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The role of corneal afferent neurons in regulating tears under normal and dry eye conditions

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Abstract

The cornea is one of several orofacial structures requiring glandular secretion for proper lubrication. Glandular secretion is regulated through a neural reflex initiated by trigeminal primary afferent neurons innervating the corneal epithelium. Corneal sensory afferents must respond to irritating and potentially damaging stimuli, as well as drying that occurs with evaporation of the tear film, and the physiological properties of corneal afferents are consistent with these requirements. Polymodal neurons are sensitive to noxious mechanical, thermal and chemical stimuli, mechanoreceptive neurons are selectively activated by mechanical stimuli, and cool cells respond to innocuous cooling. The central terminations of corneal primary afferents are located within two regions of the spinal trigeminal nucleus. The more rostral region, located at the transition between the trigeminal subnucleus caudalis and interpolaris, represents a critical relay for the regulation of the lacrimation reflex. From this region, major control of lacrimation is carried through projections to preganglionic parasympathetic neurons located in or around the superior salivatory nucleus. Dry eye syndrome may be caused by a dysfunction in the tear secreting glands themselves or in the neuronal circuit regulating these glands. Furthermore, the dry eye condition itself may modify the properties of corneal afferents and affect their ability to regulate secretion, a possibility just now being explored.

Keywords

dry eye; trigeminal ganglion; trigeminal nucleus; cornea; lacrimation

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1. Overview

The cornea requires constant secretion from multiple glands to provide lubrication, nourishment and a protective barrier to the external environment (Dartt, 2009). Although the secretory fluids from each gland vary with regards to composition, among the common constituents of glandular secretion are water, electrolytes, mucins, and other glycoproteins and proteins. Sensory innervation of the cornea is necessary to detect environmental stressors and, through brainstem circuits, regulate the flow of glandular secretion.

Neural innervation of the cornea is provided by the ophthalmic branch of the trigeminal nerve, the general sensory cranial nerve innervating the head and face. Glandular secretion to several cephalic structures, including the cornea, intraoral, and intranasal regions, are regulated through primary afferent projections to the spinal trigeminal nucleus (Vsp), where information is processed and relayed to preganglionic parasympathetic neurons located in and around the superior and inferior salivatory nuclei (Ishizuka and Murakami, 1986; Murakami et al., 1983; Murakami et al., 1982; Toth et al., 1999). The production of watery tears is through the activation of postganglionic parasympathetic neurons located in the pterygopalatine ganglion that terminate in the lacrimal gland (Dartt, 2009; Ruskell, 1971). In addition, conjunctival goblet cells, the main source of mucins, are innervated and regulated by the pterygopalatine ganglion (Dartt, 2004; Dartt et al., 1995; Kanno et al., 2003; Kessler and Dartt, 1994; Kessler et al., 1995). Preganglionic parasympathetic denervation decreases tear flow through diminished aqueous tear secretion as well as a reduction in goblet cell density (Toshida et al., 2007). Furthermore, the activation of corneal primary afferent neurons increases both aqueous and mucin secretion (Kessler et al., 1995; Yasui et al., 1997). Conjunctival and corneal epithelial cells can also modify tear composition through the secretion and absorption of electroytes and water. Fluid secretion from conjunctival epithelial cells appears to be increased primarily by sympathetic rather than parasympathetic agonists (Dartt, 2004; Kompella et al., 1996; Rios et al., 1999; Shi and Candia, 1995). Less is known regarding the neural regulation of meibomian glands, which are the main source of lipid content in tears. Meibomian glands receive parasympathetic innervation, however the significance of this innervation in their regulation remains unclear (LeDoux et al., 2001).

Primary afferent neurons innervating the cornea are able to sense whether the ocular surface is exposed to damaging or potentially damaging stimuli and evoke protective autonomic and somatic reflexes, including lacrimation and blinking, as well as irritating and painful sensations (Figure 1) (Belmonte et al., 2004b; Evinger et al., 2002; Pellegrini et al., 1995). The cornea, like other specialized tissues of the cephalic region innervated by the trigeminal nerve, is represented in multiple regions of Vsp that serve to carry out these different functions (Bereiter et al., 2000b). Lacrimation is regulated by a specialized region located in rostral Vsp, whereas other nociceptive specific functions can be attributed to more caudal regions (Bereiter et al., 2000b; Hirata et al., 2004; Meng et al., 1997).

Increases in glandular secretion are often accompanied by pain or irritation and associated reflexes. However, most secretion, particularly that precipitated by the evaporation of water from the cornea, occurs without an accompanying conscious perception. Indeed evidence indicates that basal secretion and secretion evoked by noxious stimulation of the cornea are

driven by unique, but possibly overlapping, brainstem reflex arcs (Figure 2) (Hirata and Meng, 2010; Hirata et al., 2004; Kurose and Meng, 2013a; Parra et al., 2010; Robbins et al., 2012).

