributable risk factors in a hospital-based population. *Indian J Ophthalmol* 53: 87-91.
26. Lee AJ, Lee J, Saw SM, Gazzard G, Koh D, et al. (2002) Prevalence and risk factors associated with dry eye symptoms: a population based study in Indonesia. *Br J Ophthalmol* 86:1347-1351.
27. Galor A, Feuer W, Lee DJ, Florez H, Carter D, et al. (2011) Prevalence and risk factors of dry eye syndrome in a United States veterans affairs population. *Am J Ophthalmol* 152: 377-384.
28. Galor A, Feuer W, Lee DJ (2012) Depression, Post-traumatic Stress Disorder, and Dry Eye Syndrome: A Study Utilizing the National United States Veterans Affairs Administrative Database. *American journal of ophthalmology* 154:340-346.
29. Vehof J, Zavos HM, Lachance G, Hammond CJ, Williams FM (2014) Shared genetic factors underlie chronic pain syndromes. *Pain* 155: 1562-1568.
30. Warren JW, Langenberg P, Clauw DJ (2013) The number of existing functional somatic syndromes (FSSs) is an important risk factor for new, different FSSs. *J Psychosom Res* 74: 12-17.
31. Vehof J, Kozareva D, Hysi PG, Hammond CJ (2014) Prevalence and risk factors of dry eye disease in a British female cohort. *Br J Ophthalmol* 98: 1712-1717.
32. Galor A, Felix ER, Feuer W, Shalabi N, Martin ER, et al. (2015) Dry eye symptoms align more closely to non-ocular conditions than to tear film parameters. *Br J Ophthalmol*.
33. Fernandez CA, Galor A, Arheart KL (2013) Dry eye syndrome, posttraumatic stress disorder, and depression in an older male veteran population. *Investigative ophthalmology & visual science* 54:3666-3672.
34. Labbé A, Wang YX, Jie Y, Baudouin C, Jonas JB, et al. (2013) Dry eye disease, dry eye symptoms and depression: the Beijing Eye Study. *Br J Ophthalmol* 97: 1399-1403.
35. Li M, Gong L, Sun X, Chapin WJ (2011) Anxiety and depression in patients with dry eye syndrome. *Curr Eye Res* 36: 1-7.
36. Wen W, Wu Y, Chen Y, Gong L, Li M, et al. (2012) Dry eye disease in patients with depressive and anxiety disorders in Shanghai. *Cornea* 31: 686-692.
37. E-Fadaly AB, E-Shaarawy EA, Rizk AA, Nasralla MM, Shuaib DM (2014) Age-related alterations in the lacrimal gland of adult albino rat: a light and electron microscopic study. *Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft* 196:336-351.
38. Jester BE, Nien CJ, Winkler M, Brown DJ, Jester JV (2011) Volumetric reconstruction of the mouse meibomian gland using high-resolution nonlinear optical imaging. *Anat Rec (Hoboken)* 294: 185-192.
39. Nien CJ, Paugh JR, Massei S, Wahlert AJ, Kao WW, et al. (2009) Age-related changes in the meibomian gland. *Exp Eye Res* 89: 1021-1027.
40. Villani E, Canton V, Magnani F, Viola F, Nucci P, et al. (2013) The aging Meibomian gland: an in vivo confocal study. *Invest Ophthalmol Vis Sci* 54: 4735-4740.
41. Nien CJ, Massei S, Lin G, Nabavi C, Tao J, et al. (2011) Effects of age and dysfunction on human meibomian glands. *Arch Ophthalmol* 129: 462-469.
42. McClellan AJ, Volpe EA, Zhang X, Darlington GJ, Li DQ, et al. (2014) Ocular surface disease and dacryoadenitis in aging C57BL/6 mice. *Am J Pathol* 184: 631-643.
43. Wei A, Hong J, Sun X, Xu J (2011) Evaluation of age-related changes in human palpebral conjunctiva and meibomian glands by in vivo confocal microscopy. *Cornea* 30: 1007-1012.
44. Rios JD, Horikawa Y, Chen LL (2005) Age-dependent alterations in mouse exorbital lacrimal gland structure, innervation and secretory response. *Experimental eye research* 80:477-491.
45. Williams RM, Singh J, Sharkey KA (1994) Innervation and mast cells of the rat exorbital lacrimal gland: the effects of age. *J Auton Nerv Syst* 47: 95-108.
46. Draper CE, Singh J, Adeghate E (2003) Effects of age on morphology, protein synthesis and secretagogue-evoked secretory responses in the rat lacrimal gland. *Mol Cell Biochem* 248: 7-16.
