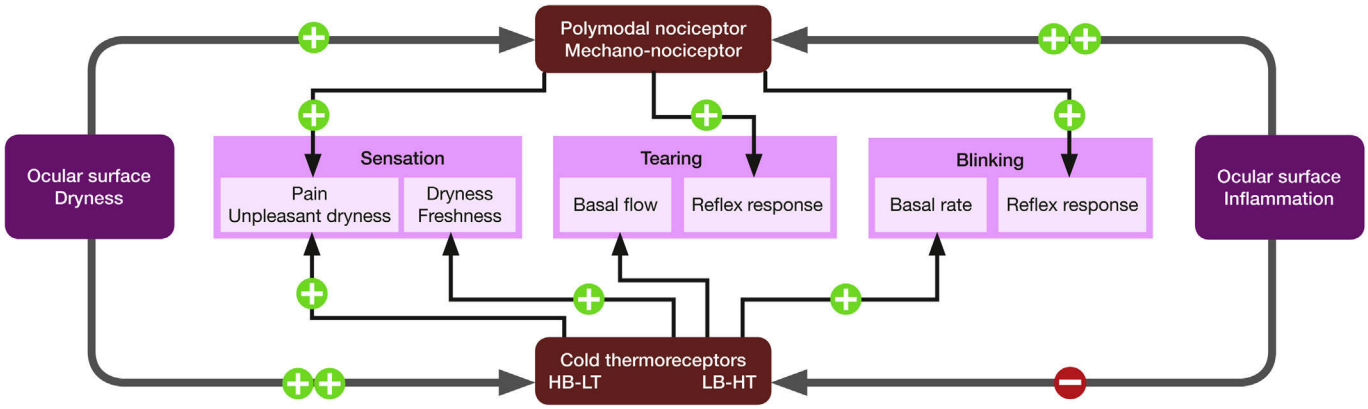

Fig. 5. Schematic diagram summarizing how ocular inflammation of various etiologies or ocular surface dryness in DED, provoke variable increases (+) or decreases (−) of nerve impulse activity in polymodal- and mechano-nociceptors and in cold thermoreceptors of the high background, low threshold (HB-LT) and low background, high threshold (LB-HT) types. Together these changes evoke conscious sensations of different quality, as well as changes in tear flow and in spontaneous and reflex blinking.

primary afferent fibers that bind to neurokinin and *N*-methyl-D-aspartate (NMDA) glutamate receptors on second-order neurons in the spinal dorsal horn, resulting in an increase neuronal excitability [338]. Sensitized spinal dorsal horn neurons project to higher brain centers and are necessary to engage thalamocortical and limbic pathways that underlie the discriminative and affective aspects of pain [384]. Considerable evidence suggests that second-order ocular neurons at the ViVc transition and VcC1 region behave, in many respects, as spinal dorsal horn neurons and are critical for the development of persistent ocular pain. In animal models for anterior uveitis [198] or photokeratitis [385], in which ocular inflammation is prominent, neurons at the VcC1 region, but not at the ViVc transition, develop hypersensitivity to ocular surface stimuli, while ocular neurons at both regions display increased convergent input from periocular skin, consistent with allodynia reported by some DED patients. Enlarged cutaneous receptive field areas of dorsal horn neurons after nerve injury or inflammation are thought to be due strictly to central brain mechanisms and to reflect spatial summation, a key component of central sensitization [386]. In a rat model for tear deficiency, ocular neurons at both the ViVc transition and VcC1 region display enhanced responsiveness to activation of ocular surface sensory neurons with hypertonic saline and enlarged convergent cutaneous receptive field areas [322]. The cellular and molecular mechanisms for hyperalgesia of ocular neurons at the ViVc transition and VcC1 region in models for DED are not known. Hypersensitivity of CNS neurons is thought to derive from a combination of increased excitatory synaptic drive and/or loss of inhibitory controls [5,338]. Central administration of a selective NMDA receptor antagonist reduces acute corneal-evoked activation of ocular neurons in both regions [387], while blockade of substance P receptors preferentially reduces activation of ocular neurons at the VcC1 region [388]. Local microinjection of the $GABA_A$ receptor agonist, muscimol, greatly reduces corneal-evoked neural activity at the ViVc transition and VcC1 region [208]. Even brief stimulation of sensory nerves at C fiber strength is sufficient to induce prolonged activation of microglia in spinal dorsal horn [389], however, similar studies have not assessed the role of microglia in animal models for ocular pain. Collectively, these studies suggest that second-order neurons at the ViVc transition and VcC1 region contribute to ocular-related hyperalgesia in addition to mediating more specialized aspects of ocular function such as lacrimation and eye blink. In future studies, it will be critical to determine how persistent tear reduction influences excitatory and inhibitory synaptic mechanisms of ocular neurons in the TBNC.

Central sensitization can develop in the setting of ongoing afferent nociceptive traffic that occurs with peripheral sensitization [390,391]. Anatomically, corneal nociceptors have their cell bodies in the TG and synapse in two main areas of the TBNC, the ViVc transition and the spinomedullary junction or the Vc/C1-2 region [100,392,393]. Dry-responsive neurons have been identified in the ViVc transition, and a subgroup of these neurons receive additional converging input from corneal afferents sensitive to other stimuli such as acidity, heat and noxious chemicals [100,190]. Cornea-responsive neurons at the ViVc transition and at the VcC1 region receive both innocuous and noxious sensory information and display increased responsiveness in an animal model for tear deficient DED [322]. This suggests that central sensitization occurs at multiple levels of the TBNC in DED.

On a molecular level, NMDA receptor activation may partially underlie the phenotypic changes seen in neurons during central sensitization. For example, NMDA receptor activation can lead to the progressive increase in the firing of second order neurons of the TBNC, even with sub-threshold noxious stimuli, clinically manifesting as hyperalgesia and allodynia [394]. *In vitro*, co-culture experiments have identified NMDA receptors as important in the communication between corneal epithelial cells and TG sensory neurons [395]. In a rat model for ocular nociception, NMDA receptors located on peripheral neurons or on postsynaptic neurons in the TBNC play a key role in transmission of nociceptive signals from the primary afferent neurons to central pain pathways [387].