Inadequate or altered tear film on the anterior ocular surface can result in dry eye syndrome (DES), a significant clinical problem affecting up to 38% of the population over the age of 50 (2007). Dry eye can result in sensations of dryness, grittyness, irritation, and burning pain, and in severe cases corneal lesions and infection can occur (2007). The causes of DES are not fully elucidated but may include inflammation and dysfunction of the lacrimal glands, resulting in aqueous tear deficiency, the meibomian glands, resulting in insufficient lipid content, or the conjunctival goblet cells, resulting in diminished mucin secretion (Javadi and Feizi, 2011; Mantelli and Argueso, 2008; Mathers, 2000; Sullivan et al., 1999). Alternatively, DES may be caused by an inability of sensory neurons in the tear reflex arc to properly regulate tearing (Dartt, 2009; Mathers, 2000; van Bijsterveld et al., 2003). In particular, dry eye associated with LASIK surgery, aging, and diabetes mellitus may involve a disruption in afferent drive, as dry eye symptoms may be accompanied by a decrease in corneal sensitivity (Ambrosio et al., 2008; De Paiva et al., 2006; Gallar et al., 2004; Konomi et al., 2008; Tavakoli et al., 2007).

The remaining sections will examine the properties of trigeminal primary afferent neurons innervating the cornea under normal and dry eye conditions, and examine the central pathways involved in controlling lacrimation. Dry eye induced alterations in the properties of corneal afferent neurons and the central processing of corneal input may have significant consequences for both the regulation of tearing and ocular pain. Understanding these changes are crucial to the development of novel interventions for the treatment of DES.

2. Cornea primary afferent neurons

The cornea is the most densely innervated tissue in the body and, based on their conduction velocity, is exclusively innervated by A-delta and C primary afferent fibers (Muller et al., 2003; Zander and Weddell, 1951). Afferent nerve bundles innervating the cornea enter the stroma from the periphery, branching to form a midstromal plexus (Marfurt et al., 2010). Continuing to branch, nerves penetrate Bowman’s layer to form the subbasal nerve plexus. In order to maintain the transparency of the cornea, the lightly myelinated A-delta corneal afferents lose their myelin sheaths as the nerve bundles enter the corneal limbus (Marfurt et al., 2010).

Corneal nerve endings originiating from the subbasal nerve plexus penetrate into all layers of the corneal epithelium and respond to multiple stimulus modalities, including mechanical, chemical and thermal stimuli (Belmonte et al., 1991; Belmonte and Giraldez, 1981; Gallar et al., 1993; Hirata and Meng, 2010; MacIver and Tanelian, 1993a; MacIver and Tanelian, 1993b; Parra et al., 2010; Robbins et al., 2012). Although irritation and pain are the primary, and possibly only, sensations evoked by stimulation of the cornea, not all sensory neurons that innervate the cornea should be considered nociceptors (Acosta et al., 2001b; Beuerman and Tanelian, 1979; Kenshalo, 1960). As evidence, innocuous stimulation of the cornea with

mild cooling or menthol activates corneal afferents and induces tearing without causing irritation or pain (Parra et al., 2010; Robbins et al., 2012).

Similar to other primary afferent neurons, corneal afferents store and release the excitatory amino acid neurotransmitter glutamate (Hegarty et al., 2010). Approximately half of these neurons are peptidergic nocicptors containing substance P and/or calcitonin gene-related peptide (CGRP) (Felipe et al., 1999; Jones and Marfurt, 1998). In addition to their role in relaying sensory information to the central nervous system, corneal afferent neurons provide neurotrophic factors important for wound healing and the general health of the cornea. Both substance P, in combination with insulin-like growth factor-1, and CGRP have been shown to increase the rate of corneal wound healing (Mikulec and Tanelian, 1996; Nakamura et al., 1997). Furthermore, denervation of the cornea leads to corneal degeneration indicated by stromal thinning and perforation of the corneal epithelium, which may be due to a reduction in corneal epithelial stem cells (Ferrari et al., 2011; Ferrari et al., 2013; Ueno et al., 2012).

With nerves that terminate as free endings, the properties of corneal afferent neurons depend on the expression of channels that transduce mechanical, thermal and chemical stimuli into electrical potentials (Figure 2), although the importance of corneal epithelial cells in the responses properties of corneal afferent neurons should not be discounted (Pan et al., 2011b). In many instances, the transduction process involves the opening of transient receptor potential (TRP) channels (Huang et al., 2006). There are approximately 30 members in the mammalian TRP family, nine of which are thermosensitive cation channels (TRPV1-4, TRPM2-4, 8, and TRPA1) expressed on sensory primary afferent neurons. In the nerve terminals, opening of cation channels allows for the influx of sodium and calcium ions. This influx produces membrane depolarization, activation of voltage gated sodium channels, and the initiation of action potentials.

Of particular importance in nociceptive transmission, TRPV1 is opened by noxious heat stimulation (Caterina et al., 2000; Tominaga et al., 1998). Also physiologically relevant is the sensitivity of TRPV1 to low pH and capsaicin, the active ingredient in hot chili peppers. TRPV1 is not the only channel responsive to low pH, as acid sensing ion channels (ASICs) are also found on trigeminal sensory neurons, often co-expressed with TRPV1 (Ugawa et al., 2005a; Ugawa et al., 2005b). This combination of thermal and chemosensitivity found in TRPV1 channels is a property shared by TRPA1 and TRPM8 channels (Bandell et al., 2006; Bautista et al., 2007; de la Pena et al., 2005; Jordt et al., 2004; Madrid et al., 2006). TRPA1 channels are activated by noxious cold and allyl isothiocynate, the pungent chemical in mustard oil and wasabi, and TRPM8 channels are activated by non-noxious cooling and menthol. In addition, potassium channels such as members of the KCNK channel family may be involved in cold transduction (Thut et al., 2003). A reduction in potassium conductance would result in membrane depolarization and increased action potential generation.