47. Massingale ML, Li X, Vallabhajosyula M, Chen D, Wei Y, et al. (2009) Analysis of inflammatory cytokines in the tears of dry eye patients. *Cornea* 28: 1023-1027.
48. Yoon KC, Jeong IY, Park YG, Yang SY (2007) Interleukin-6 and tumor necrosis factor-alpha levels in tears of patients with dry eye syndrome. *Cornea* 26: 431-437.
49. Niederkorn JY, Stern ME, Pflugfelder SC, De Paiva CS, Corrales RM, et al. (2006) Desiccating stress induces T cell-mediated Sjögren's Syndrome-like lacrimal keratoconjunctivitis. *J Immunol* 176: 3950-3957.
50. St-Jacques B, Ma W (2014) Peripheral prostaglandin E2 prolongs the sensitization of nociceptive dorsal root ganglion neurons possibly by facilitating the synthesis and anterograde axonal trafficking of EP4 receptors. *Experimental neurology* 261:354-366.
51. Stemkowski PL, Smith PA (2012) Sensory neurons, ion channels, inflammation and the onset of neuropathic pain. *Can J Neurol Sci* 39: 416-435.
52. Decorsis J, Saumet JL, Sommer P, Sigaudou-Roussel D, Fromy B (2014) Effect of ageing on tactile transduction processes. *Ageing Res Rev* 13: 90-99.
53. Yezierski RP (2012) The effects of age on pain sensitivity: preclinical studies. *Pain Med* 13 Suppl 2: 27-36.
54. Taguchi T, Ota H, Matsuda T, Murase S, Mizumura K (2010) Cutaneous C-fiber nociceptor responses and nociceptive behaviors in aged Sprague-Dawley rats. *Pain* 151: 771-782.
55. Namer B, Barta B, Ørstavik K, Schmidt R, Carr R, et al. (2009) Microneurographic assessment of C-fibre function in aged healthy subjects. *J Physiol* 587: 419-428.
56. Taguchi T, Mizumura K (2011) Augmented mechanical response of muscular thin-fiber receptors in aged rats recorded in vitro. *Eur J Pain* 15: 351-358.

57. Niederer RL, Perumal D, Sherwin T, McGhee CN (2007) Age-related differences in the normal human cornea: a laser scanning in vivo confocal microscopy study. *Br J Ophthalmol* 91: 1165-1169.
58. Gambato C, Longhin E, Catania AG, Lazzarini D, Parrozzani R, et al. (2014) Aging and corneal layers: an in vivo corneal confocal microscopy study. *Graefes's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*.
59. Erie JC, McLaren JW, Hodge DO, Bourne WM (2005) The effect of age on the corneal subbasal nerve plexus. *Cornea* 24: 705-709.
60. Dennis EL, Thompson PM (2014) Functional brain connectivity using fMRI in aging and Alzheimer's disease. *Neuropsychol Rev* 24: 49-62.
61. Kurose M, Meng ID (2013) Corneal dry-responsive neurons in the spinal trigeminal nucleus respond to innocuous cooling in the rat. *J Neurophysiol* 109: 2517-2522.
62. Sarantopoulos C (2013) Burning mouth syndrome: a misunderstood, underinvestigated, and undertreated clinical challenge. *Reg Anesth Pain Med* 38: 378-379.
63. Miller C, Utter ML, Beech J (2013) Evaluation of the effects of age and pituitary pars intermedia dysfunction on corneal sensitivity in horses. *Am J Vet Res* 74: 1030-1035.
64. Roszkowska AM, Colosi P, Ferreri FM, Galasso S (2004) Age-related modifications of corneal sensitivity. *Ophthalmologica Journal international d'ophtalmologie International journal of ophthalmology Zeitschrift fur Augenheilkunde* 218:350-355.
65. Golebiowski B, Papas EB, Stapleton F (2008) Factors affecting corneal and conjunctival sensitivity measurement. *Optometry and vision science : official publication of the American Academy of Optometry* 85:241-246.
66. Gonzalez A, Ehrmann K, Rowaan C (2014) Age-Related Corneal Sensitivity assessed with a modified BHVI-Belmonte Aesthesiometer: a preliminary study. *Association for Vision and Research in Ophthalmology meeting* 2014.
67. Acosta MC, Alfaro ML, Borrás F, Belmonte C, Gallar J (2006) Influence of age, gender and iris color on mechanical and chemical sensitivity of the cornea and conjunctiva. *Exp Eye Res* 83: 932-938.
68. Bourcier T, Acosta MC, Borderie V, Borrás F, Gallar J, et al. (2005) Decreased corneal sensitivity in patients with dry eye. *Invest Ophthalmol Vis Sci* 46: 2341-2345.