Interactions between glial cells and neurons likely have an important role in the pathophysiology of chronic pain [396,397]. Preclinical studies have found that activated microglia and astrocytes mediate the generation and maintenance of several pain states [398] in a fashion modulated by specific genetic polymorphisms and circulating pro-inflammatory cytokines [399]. Glial activation in the brain as a consequence of stress (eg traumatic brain injury or systemic inflammatory responses) can induce the expression of pro-inflammatory cytokines that directly amplify spinal cord synaptic transmission and induce central sensitization to pain via signal amplification [397]. Peripheral and systemic inflammatory responses can also lead to microglial activation and depression via monoaminergic, glutamatergic and neurotrophic mechanisms [400].

5.3. Descending mechanisms

The activity in ascending excitatory nociceptive pathways is modulated by descending control pathways from higher brain centers that may exert facilitatory or inhibitory effects on spinal

and trigeminal sensory input [384,401]. However, the role of descending control systems in DED is not known. Normally, interneurons within the central pain pathway release neurotransmitters including gamma amino butyric acid (GABA) and glycine, which are involved in the inhibition of nociceptive signaling [402]. However, after a noxious insult, the ensuing inflammatory cascade in the spinal cord may reduce the GABA-mediated inhibitory influence on the ascending pathway or even make the GABA inputs excitatory [5]. In a rat model for ocular nociception, application of muscimol, a GABA receptor agonist, inhibited corneal input to both ViVc transition and VcC1 neurons [208]. A loss of the inhibitory GABA-mediated chloride current may allow for an upregulation of ascending pain pathway signals and thus a chronic neuropathic pain state [403,404].

Quantitative sensory testing can assess abnormalities in the ascending and descending pain pathways. Chronic pain patients (not involving the eye) often display greater temporal summation following repetitive presentations of a noxious stimulus ("wind up") [405,406] and reduced descending controls, or conditioned pain modulation, as compared to normal subjects [407].

5.4. Contribution of peripheral and central mechanisms to DED discomfort and pain

Peripheral and central neural mechanisms participate in the development of adverse symptoms of discomfort, dryness or burning pain in DED patients and their relief is the main reason for them to seek medical attention [404,408,409]. However, efforts to manage symptoms in chronic moderate to severe DED by ocular treatments alone have been inadequate [410,411]. Peripherally mediated DED pain or discomfort symptoms are presumed to originate by noxious stimulation of sensory neurons supplying the ocular surface (see Section 2.1). In DED, an inadequate or unstable tear film is the likely cause of tear hyperosmolarity and local surface drying leading to damage of the ocular surface tissues, including nerve terminals. Indeed, tear breakup is associated with increased sensation [141,412] and repeated episodes of tear breakup have been shown to lead to DED-like symptoms of ocular irritation [413]. Noxious stimulation arising from an inadequate tear film may also lead to inflammation which results in sensitization of the sensory terminals at the ocular surface (see Section 5.1), rendering previously non-noxious or low power stimuli able to evoke sensation [414]. If the underlying cause is not addressed and ameliorated, the increased activity of peripheral sensory neurons may lead to central sensitization. Although in most cases ocular surface pain has a proximate physical cause, it may be reported in the absence of tissue damage or any likely pathophysiological cause, but still should be accepted as pain [415]. Indeed, the weak correlation between signs and symptoms in DED [416–418] is consistent with the notion that sensitization of eye sensory pathways is triggered by events that may occur well before the patient enters the clinic. When peripheral nerve injury/inflammation due to disturbances of ocular surface homeostasis generates functional and anatomical alterations at higher levels of eye pain pathways, central pain largely independent of the original cause may develop and persist without an obvious relationship with the peripheral nociceptive input. Instillation of a topical anesthetic at the surface of the eye has been suggested as a simple and immediate way to differentiate pain arising from activation of peripheral sensory nerve terminals from that arising at a more proximal site in the sensory neuron or in the CNS [419].

Fig. 6 summarizes the peripheral and central neural mechanisms involved in the generation of perceptual, autonomic and motor responses in DED.