Based primarily on *in vivo* electrophysiological recordings performed in cats, guinea pigs, and rats, corneal primary afferents fall into three main categories: mechanoreceptors, polymodal receptors, and cold receptors (Belmonte et al., 2004b). Corneal mechanoreceptors respond exclusively to mechanical stimulation, polymodal receptors are

activated by noxious thermal and chemical stimuli as well as mechanical stimulation, and cold receptors respond to innocuous cooling. The overall composition of corneal afferents has been estimated as 70% polymodal nociceptors, 20% mechanoreceptors, and 10% cold receptors (Belmonte et al., 2004a). However, the true representation of these different afferent populations is unknown as these values depend on the sampling methods used in the various studies.

Polymodal nociceptors innervating the cornea express several different thermal and chemosensitive channels, most from the TRP family, that account for their physiological properties (Figure 2). Corneal polymodal nociceptors have been characterized that respond to noxious thermal stimulation and low pH, indicating the presence of TRPV1 and ASICs (Belmonte et al., 1991; Belmonte and Giraldez, 1981; Gallar et al., 1993). The response to acidity has been exploited with the use of CO_2 stimulation in human and animal studies. CO_2 pulses directed at the cornea activate polymodal corneal afferent neurons in animal studies, and produce irritation and lacrimation in humans (Acosta et al., 2004; Belmonte et al., 1999; Chen et al., 1995).

Unlike polymodal nociceptors, less is known regarding the transduction mechanism for corneal mechanoreceptive neurons (Figure 2). Several putative mechanotransduction molecules have been proposed for nociceptive neurons with free nerve endings, such as TRPA1, TRPV4, ASICs, and members of the KCNK family (Dubin and Patapoutian, 2010; Hu et al., 2006; Story and Gereau, 2006). However, the sensitivity of these channels to noxious chemicals, low pH, or thermal stimuli indicates that they are more likely to be relevant to mechanotransduction mechanisms in polymodal nociceptors rather than mechanoreceptive neurons. Additional candidates for mechanotransduction proteins include piezo proteins, recently identified as a unique class of cation channels, and epithelial sodium channels, ENaC (Garcia-Anoveros et al., 2001; Hao et al., 2013). Further study is needed to determine the role of these channels in corneal mechanotransduction.

Cold receptors represent a distinct population of corneal sensory neurons that respond to moderate, non-noxious reductions in temperature (Figure 2). Similar to cutaneous cold receptors, corneal cold receptors respond vigorously to cooling of the cornea and, when the cornea is warmed, activity is suppressed (Belmonte and Gallar, 2011; Brock et al., 2001; Carr and Brock, 2002; Carr et al., 2003; Gallar et al., 1993; Hirata and Meng, 2010; Madrid et al., 2006). In contrast to polymodal and mechanoreceptive neurons, cold sensitive neurons have relatively high ongoing activity at room temperature. Menthol, a TRPM8 channel agonist, is a potent activator of corneal cold receptors (Hirata and Meng, 2010; Kurose and Meng, 2013b; Madrid et al., 2006; Robbins et al., 2012).

In a property particularly relevant to the cornea, both polymodal and cold receptors are activated by hyperosmotic stimuli (Gallar et al., 1993; Hirata and Meng, 2010). The membrane channel responsible for the response to hyperosmotic stimuli is presently unknown, but may involve the expression of a TRPV1 splice variant (Pan et al., 2011a; Sharif Naeini et al., 2006). Thus, continuous evaporation of tears from the ocular surface causes cooling and increased tear osmolarity, ideal conditions for activating cold receptors. This sensitivity to mild cooling and hypertonicity would explain the previously described

response of cold receptors to drying of the ocular surface (Hirata and Meng, 2010; Hirata et al., 2004).

Based on the properties of each subtype of corneal primary afferent neuron, it is possible to infer their distinct role in lacrimation. Mechanoreceptive neurons are often preferentially activated by movement tangential to the corneal surface rather than by punctate stimuli (MacIver and Tanelian, 1993a; MacIver and Tanelian, 1993b), suggesting that activation of mechanoreceptors in the cornea induces lacrimation and blinking to clear particulate matter from the eye (Acosta et al., 2004; Belmonte et al., 2004b). Polymodal nociceptors promote similar reflexes vital to protecting the corneal epithelium, typically in response to noxious thermal or chemical stimuli. This activation of mechanoreceptors and polymodal nociceptors by mechanical, chemical, and thermal stimuli induces glandular secretion while also producing the sensation of irritation or pain (Acosta et al., 2001a; Acosta et al., 2004; Belmonte et al., 2004b).

In contrast to mechanoreceptors and polymodal receptors, cold receptor activation appears to evoke secretion that is not accompanied by ocular pain. As evidence, cold receptors are the only known corneal primary afferent neuron with spontaneous activity at room temperature (Gallar et al., 1993). Diminished tearing observed after application of a topical analgesic to the corneal surface demonstrates the importance of this corneal afferent neural activity in tear production (Herreras et al., 1997). Furthermore, activation of cold receptors with cold room air increases lacrimation in human subjects (Parra et al., 2010). In animal studies, application of menthol to the ocular surface increases tearing in a TRPM8-dependent fashion, yet fails to elicit nociceptive behaviors (Robbins et al., 2012).

Each class of primary afferent neuron has the potential to induce tear secretion by responding to different environmental stimuli. Tear composition, however, may differ depending on the class of primary afferent neuron that is activated, a possibility that requires further study. For example, it is possible that activation of cold receptors increases lacrimal gland secretion, whereas activation of polymodal nociceptors may also induce mucin secretion from goblet cells (Kessler et al., 1995). Distinct central pathways processing corneal afferent information would be required in order for these divergent responses to occur.

3. Central Processing within the Spinal Trigeminal Nucleus

Trigeminal primary afferent neurons innervating the head and face carry sensory information to Vsp, which is divided into three primary subdivisions: subnucleus oralis (Vo), interpolaris (Vi), and caudalis (Vc), from rostral to caudal (Olszewski, 1950). The processing of sensory information in Vsp is unique from that of the spinal cord dorsal horn in that there are multiple representations of the same orofacial region in different subnuclei of Vsp (Bereiter et al., 2000a). As might be expected, multiple representations indicate a specialization of function amongst these regions (Figure 1).

Tract tracing studies of corneal primary afferent neurons have identified two discrete terminal fields located within Vsp in numerous animal species (Marfurt, 1981; Marfurt and Del Toro, 1987; Marfurt and Echtenkamp, 1988; Panneton and Burton, 1981). These two

regions, one located at the transition between Vi and Vc (Vi/Vc) and the other located further caudally at the transition between Vc and the first cervical vertebra (Vc/C1), are also labelled with c-Fos protein following noxious stimulation of the cornea (Meng and Bereiter, 1996; Strassman and Vos, 1993). Neurons with corneal receptive fields have been characterized in each of these regions in the rat and noted differences in the receptive field properties have been described, a first indication that the Vi/Vc and Vc/C1 transition regions are functionally distinct (Hirata et al., 1999; Hirata et al., 2004; Meng et al., 1997; Meng et al., 1998).

The trigeminal subnucleus caudalis is often referred to as the medullary dorsal horn, emphasizing the similar anatomical and functional properties with the spinal cord dorsal horn (Dubner and Bennett, 1983; Gobel et al., 1977). As with the spinal cord dorsal horn, neurons in Vc process and relay nociceptive signals to brainstem and thalamic nuclei important in producing a wide array of nociceptive responses, including pain sensation and autonomic responses such as increased heart rate and blood pressure (Figure 1). Corneal units recorded at the Vc/C1 region typically posses nociceptive receptive fields that are contiguous with the cornea and have properties similar to other nociceptive neurons located in the medullary dorsal horn (Meng et al., 1997). They are excited by noxious heat, chemical (mustard oil), and acidic (CO_2) stimuli, and project to the thalamus and parabrachial area, relays to sensory and autonomic output regions, respectively (Hirata et al., 1999; Hirata et al., 2000; Meng et al., 1997).

The properties exhibited by Vc/C1 cornea sensitive neurons suggest that this region performs a similar function in nociception as other medullary and spinal cord dorsal horn neurons (Sessle, 1999; Sessle, 2000). Additionally, the Vc/C1 region does not appear to directly regulate noxious stimulation-evoked tearing. In support of this view, direct activation of the Vc/C1 region with glutamate did not elicit tearing, and inactivation of the Vc/C1 transition region had no effect on tearing evoked by CO_2 or bright light stimulation (Hirata et al., 2004; Okamoto et al., 2012). Instead, evidence has accumulated to indicate a critical role of the Vi/Vc transition region in regulating lacrimation.

Cornea sensitive neurons recorded at the Vi/Vc transition region possess several unique features that distinguish this region as one that is specialized for processing information unique to the cornea. Foremost among these features, many Vi/Vc neurons have excitatory receptive fields that include only the cornea (Hirata et al., 2004; Meng et al., 1997). In addition, compared to Vc/C1 cornea sensitive neurons, Vi/Vc neurons preferentially project to the superior salivatory nucleus (SSN) and the facial motor nucleus, regions involved in the efferent pathway for lacrimation and blinking (Figure 2) (Henriquez and Evinger, 2007; Hirata et al., 2004; Hirata et al., 2000; Toth et al., 1999). Presumably, emotional tears, a uniquely human response, are mediated by limbic projections to the SSN. Direct projections from the amygdala and hypothalamus to the SSN have been described in the cat and rat, respectively, providing a potential anatomical substrate for this response (Hosoya et al., 1983; Spencer et al., 1990; Takeuchi et al., 1991). In contrast to Vc/C1 corneal neurons, relatively few Vi/Vc neurons projected to thalamus and none could not be antidromically activated from the parabrachial area (Hirata et al., 1999; Hirata et al., 2000; Meng et al., 1997).