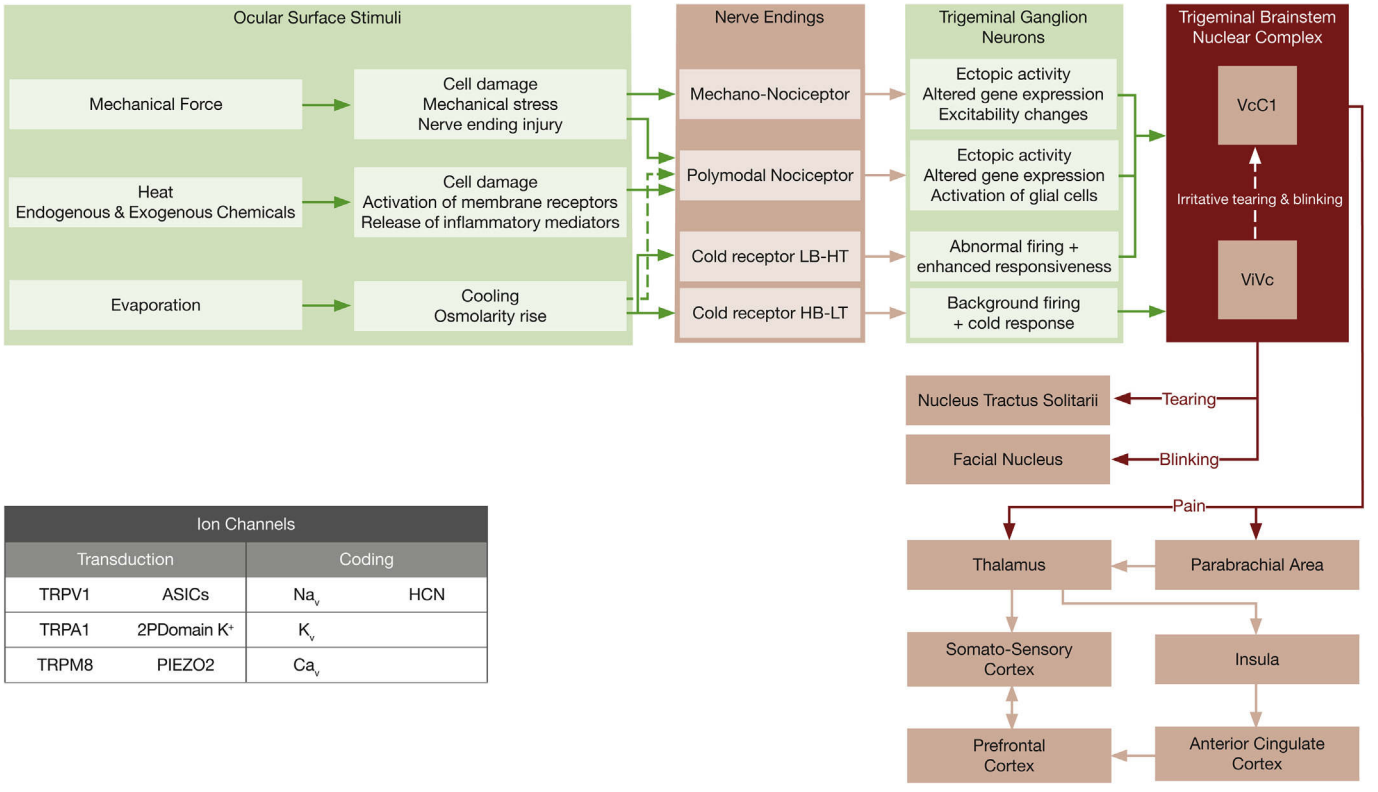

Fig. 6. Peripheral and central neural mechanisms involved in the sensory and autonomic responses evoked by ocular surface dryness. The main types of ion channels involved in the transduction and coding of mechanical, thermal and chemical stimuli are represented in the inset.

Clinical identification of the neurological mechanisms underlying pain is important in order to define therapies. This is required to distinguish nociceptive from neuropathic pain, whose definition has been restricted to lesion or disease affecting the somatosensory system [420]. Precise identification of the neural mechanism underlying discomfort or pain reported by patients with a diagnosis of DED is often difficult with the exploration tools available today to evaluate the neurobiology of the ocular surface and the functional state of central neural pathways involved in eye pain (see TFOS DEWS II Diagnostic Methodology report) [545].

6. Evaluation of ocular surface neurobiology

6.1. Patient-reported characteristics (surveys, questionnaires)

Numerous questionnaires have been developed for DED (see TFOS DEWS II Diagnostic Methodology and Epidemiology reports) [545, 546]. Most of these DED questionnaires were developed from a clinical perspective, aiming to understand the symptoms associated with the condition and develop diagnostic tools based on symptoms. Using this approach, the discomfort category of DED symptoms has been characterized as ocular dryness, irritation, soreness, grittiness, scratchiness or achiness, but may also contain questions about burning and stinging. Many DED questionnaires also include questions on foreign body sensation (feeling like 'something is in the eye') and light sensitivity [421–424]. Interestingly, dryness is not always queried in DED questionnaires [421]. It is also important to note that the words chosen as symptoms in these questionnaires depend heavily on language, which may take seemingly different meanings when translated. For example, "tired eyes" has been reported as the most common symptom of DED in Japan [425], but is included in only a few questionnaires in English [422,426].

Ocular pain and discomfort have also been measured by other questionnaires that were not specifically developed for DED. The National Eye Institute Visual Functioning Questionnaire (NEI VFQ-25) queries pain and discomfort around the eyes and vision-related quality of life, which is appropriate for the dry eye condition due to its effects on vision [427,428]. In one study, the ocular pain subscale of the NEI VFQ-25 was shown to be substantially lower (worse) for patients with DED than for eight other ophthalmic conditions [428]. The Eye Sensation Scale was developed to measure ocular pain, specifically to assess pain relief following corneal transplantation surgery [429]. Recently, a new questionnaire, the Ocular Pain Assessment Survey (OPAS), was validated to assess ocular pain in a variety of ocular conditions, from corneal ulcers to DED [430]. In addition to the eye, the OPAS queries the location of pain elsewhere in the body, supporting recent findings that DED symptoms closely align with non-ocular pain [409,431]. A grading system based on clinical judgement, to define the level of certainty that the pain in an individual is neuropathic in nature was proposed in 2008 by a Special Interest group of IASP [432] and revised recently [420]. This system distinguishes Possible, Probable and Definite Neuropathic Pain grades. An extension of these criteria to DED or other pain conditions in the eye has not been yet made, but may serve as a tool to distinguish definite ocular neuropathic pain from the discomfort and pain experienced in many eye pathologies.

6.2. Psychophysical characteristics

While corneal and conjunctival sensory function has been evaluated using electrophysiological recording in animals, this experimental approach cannot be used in humans. Consequently, the evaluation of subjective responses to controlled stimulation of the ocular surface has been applied to capture human ocular sensory information. The use of mechanical, chemical or thermal stimuli has been enabled by various esthesiometer designs, including the Cochet Bonnet esthesiometer and the gas jet esthesiometer [433–436].

Depending on the experimental or psychophysical paradigm and instrument used, different qualities of the sensitivity or sensation response can be determined. Measurement of detection thresholds is the most common method, allowing ease of comparison between conditions and analysis of change. Thresholds to mechanical, chemical and thermal stimuli have been measured using a variety of psychophysical techniques, including method of limits [437], method of constant stimuli [129] and staircasing techniques [438]. As with other sensory systems, which may show adaptation or sensitization, different psychophysical methods result in different threshold values. Discrepancies in threshold may also result from physiological variations in sensitivity, variations due to disease, as well as method-related variations including stimulus duration, distance of probe from the ocular surface and characteristics of the gas stimulus.

A number of investigators have also utilized subjective grading of suprathreshold stimuli in order to determine the relationship between the magnitude of the stimulus presented and its perceived intensity [63,64,317,435,439], and some have made observations of the quality and attributes of the evoked sensations [63,64,435,439–441]. Other suprathreshold approaches have included grading of just noticeable differences in sensation [442], threshold differences in sensation [443], matching detection thresholds to the discomfort experienced and grading the intensity or assigning a descriptor to suprathreshold stimuli. Sensations evoked can be evaluated quantitatively and qualitatively and compared with the receptor properties established in animal models [63], providing a clearer understanding of the processes involved in human ocular surface sensitivity.

Psychophysical studies have demonstrated that the eyelid margins are sensitive to mechanical stimuli [444–446], but responses to other types of stimuli have not been assessed. Careful assessment of the tactile sensitivity of the eyelid margins indicates that the occlusional surface has lower sensitivity than the marginal angle where the eyelid margin contacts the surface of the eye [445]. Studies also indicate that the tactile sensitivity of lower eyelid margin is higher than that of upper eyelid margin [445,446]. Interestingly, in healthy subjects, the tactile sensitivity of the lower eyelid margin was positively correlated with tear osmolarity [446], however, neither eyelid sensitivity nor tear osmolarity correlated with symptoms of DED.