The Vi/Vc region contains a heterogeneous population of neurons, with at least four broad classes of cornea-responsive neurons that have been characterized based on their responses to mechanical, thermal, and acidic conditions. The first category responds only to mechanical stimulation, indicating selective input from mechanoreceptive primary afferent neurons (Meng et al., 1997). A second category responds to mechanical stimulation, innocuous cooling, and hyperosmotic stimulation, suggesting input from cold receptors and possibly mechanoreceptors (Kurose and Meng, 2013a). A third group is sensitive to mechanical stimulation, innocuous cooling, hyperosmotic stimulation, noxious heat, and low pH. This combination of responses indicates a convergence of input from all three categories of corneal primary afferent neurons (Kurose and Meng, 2013a). The final class of neuron is activated by mechanical, noxious heat, and low pH, likely receiving selective input from polymodal receptors (Hirata et al., 1999; Hirata et al., 2000; Meng et al., 1997). These labelled lines of transmission through the Vi/Vc region may reflect distinct pathways involved in the regulation of tearing and blinking.

Given their receptive field properties and projection targets, it has been proposed that cornea sensitive neurons at the Vi/Vc region are involved in the regulation of tearing, blinking, and other homeostatic functions unique to the cornea (Henriquez and Evinger, 2007; Hirata et al., 2004; Pellegrini et al., 1995). In support of this idea, glutamate microinjections into the Vi/Vc region have been shown to increase lacrimation (Hirata et al., 2004). Furthermore, tearing elicited by CO_2 and bright light stimulation was blocked by prior inactivation of the Vi/Vc transition region (Hirata et al., 2004; Okamoto et al., 2012). These results demonstrate the importance of the Vi/Vc region in noxious stimulation evoked tearing. This region may also represent a critical relay in tearing induced by simple evaporation of the tear film, as suggested by the select class of neurons activated by innocuous cooling (Figure 2) (Kurose and Meng, 2013a).

In summary, the cornea, like other specialized tissues of the cephalic region innervated by the trigeminal nerve, has its own requirements for health. The available evidence indicates that the Vi/Vc transition region is unique in its ability to regulate lacrimal gland secretion and blinking. As illustrated in Figure 1, we propose that this region is also involved in the regulation of intranasal and intraoral secretion. The convergence of input from the cornea, intranasal mucosa, and intraoral region onto individual neurons in the Vi/Vc transition region may explain why stimulation of one region can affect secretion in another (Drummond, 1995; Gupta et al., 1997; Nicolodi, 1994; Philip et al., 1994; Pramanik and Ghising, 2009; Togias et al., 1990). Despite the progress that has been made in understanding the neural regulation of secretion, relatively little is known regarding the function of neurons under pathophysiological conditions such as dry eye.

4. Properties of corneal sensory neurons in dry eye

In vivo confocal microscopy in humans has allowed for the examination of the subbasal nerve plexus in dry eye patients (Cruzat et al., 2010). Results have not been entirely consistent with regards to differences in the overall density of the subbasal plexus, which may be the result of the varying causes and stages of dry eye in these studies. However, an increase in subbasal nerve tortuosities and bead-like formations has been consistently

described (Benitez-Del-Castillo et al., 2007; Erdelyi et al., 2007; Tuisku et al., 2008; Tuominen et al., 2003; Villani et al., 2007; Zhang et al., 2005). The correlation between these morphological differences and potential sensory changes in dry eye is still unclear. An examination of the excitability of nerve endings in dry eye would allow for these morphological alterations to be associated with potential functional changes.

Animal models of dry eye typically use tear levels and corneal fluorescein staining as end points for evaluating the potential therapeutic effects of various interventions (Barabino et al., 2004; Barabino and Dana, 2004; Fabiani et al., 2009; Fujihara et al., 2001; Higuchi et al., 2010; Lin et al., 2011; Zhu et al., 2009; Zhu et al., 2012). However, improvements in the condition of the corneal epithelium may not be indicative of changes in the properties of corneal sensory neurons, and it could be argued that the most relevant outcome to dry eye treatment is an overall improvement in irritation and pain. Therefore, the assessment of potential therapeutics for treating dry eye requires an understanding of how the dry eye condition itself may affect corneal sensory processing.

The effect lacrimal gland excision on the properties of corneal primary afferent cold receptors has recently been explored in the rat (Kurose and Meng, 2013b). In this study, unilateral removal of the exorbital and infraorbital lacrimal glands resulted in diminished tear production and elevated fluorescein staining over an 8-week observation period. In addition, spontaneous blink rates on the side ipsilateral to lacrimal gland excision were elevated. These findings are consistent with previous studies that have found increased fluorescein staining and spontaneous blinking after removal of the exorbital lacrimal gland (Fujihara et al., 2001; Higuchi et al., 2012; Higuchi et al., 2010; Kaminer et al., 2011). The increase in blinking, while referred to as “spontaneous” (Kaminer et al., 2011), likely requires the activation of corneal primary afferent neurons. In preliminary studies, application of a topical anaesthetic to the cornea caused a notable reduction in the spontaneous blink rate in dry eye animals (Meng et al., 2013). While the class of primary afferent neuron driving these spontaneous blinks in the dry eye condition are still unknown, it may be indicative of ongoing irritation or pain.

Cold receptors recorded 8 weeks following lacrimal gland excision had warmer activation temperature thresholds and an increase in cold-evoked activity (Kurose and Meng, 2013b). In addition, cold receptors demonstrated an increased sensitivity to menthol, suggesting an upregulation of TRPM8 receptors. This type of cold receptor sensitization in dry eye would most likely lead to an increase in afferent drive for both tearing and blinking. These results also run counter to the cornea and lacrimal gland feedback model proposed by Mathers (Mathers, 2000), in which it was hypothesized that dry eye produces a decrease in the sensitivity of corneal primary afferent neurons. The effect of dry eye on the properties of mechanoreceptive and polymodal neurons has not been examined, yet sensitization of these neurons would be consistent with studies that have reported significant hyperesthesia in individuals symptomatic for ocular dryness (Chen and Simpson, 2011; De Paiva and Pflugfelder, 2004; Situ et al., 2008a; Situ et al., 2008b; Tuisku et al., 2008)(but see (Benitez-Del-Castillo et al., 2007; Bourcier et al., 2005; Stapleton et al., 2006)).

The sensitization of corneal afferents in dry eye may involve inflammatory mediators, which have been shown to sensitize polymodal nociceptors innervating other tissues (Schaible et al., 2011). Under dry eye conditions, increased prostaglandins and inflammatory cytokines such as IL1-beta and TNF-alpha increase in corneal tissue, and could potentially sensitize corneal polymodal receptors (LaMotte et al., 1992; Liang et al., 2001; Lin et al., 2011; Massingale et al., 2009; Shim et al., 2012; Stevenson et al., 2012; Szolcsanyi, 1987; Zhu et al., 2009). However, similar factors have been shown to desensitize cold receptors (Linte et al., 2007; Zhang et al., 2012). This area of research requires further study, since any effect of dry eye on corneal primary afferent neurons would alter their ability to regulate lacrimation.

Dry eye may also affect the function of trigeminal brainstem neurons that regulate secretion. The properties of Vsp cornea-responsive neurons in the dry eye condition have not been explored, yet studies indicate neurons located at the Vi/Vc and Vc/C1 transition regions respond differently to repeated noxious stimulation and ocular inflammation or injury. At the Vc/C1 transition region, noxious heat applied repeatedly to the cornea caused an increase in neuronal responses, whereas Vi/Vc neurons desensitized to the same stimulus (Meng et al., 1997).

These early results are consistent with more recent studies characterizing cornea-responsive neurons following ocular inflammation (Bereiter et al., 2005; Tashiro et al., 2010). In an animal model of photokeratitis, ultraviolet irradiation of the eye resulted in the sensitization of neurons recorded at the Vc/C1 but not Vi/Vc transition region (Tashiro et al., 2010). The sensitization of Vc/C1 neurons correlated with an increase in hypertonic saline evoked eye wipe behavior. Furthermore, only Vc/C1 neurons were sensitized to CO_2 stimulation one-week after endotoxin-induced uveitis (Bereiter et al., 2005). The enhanced neural activity correlated with an increase in CO_2-induced blinking. At this same time-point, however, there was no change in CO_2-induced lacrimation. Taken together, these results indicate that differences in central processing of corneal input at the Vi/Vc and Vc/C1 transition regions can result in distinct functional consequences with respect to lacrimation and sensation. In dry eye, even though ocular conditions may sensitize primary afferent neurons, the ability of Vi/Vc neurons to stimulate secretion may still be impaired.

5. Future Directions

At present, the neural regulation of tearing under normal conditions is relatively well characterized. Noxious mechanical, thermal, and chemical stimuli activate corneal primary afferent polymodal receptors and mechanoreceptors, whereas innocuous cooling, such as that which occurs during minute-to-minute evaporation of the tear film, increases corneal cold receptor activity. These primary afferent neurons project to the Vi/Vc transition region in the brainstem, a critical relay in the lacrimation circuit. At the Vi/Vc region, select labelled-lines remain as distinct categories of second-order neurons, although convergence of several different classes of primary afferent neurons is also apparent. Corneal input at the Vi/Vc region is processed and modified before information is sent to pre-ganglionic parasympathetic neurons located in the salivatory nuclei.

While the basic circuitry involved in the neural regulation of glandular secretion has been described, several unanswered questions remain. In particular, relatively little is understood regarding the peripheral and central changes that occur under pathophysiological conditions such as dry eye. Modifications in the properties of cornea-responsive neurons could have a severe impact on the regulation of tearing. Already, dry eye induced by lacrimal gland excision has been shown to sensitize corneal cold receptors (Kurose and Meng, 2013b). Understanding the mechanisms underlying these changes may prove important in developing novel treatments for dry eye. In such an approach, increasing the sensitivity of cold receptors could enhance tearing elicited by evaporative cooling.

In addition to the sensitization of cold receptors observed under dry eye conditions, it is now clear that cold receptors are also susceptible to desensitization (Kurose and Meng, 2013b; Robbins et al., 2012). Following low concentrations of menthol, cold-evoked neuronal activity is increased, yet after high concentrations of menthol these same neurons will desensitize. Lacrimal gland excision also increased the propensity for menthol to desensitize cold receptors (Kurose and Meng, 2013b). The process of desensitization is unknown, but may involve changes in membrane phospholipids (Daniels et al., 2009; Rohacs et al., 2005; Yudin et al., 2011; Yudin and Rohacs, 2012). Thus, while it may be advantageous to activate cold receptors to increase lacrimation, the potential to desensitize these neurons also must be considered.