6.2.1. Ocular surface sensitivity and DED

Table 1 summarizes studies that have evaluated ocular surface sensitivity in various populations of DED subjects, and the associations between sensitivity and symptoms reported. While subject numbers are frequently low in individual studies, corneal sensitivity to a pure mechanical stimulus, such as the Cochet Bonnet instrument, is consistently reduced in DED [372,447–453]. Of the different subclasses of DED patients, those presumed as demonstrating aqueous deficient DED consistently present with reduced corneal sensitivity using the Cochet Bonnet instrument, and this has been attributed to the greater corneal epithelial disruption assessed by corneal staining [454]. In Sjögren syndrome, the decrement in corneal sensitivity to mechanical stimuli was similarly associated with the degree of corneal staining [455]. In a cohort with DED defined by clinical signs and symptoms, the degree of reduction in corneal mechanical sensitivity was associated with severity of clinical signs, including tear film signs [453].

Some studies, particularly those using a gas jet aesthesiometer

Table 1
Corneal sensitivity and symptoms report in DED.

Author(s)/Year	Subjects	Sensitivity (with ↑symptoms)	Esthesiometer	Symptoms questionnaire
Xu et al. (1996) [447]	SS (n = 15) + DED (n = 44)	↓	COBO	not stated
Versura et al. (2007) [448]	SS (n = 66) + DED (n = 59)	↓	COBO	OSDI
Barboza et al. (2008) [449]	SS (n = 17)	↓	COBO	OSDI
Toker and Asfuroglu (2010) [450]	SS (n = 23) + DED (n = 14)	↓cornea + conjunctiva	COBO	OSDI
Bourcier et al. (2005) [466]	SS (n = 14) + DED (n = 30)	↓*	BGE	burning, itching, stinging
Benitez-del-Castillo et al. (2007) [460]	SS (n = 11) + DED (n = 10)	↓*	BGE	not stated
De Paiva and Pflugfelder (2004) [457]	DED (n = 20)	↑	modified BGE	11 items
Situ et al. (2008) [456]	DED (n = 43)	↑* cornea + conjunctiva	modified BGE (20 °C)	OSDI, SeSOD
Tuisku et al. (2008) [371]	SS (n = 20)	↑*	modified BGE	OSDI
Labbe et al. (2012) [452]	DED (n = 12)	↓	COBO	not measured
Kim et al. (2012) [451]	RA DE (n = 106)	↓	COBO	OSDI
Labbe et al. (2013) [372]	DED (n = 43)	↓	COBO	OSDI
Nepp and Wirth (2015) [453]	DED (n = 46)	↓	COBO	not measured
Rahman et al. (2015) [454]	MGD (n = 11) SS (n = 3) DED (n = 7) CC (n = 12)	↓ (DED only)	COBO and Jet aesthesiometer (28 °C)	OSDI, VAS
Spierer et al. (2016) [458]	DED (n = 129)	↑	modified BGE (23–26 °C)	DEQ-5, OSDI
Kaido et al. (2016) [462]	DED (n = 21)	No change in touch sensitivity ↑ pain sensitivity ↑ blink sensitivity	COBO	12 item questionnaire

Key: SS, Sjögren's Syndrome; DED, dry eye disease; BGE, Belmonte's gas esthesiometer; COBO, Cochet-Bonnet esthesiometer; MGD, meibomian gland dysfunction; OSDI, ocular surface disease index; CC, conjunctivochalasis.

with mechanical stimuli delivered either at eye temperature or at room temperature have shown an increased [371,456–458] or decreased [454,459,460] corneal sensitivity in DED. This apparent dichotomy could be related to the type of stimulus, where a gas jet aesthesiometer delivers a more complex stimulus than the pure mechanical stimulus of the Cochet Bonnet aesthesiometer; gas jet and contact aesthesiometers vary in stimulus composition and mode of stimulation and are therefore likely to assess different aspects of the neural response [461]. Aside from a mechanical stimulus, the gas jet stimulus may induce a cooling/evaporative/tear thinning effect that may stimulate cold thermoreceptors in addition to mechano-nociceptors and polymodal nociceptors, even with stimuli delivered at eye temperature. Given the likely relevance of corneal cold themoreceptors in DED, it is conceivable that augmented activity of these fibres in DED is associated with an increased corneal sensitivity to cooling stimuli.

An additional cause of the differences in corneal sensitivity among DED patients is that sensitivity may vary between DED subtypes or with disease severity. Recent studies have shown that patients exhibiting high DED symptom severity scores and neuropathic pain symptom inventory scores have lower mechanical thresholds and pain thresholds measured with a gas esthesiometer [458]. Also, in patients showing DED symptoms, touch sensitivity measured with the Cochet-Bonnet esthesiometer was not significantly altered, but the mechanical threshold required to evoke blinking and to report pain was lower than in asymptomatic subjects [462]. These observations suggest that in DED the reduction of corneal sensitivity, caused by damage to the sensory nerve endings, may be accompanied by central sensitization due to abnormal ongoing activity in injured corneal nerve fibers, trigeminal neurons and higher order neurons of the central ocular pain pathways leading to neuropathic pain symptoms. This mechanism may also underpin the report of eye discomfort symptoms in the presence of reduced corneal sensitivity to external stimuli. The relationship between corneal sensitivity in DED and disease severity is often confounded by a lack of knowledge of the time of disease onset [463]. For example, persistent ocular surface damage may ultimately cause central nervous system sensitization and the involvement of neuropathic mechanisms.

Corneal sensitivity has been discussed as a potential biomarker in DED [464]. One study has demonstrated an improvement in corneal sensitivity following cyclosporine therapy [450]. There are however concerns for the repeatability of corneal sensitivity measurements in DED over a three month period without intervention [453], although in normal subjects, good repeatability has been demonstrated [465].

6.2.2. Neuropathic pain

Neuropathic pain (neuralgia) is pain caused by damage or disease affecting the somatosensory nervous system and is often chronic in nature [467]. This pain can be associated with any part of the body including the eyes, but as it is not caused by the pathophysiology of DED (see TFOS DEWS II Pathophysiology report [544]), it should not be diagnosed as DED (see TFOS DEWS II Diagnostic Methodology report [545]). As commented above (Section 5.1.2) neuro-sensory dysfunction is a recognized feature of DED [404,468], but this aspect of the disease is not routinely evaluated or considered in clinical practice. DED-related neurosensory dysfunction may account for the lack of association between signs and symptoms and those DED patients who remain symptomatic despite adherence to therapy.

6.3. Objective metrics

6.3.1. Blink parameters

Blinking is commonly quantified by measuring the blink rate [315,320,321,323,329,331,469] or its reciprocal value, the interblink interval [318,324,333]. Neurologically, the blink rate is theoretically set by an endogenous spontaneous blink generator located in the spinal trigeminal complex that is modulated by afferent input from the cornea, dopamine levels in the brain and cognitive state [333]. (see Section 4).

It is well established that stimulation of the ocular surface leads to an increased blink rate [315,318], whereas surface anesthesia leads to a decreased blink rate [319]. This observation has been used to explain the increased blink rate in DED, presumably caused by ocular surface irritation due to surface dryness or an unstable tear film [196,333]. Experimental evidence in animals has shown

that chronic reduction of basal tearing produced by surgical removal of the main lacrimal gland increases background activity of corneal cold thermoreceptors [28]; conversely, basal blinking of TRPM8 null mice, whose cold thermoreptor background activity is absent, is very low [101]. These findings indicate that cold thermoreceptors contribute to the peripheral tonic drive maintaining basal blinking. Although corneal nerve terminals in DED patients are often reported to be less sensitive to external mechanical, thermal and chemical stimuli [455,459], this does not exclude the possibility that, in parallel, they display enhanced spontaneous firing (see Section 5.1.2). An increase in spontaneous activity may explain ongoing discomfort and an augmented basal blink rate in DED patients [28,196,333], while a low blink rate may be a causative factor in DED [470]. The Ocular Protection Index (OPI) is based on the idea that the blink rate can be too slow to compensate for more rapid tear breakup [471]. Thus, changes in the blink rate can be considered both a cause and an effect of DED.

Aside from blink rate, other blink parameters include the amplitude, duration and velocity of the upward and downward phases of the blink (see section 4). Regardless of whether the blinks are spontaneous, reflex or voluntary, all blinks show a similar pattern. The down phase is very rapid and the up phase is slower, with the maximum velocity of the down phase roughly double that of the up phase [314]. In a group of normal subjects, Evinger et al. [314] showed that the maximum velocity of the down and up phase show a linear relationship to blink amplitude, meaning that a fuller blink tends to be faster and vice versa. In addition, they found that the duration of most blinks changed little with blink amplitude.

However, some factors are known to alter blink duration. Wu et al. found that ocular surface stimulation by air tended to increase down phase blink duration and some subjects demonstrated cluster blinking [318]. This supports the hypothesis that ocular surface irritation can prolong blink duration and enhance blink excitability, perhaps for protective purposes [312]. However, concentration on a task descreased blink duration, presumably to minimize interruption of vision by the lid. In addition, an increased variability in the relationship between maximum blink velocity and amplitude for DED subjects has been reported, suggesting that individual subjects may respond differently [318].

Partial or low amplitude blinking is common and some studies have found that blink amplitude and blink rate decreased with concentration on a visual task [324,332]. Twenty-nine percent of blinks were partial or lower than 100% amplitude, but most (71%) covered the pupil, suggesting that blinks are necessary to wet the cornea over the pupil to provide a smooth tear film surface for good vision [317].

Blinking can be affected by several factors other than ocular surface stimulation or spontaneous firing of ocular surface nerves. The effect of cognitive input on the blink rate is marked, especially during a visual task, when the blink rate can markedly slow [316,320,321,331,472]. Blink rate can be altered by task, lighting, time of day and time period of data collection. Given the large percentage of the population that works on computers, this is likely to be a major cause of the increasing incidence of ergonomic dry eye complaints. Engaging in conversation and daydreaming can also affect blink rate [328–330], so that any clinical measure of blink rate should include information about task and lighting because blink rate varies so widely with mood and task. Time of day should also be included as blink rate is known to vary diurnally, presumably due to changing dopamine levels over the day [333]. Another aspect that affects measurement of the blink rate is the length of the time period of data collection. Kaminer et al. showed that shorter periods of data collection generated a higher blink rate than did longer periods, attributed to the increased probability of smaller inter-blink intervals when short periods of eye blink data were data collected [333].

6.3.2. *In vivo confocal microscopy of corneal nerves and immune cells*

With increased focus on corneal neurobiology in DED, there is an urgent need for the development of new biomarkers in this area. However, objective assessment of ocular surface neurobiology has been challenging for clinicians, given that corneal nerves cannot be visualized in detail by slit-lamp examination and that accurate functional tests are not widely available. Corneal IVCM, allows high-resolution *in vivo* visualization of subbasal corneal nerves and immune cells at a cellular level, providing an image resolution closer to the one obtained with histochemical methods. In particular, the Heidelberg Retina Tomograph with the Rostock Cornea Module (HRT/RCM, Heidelberg Engineering, Heidelberg, Germany), is a laser-scanning IVCM that uses a 670 nm diode laser [473], and allows real-time imaging of the cornea, generating a 400 × 400 μm images and a lateral resolution of 1μm/pixel. Recent studies demonstrated that there are no significant differences in the mean nerve and immune cell densities in the central cornea between representative standard IVCM images and wide-field composite images, confirming that standard images can be used in clinical studies to accurately assess cellular structures [474]. IVCM allows detection of changes in the subbasal nerve plexus in patients with corneal neuropathy and corneal neuralgia from DED or other ocular and systemic conditions, which can be monitored for disease severity and response to treatment [475,476].

6.3.2.1. Corneal nerves. There are several published qualitative and quantitative IVCM studies of the central corneal nerve plexus in patients with DED that attempt to elucidate alterations in corneal innervation and their clinical significance [477,478]. These studies have demonstrated rather conflicting results regarding nerve density. Most studies have reported a decrease in nerve density in both Sjögren and non-Sjögren DED patients [452,460,474,479], correlating to decrease in corneal sensitivity [372,447,452,460,480]. In contrast Hoşal et al. [481] and Tuominen et al. [368] observed no change in subbasal nerve density in DED patients compared with controls, while Zhang et al. reported increased corneal nerve density in patients with Sjögren's syndrome (Table 2) [482]. The latter study corresponds to studies showing hypersensitivity of the cornea [371,483]. The discrepancies in findings related to changes in nerve density may be attributed to either different stages and severity of DED that induce different degeneration/regeneration patterns of nerves, levels of inflammation, or levels of corneal hyperalgesia and allodynia after repeated insults to corneal nerves. More consistency has been shown regarding other morphological nerve parameters, such as increased tortuosity, reflectivity and beading [368,369,460,480,482,484,485]. These changes are believed to arise from initial damage and subsequent regeneration of subbasal corneal nerves.