Modifying cold receptor activity may be a useful strategy in promoting tearing without inducing irritation or pain (Parra et al., 2010; Robbins et al., 2012). However, the development of better treatments for irritation and pain often associated with dry eye and other ocular conditions will require a greater understanding of alterations that occur in mechanoreceptive and polymodal neurons under these conditions. Importantly, it should be recognized that changes in the properties of primary afferent neurons are not necessarily reflected in central neurons that regulate lacrimation. The effect of dry eye on the processing of corneal input at the Vi/Vc transition region has not been explored, yet modifications in the properties of these neurons would have a severe impact on tear secretion.

Novel therapies must address both ocular pain, produced by activation of polymodal receptors, and insufficient tearing, which may be caused by inadequate cold receptor activity. In order to assess the potential efficacy of novel therapies for dry eye and ocular pain, it is essential to determine their effect on the properties of corneal afferent neurons in the dry eye condition.

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Highlights

- Tearing is regulated through a neural reflex initiated by trigeminal primary afferent neurons.
- The properties of corneal sensory afferents are consistent with their ability to sense potentially damaging stimuli and drying that occurs with evaporation of the tear film.
- Projections from a specialized region in the spinal trigeminal nucleus to preganglionic parasympathetic neurons located in or around the superior salivatory nucleus control secretions.
- The dry eye condition may modify the properties of corneal primary afferent and second-order neurons, affecting their ability to regulate secretions.

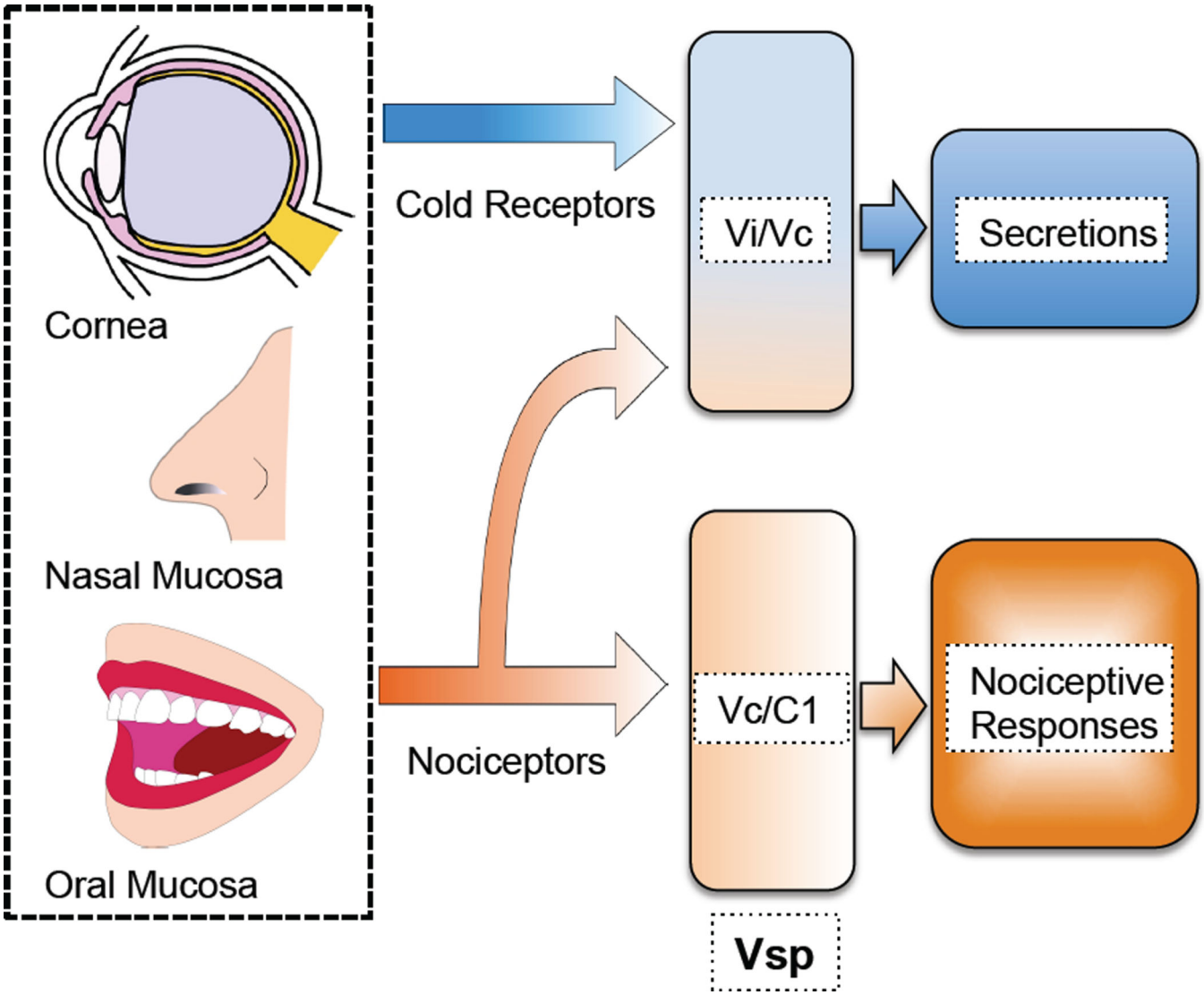

Figure 1.