Correlation of nerve alterations by IVCM to clinical signs and symptoms has been shown in several studies. Benitez et al. found that subbasal nerve density and corneal sensation correlated with Schirmer test results [460]. Further, Zhang et al. demonstrated that beading of corneal nerves was inversely related to corneal damage assessed with rose bengal staining [484]. Moreover, Labbe et al. revealed that both subbasal nerve density and corneal sensitivity were negatively correlated with the severity of DED [372]. Finally, a recent randomized clinical trial demonstrated that only patients with near-normal corneal nerve density showed improvements in both symptoms and signs after one-month therapy with artificial tears or the topical steroid, loteprednol, while patients with low corneal nerve density did not demonstrate changes in signs or symptoms, providing one possible explanation for the variability in

Table 2
Nerve morphology changes described in DED and Sjögren syndrome, and associations with sensitivity.

Author(s)/Year	Subjects	Control	Nerve morphology parameters					Association with sensitivity
			Density/number	Tortuosity	Branching	Beading	Other	
Tuominen et al., 2003 [368]	SS (n = 10)	normal (n = 10)	–	altered	–	–	↑sprouting	–
Benitez del Castillo et al., 2004, 2007 [369,460]	DED (n = 10) SS (n = 11)	normal (n = 20)	↓	↑	no Δ	↑	no Δ thickness, reflectivity	Sensitivity associated with density (BGE)
Zhang et al., 2005 [484]	DED (n = 30) SS (n = 8)	normal (n = 30)	no Δ (DED) ↑ (SS)	↑	↑	no Δ	no Δ thickness,	–
Hosal et al., 2005 [481]	DED (n = 6) SS (n = 10)	normal (n = 10)	no Δ	no Δ	–	–	no Δ thickness, reflectivity	–
Villani et al., 2007 [485]	SS (n = 35)	normal (n = 20)	↓	↑	–	–	–	Sensitivity associated with tortuosity (COBO)
ErDEDlyi et al., 2007 [370]	DED (n = 10)	normal (n = 10)	no Δ	–	no Δ	no Δ	–	–
Tuisku et al., 2008 [371]	SS (n = 20)	normal (n = 10)	no Δ	–	–	–	↑sprouting	No association (modified BGE)
Zhang et al., 2011 [482]	DED (n = 40)	normal (n = 20)	–	↑	–	–	Subbasal nerve rupture (moderate/severe DED)	–
Labbe et al., 2012 [452]	DED (n = 12)	normal (n = 10)	↓	no Δ	no Δ	no Δ	no Δ thickness, reflectivity	Sensitivity associated with density (COBO)

Key: SS, Sjögren Syndrome; DED, dry eye disease; BGE Belmonte esthesiometer; COBO, Cochet-Bonnet esthesiometer.

therapeutic response [474].

Corneal nerve injury due to inflammatory processes, followed by altered excitability in regenerated nerves [475,476,486], may result in the development of hyperalgesia or allodynia in patients with DED. These findings potentially explain the variability of their response to therapy and the different effects of DED on subbasal nerve density observed in various IVCM studies. In these patients, the formation of microneuromas, abrupt swellings of injured nerve endings formed during regeneration, is caused by sprouting from endbulbs [368,371,404].

A new and highly promising use for IVCM is the clinically challenging differentiation between DED-induced discomfort and light sensitivity from corneal neuralgia or photoallodynia in patients with corneal neuropathy, given their similar symptomatic presentation and potential clinical overlap [404,408,409,468]. Particularly in patients with ocular pain, with notoriously poor correlation between clinical signs and symptoms [417,487,488], IVCM may allow the diagnosis of corneal neuropathy with quantifiable changes in corneal subbasal nerve metrics [475,476,486]. IVCM in patients with corneal neuropathy demonstrates the presence of microneuromas, increased beading and reflectivity, as well as a more profound loss of subbasal nerves [475,476]. In recent studies, the treatment of patients with corneal neuropathy-induced photoallodynia or neuralgia with autologous serum tears demonstrated restoration of nerve topography through nerve regeneration, correlating with decreased symptoms of photoallodynia and pain scores [475,476]. While IVCM does not distinguish causality from secondary effects, additional IVCM studies in more homogenous populations would shed light on the pathophysiology of corneal neuropathic disease and DED. IVCM also shows promise in monitoring the corneal neurogenerative response to treatment. Methods to automate quantitative IVCM meausures would greatly enhance research methodology and interpretion of results.

6.3.2.2. Corneal immune cells. Recent studies show that inflammation plays a significant role in the development of DED [349]. One of the major participants of the immune system in DED are the APCs that induce T cell activation and thereby initiate an inflammatory cascade [489,490]. Among APCs, corneal DCs are involved in the development of DED [349,491,492]. To evaluate immune cell alterations in patients with DED, IVCM has recently been used to visualize DCs in the cornea. Results from these studies are consistent with immunohistochemical findings [493,494] showing that epithelial DCs are primarily located in close proximity to the subbasal nerve plexus [148].

Several IVCM studies have assessed the density and distribution of DCs and other immune cells in DED and demonstrated an increased density of DCs [371,477–480,495–500]. Lin et al. also showed that central and peripheral corneal DCs were significantly increased in both non-Sjögren and Sjögren syndrome DED, as compared to normal subjects [495]. Further, they showed putative activation of DCs as documented by the increased presence of dendrites on these cells. Similarly, increased density of purportedly mature DCs in DED patients with underlying systemic immune diseases has been reported [371,474]. Moreover, comparison between patients with presumed aqueous-deficient and evaporative DED showed that DC density is significantly higher in aqueous-deficient DED [474].

Alterations of epithelial DC density correlate with clinical signs and symptoms of DED [499,501]. Thus IVCM may serve as a useful supplementary assessment tool for clinical diagnosis of DED and for determining the need for anti-inflammatory therapy. Further, IVCM can be used serially to objectively assess the therapeutic success of an anti-inflammatory therapy [499,501]. However, additional studies are required to validate utility of IVCM imaging of DC in clinical practice, including the development of analytical tools to automate and standardize image analysis. Evaluation of DCs could also be used for treatment stratification and measurement of therapeutic efficacy when used with clinical tests (see TFOS DEWS II Management report) [547].

6.3.3. Biomarkers in tears

Biomarkers in tears can potentially be used as an indicator of the status of ocular surface innervation, DED severity or as a measure of disease progression or response to treatment.

6.3.3.1. Nerve growth factor. NGF and its receptors are upregulated following damage to the ocular surface or its innervation [502,503] and the levels return to normal following wound healing [504]. Tear levels of NGF are elevated immediately post-laser-assisted in situ keratomileusis (LASIK) and remain increased until at least 6 months post-surgery [505]. Table 3 shows the levels of NGF in tears are also elevated in non-Sjögren DED [506] and in contact lens related DED [507].

Table 3
Changes in tear concentration of neuromediators after refractive surgery and with DED compared with normal eyes.

	Tear neuropeptide levels				
	Nerve Growth Factor (NGF)	Substance P	CGRP	Neuropeptide Y	Vasoactive intestinal peptide
Refractive surgery	Increased with PRK > LASIK Mean NGF/total tear protein PRK 89.2.8 ± 10.2 pg/μg, LASIK 55.4 ± 11.7 pg/μg at 1 month [505]	Increased after LASIK 9.6 ± 2.6 ng/ml at 1 month [516]	Increased with PRK 377 ± 83.2 ng/ml at day 7 [517] No change with LASIK 93.7 ± 59.6/ml [516]	n/a	n/a
Non-Sjögren syndrome	Increased 186.5 ± 64.8 pg/ml [507,518]	No change [518]	Reduced 3.0 ± 1.7 ng/ml [518]	No change 4.6 ± 3.9 ng/ml [518]	No change [518]
Sjögren syndrome	No change 54.5 ± 61.8 pg/ml [518]	No change [518]	No change 6.0 ± 2.4 ng/ml [518]	Reduced 1.5 ± 0.3 ng/ml [518]	No change [518]
Ocular cicatrizing pemphigoid	Increased 120.8 ± 53.3 pg/ml [518]	Not reported [518]	Reduced 2.3 ± 1.2 ng/ml [518]	Reduced 1.5 ± 2.0 ng/ml [518]	No change [518]
Normal eyes	Mean 64.7 ± 48.0 pg/ml [518] Mean NGF/total tear protein 32.8 ± 6.2 pg/μg [505]	Mean 2300 pg/ml [518] Range 306-332 7.5 ± 1.7 ng/ml [516]	77.4 ± 75.7 ng/ml [516] 6.0 ± 2.2 ng/ml [518] 198 ± 36.6 ng/ml [517]	4.3 ± 1.9 ng/ml [518]	Not reported [518]

CGRP, calcitonin gene-related peptide; PRK, photorefractive keratectomy; LASIK, laser in situ keratomileusis.

6.3.3.2. Substance P and calcitonin gene-related peptide. A substantial percentage of the sensory neurons supplying the ocular surface contain neuropeptides including substance P, CGRP and galanin [19,56,78,508]. These neuropeptides modulate epithelial and immune cell function in normal and damaged cornea [56], and play a role in local inflammation, wound healing and in the initiation and maintenance of pain (see Section 2.4) [509]. At the ocular surface specifically, CGRP induces epithelial cell differentiation and substance P stimulates epithelial cell proliferation [86].

One study has shown that tear levels of CGRP are reduced in DED patients and that tear CGRP levels are inversely associated with DED severity, corneal fluorescein staining and Schirmer test results (shown in Table 3) [506]. Exogenously delivered CGRP facilitates corneal epithelium repair *in vivo* in animals and *in vitro* in cell culture models [510,511], which may be consistent with reduced tear levels and increased ocular surface damage in DED. There are no reported studies in humans that have evaluated the effects of exogenous CRGP on nerve morphology, corneal sensitivity or DED.

In contrast, the role of substance P in human DED is equivocal. As can be seen in Table 3, there are a small number of studies available with varying methodology and generally small sample sizes. No studies have determined relationships between tear levels of substance P and disease severity. The level of substance P in tears appears to be unchanged in both Sjögren and non-Sjögren DED [506], but it is reduced in patients with corneal hyperesthesia [512], compared to those with normal corneal sensitivity [506,513,514].

One study has investigated the levels of VIP and NPY in tears of DED patients and found no differences in VIP levels, but reduced levels of NPY compared to those of subjects with normal eyes [506]. NPY is released from sympathetic nerves and it is not known whether the changes in tear NPY levels reflect changes in the lacrimal gland (or other glandular tissues contributing the tears) or arise as a consequence of ocular surface damage. NPY inhibits T cell type I-driven inflammatory responses [515]. The lack of change in VIP with DED may reflect the very limited innervation of the cornea by VIP-expressing parasympathetic nerves [511].

There are other potential tear markers of inflammation and/or ocular surface demage that have not yet been assessed in DED. Tear levels of TNF-α, transforming growth factor (TGF-β), VEGF and hepatocyte growth factor (HGF) are increased immediately after surface refractive surgery procedures and decline thereafter [519–521]. These biomarkers are all likely to be relevant in wound healing, and VEGF in particular may be relevant for corneal reinnervation as sequestration of this growth factor reduced recovery of subbasal fibers in a mouse model of corneal injury [522].

7. Future directions

Understanding the characteristics of adverse sensations associated with the ocular surface is important since symptom presentation is often the driving force behind treatment in DED [523]. Research to date has uncovered the complexity of peripheral and central neural mechanisms associated with ocular surface sensations and tissue homeostasis; however, a thorough understanding of this complexity in relation to DED is still not fully achieved. Application to the eye, of the technological and conceptual advances to the understanding of pain made at the genetic, molecular, cellular and integrative level in other tissue pathologies, could help extend our knowledge of neural mechanisms underlying DED dysesthesias. Also, development of adequate animal models aimed at reproducing this pathology, and analyzing experimentally integrative neural mechanisms, will help to refine the present knowledge on how unpleasant sensations originate in DED. New instruments and procedures designed to quantify eye sensations and pain need to be developed both in experimental animals and in humans, to correlate experimental and clinical data and to obtain reliable, objective information on the psychophysical parameters of normal and pathological sensations.

A second area of research important in furthering the understanding of pain and sensation at the ocular surface is the definition and characterization of pain, referred to the eye, that has a neuropathic origin. The lack of correlation between signs and symptoms of DED has made research and clinical practice challenging for many [417]. Differentiating DED-evoked nociceptive pain from peripheral and central neuropathic ocular pain is important in successful treatment of patients and in defining research approaches. The investigation of these questions using basic research technology will help promote better understanding of the molecular and cellular modifications taking place in the peripheral and central ocular pain pathways in DED and how they develop, progress and eventually perpetuate. From a clinical perspective,

neuropathic pain from a wide range of etiologies has been studied in large samples of patients collected in multinational pain research networks, and classified according to their intrinsic pain-related sensory symptoms and signs which were associated with pathophysiological mechanisms [420,524]. To extend this approach to neuropathic pain associated with ocular sensory pathways could help to extend the advances in diagnosis and therapy, made for neuropathic pain affecting other body territories, to eye pain.