Activation of nociceptors innervating the cornea, nasal mucosa and oral mucosa induces secretion and nociceptive responses, including pain sensation. In contrast, cold receptors, activated by evaporative cooling of the ocular surface, increases secretion without evoking nociceptive responses. Cold and nociceptor evoked secretion is regulated by neurons located at the transition between subnucleus interpolaris and caudalis (Vi/Vc) in the spinal trigeminal nucleus (Vsp), whereas nociceptive responses are controlled by neurons located at Vc and the first cervical vertebra (C1).

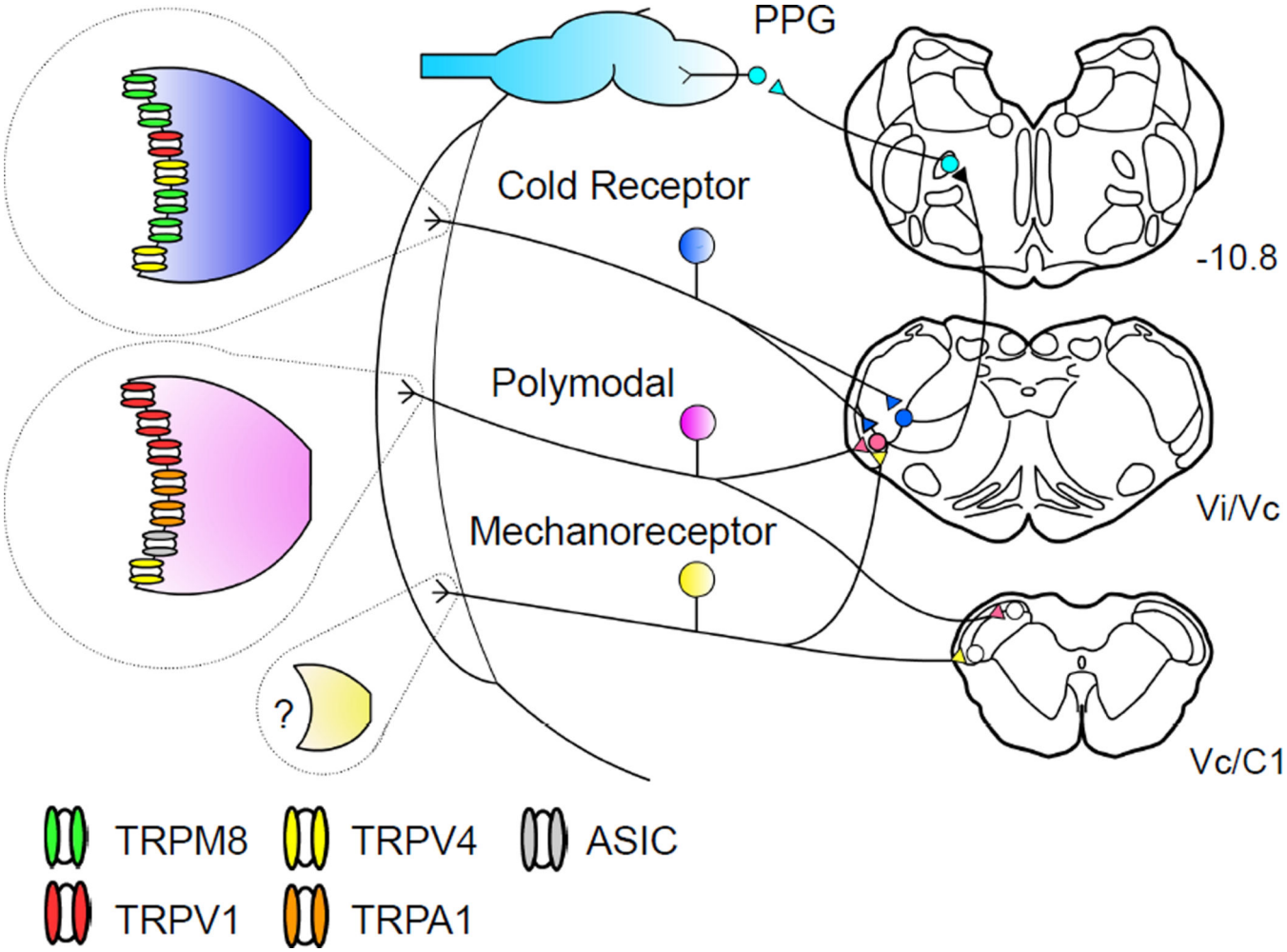

Figure 2.

Model for neural control of lacrimation. Corneal primary afferent neurons express a range of membrane channels, which corresponds to their physiological characteristics. The primary afferent neurons innervating the cornea regulate secretion of basal tearing with a relay through the spinal trigeminal nucleus. It has been proposed that polymodal nociceptors express channels responding to noxious chemical, thermal, and mechanical stimulation, including TRPV1, TRPA1, TRPV4, and acid sensing ion channels (ASIC) channels. In contrast, cold receptors express TRPM8 channels, which are sensitive to innocuous cooling. The channels responsible for mechanical responses in mechanoreceptive neurons and hyperosmotic responses in cold receptors have yet to be identified. PPG: Pterygopalatine ganglion