Neuropathic pain should not be diagnosed as DED (See section 6.2.2), but management when it manifests as dry eye symptoms, needs further research. For patients who report pain in multiple body parts as well as the eye, management of or referral for neuropathic pain interventions should be evoked such as neuropathic pain therapy [408,419], a diet rich in anti-oxidants [525,526], systemic pharmacological agents traditionally used to manage pain (such as anticonvulsants, tricyclic antidepressants, opioids) [486,527,528,530] topical analgesia [531], neuromodulators (such as diclofenac, gabapentin and pregabalin) [528,532–540], GABAergics in late stage pain [529], and using stimulation therapies and psychological treatments (including exercise, acupuncture, “scrambler” or peripheral stimulation therapy, transcranial magnetic stimulation, transcranial direct current stimulation and cognitive behavior therapy) [541–543]. These have mostly not been investigated specifically for ocular neuropathic pain, but there is evidence of effectiveness in neuropathic pain and/or chronic pain syndromes, so further research is warranted.

8. Summary

The TFOS DEWS II Pain and Sensation Subcommittee report provides a perspective of DED focused on pain. Pain can be divided into nociceptive and neuropathic pain. Nociceptive pain occurs in response to actual or threatened damage to tissues. However, neuropathic pain occurs due to a lesion within the somatosensory nervous system and does not have biological value.

Pain associated with DED is transmitted via the peripheral axons of TG neurons innervating the cornea and conjunctiva. They form a subepithelial nerve plexus in the stroma whose ascending branches penetrate Bowman's layer and ramify extensively to terminate within the surface epithelium layers. Functionally, corneal sensory neurons can be classified as polymodal nociceptors, specific mechano-nociceptors or cold thermoreceptor neurons. Polymodal nociceptors are normally silent and respond to chemical, mechanical and thermal stimuli. They become sensitized by the inflammatory mediators released by ocular surface injury. TRPV1 channels are important for sensory transduction and sensitization of polymodal nociceptors. Mechano-nociceptors are normally silent at rest, and respond only to mechanical forces through mechanosensitive ion channels such as Piezo2. Most cold thermoreceptors discharge continuously at tnormal eye surface temperature with an increase or decreasing the firing frequency upon cooling or warming, respectively. TRPM8 is the main transduction channel for cooling or cold, and is also sensitive to changes in osmolarity. Inter-blink tear evaporation causes discrete cooling of the ocular surface and tear osmolarity rises, thereby augmenting basal activity of cold thermoreceptors. This is consistent with the hypothesis that cold-sensitive fibers contribute to the reflex control of basal tear production and blinking (Figs. 5 and 6).

The TG neurons that supply the ocular surface project primarily into two spatially discrete regions within the TBNC: the transition region between caudal Vi and Vc (ViVc transition) and at the Vc/upper cervical cord junction (VcC1 region). Evidence suggests that the VcC1 region plays a dominant role in sensory-discriminative aspects of ocular pain. ViVc transition and VcC1 neurons are excited by bright light whereas only ViVc transition neurons are activated by changes in the moisture status of the ocular surface. Ocular neurons at the ViVc transition are more likely to project to brain regions that control lacrimation (superior salivatory nucleus) and eye blink (facial motor nucleus), while cornea-responsive neurons in both regions project to the sensory thalamus. Thus, it is suggested that ocular neurons at the ViVc transition play a significant role in maintaining ocular surface homeostasis, while neurons at VcC1 may be more concerned with the expression of adverse symptoms (Fig. 6).

Autonomic sympathetic and parasympathetic nerves, whose activity is regulated by reflex influences from sensory neurons supplying the ocular surface, regulate the secretory activity of the main lacrimal gland. Parasympathetic innervation of the main lacrimal gland is extensive, while little is known about the neural control of accessory lacrimal glands. While nerves are present around the meibomian glands, there are no studies examining the role of sensory or autonomic nerves and their neurotransmitters in regulating the holocrine secretion of the meibomian gland. Activation of sensory nerves supplying the rat cornea evokes goblet cell mucous secretion; however efferent nerve type(s) involved in this reflex response remain to be established. Several non-neural processes regulate the release of mucins from stratified squamous cells, but to date no regulatory role for nerves or neurotransmitters has been identified.

In addition to regulation of tear production, ocular surface nerves mediating sensations contribute to blinking behavior. It has been suggested that spontaneous blinking is maintained, at least in part by the continuous nerve impulse firing of eye surface cold thermoreceptors, an effect likely mediated by the connections of TG neurons with brainstem ViVc neurons which in turn project to the facial motoneurons (Cranial nerve VII). Nociceptive sensory input to neurons at the VcC1 region initiates reflex eye blinking and, through their projections to ViVc transition neurons, sets the blink amplitude and peak velocity of corneal reflex blinks.

In DED, reduced tear secretion leaves the corneal epithelium exposed to adverse environmental conditions that may result in inflammation of the ocular surface and peripheral nerve damage. Inflammation may sensitize polymodal nociceptors and mechano-nociceptors, while depressing cold thermoreceptor activity. However, in experimental models of DED, sensitization of nociceptor fibers is minor, whereas a prominent and abnormal increase in cold thermoreceptor nerve activity occurs that parallels the morphological changes in corneal innervation. In trigeminal brainstem, ocular surface-responsive neurons at both ViVc and VcC1 regions display enhanced excitability.

Several questionnaires are available to assess pain and sensation associated with DED. These questionnaires vary widely in wording, the symptoms investigated, and scaling. In addition to questionnaires, esthesiometry can be used to assess the functional status of the corneal nerves.

IVCM allows for visualization of nerves and inflammatory cells in the corneal surface. DED is associated with morphological abnormalities in nerve terminals, such as increased tortuosity, reflectivity and beading, while changes in nerve density are not consistent. In addition, an increased density of inflammatory cells in DED has been reported. Tear components may also help to objectively assess DED. NGF is increased in DED while CGRP is reduced. Substance P, neuropeptide Y and VIP appear to be unchanged.

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