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Review

Neural basis of sensation in intact and injured corneas

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This paper is dedicated to the memory of David Maurice. As in so many other aspects of corneal research, David realized in the early 1970s the need of new methods and approaches to fully understand the mechanisms of corneal sensitivity, and led one of the first attempts to record electrical activity from corneal nerve fibres 'in vitro' (Mark and Maurice, 1977) as well as to study human corneal sensation using different modalities of stimuli (Beuerman et al., 1977). Twenty-five years later, similar techniques are being used routinely to extend our knowledge of the functional properties and roles of corneal sensory receptors in normal and injured corneas with the aim of understanding corneal pain, one of the many scientific problems that excited David's insatiable curiosity.

Abstract

A renewed interest in the characteristics and neural basis of corneal and conjunctival sensations is developing in recent years due to the high incidence of discomfort and altered sensitivity of the cornea following refractive surgery, use of contact lenses and dry eyes. Corneal nerves are functionally heterogeneous: about 20% respond exclusively to noxious mechanical forces (mechano-nociceptors); 70% are additionally excited by extreme temperatures, exogenous irritant chemicals and endogenous inflammatory mediators (polymodal nociceptors), and 10% are cold-sensitive and increase their discharge with moderate cooling of the cornea (cold receptors). Each of these types of sensory fibres contributes distinctly to corneal sensations. Mechano-nociceptors mediate, sharp acute pain produced by touching of the cornea. Polymodal nociceptors elicit the sustained irritation and pain that accompany corneal wounding; cold receptors evoke cooling sensations. Depending on the relative activation by the stimulus of each subpopulation of corneal sensory fibres, different subqualities of irritation and pain sensations are evoked. Corneal sensations can be explored experimentally in humans with a gas esthesiometer that applies controlled mechanical, chemical and thermal stimuli to the corneal surface. When the cornea is wounded, corneal nerves are excited and eventually severed in a variable degree and local inflammation is produced. Activated corneal nerves release neuropeptides (SP, CGRP) that contribute to the inflammatory reaction (neurogenic inflammation). They also become sensitized by local inflammatory mediators, such as prostaglandins or bradykinin and thus exhibit spontaneous activity, lowered threshold and enhanced responses to new stimuli. This leads to spontaneous pain and hyperalgesia. Nerves destroyed by injury soon start to regenerate and form microneuromas that exhibit abnormal responsiveness and spontaneous discharges, due to an altered expression of ion channel proteins in the soma and in regenerating nerve terminals. Presumably, this altered excitability is the origin of the lowered sensitivity and the spontaneous pain, dry eye sensations and other dysaesthesias reported in patients following refractive surgery.

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1. Introduction

Ophthalmologists have traditionally paid little attention to the mechanism of pain arising from the eye. Trigeminal nerve injuries, which lead to neuropathic pain referred to the eye, are relatively infrequent and in general handled by

neurologists. Some common clinical conditions, such as ocular dryness or conjunctivitis normally proceed with moderate levels of ocular discomfort that are in general considered 'tolerable' by the clinician. Intense pain may appear in certain ocular disturbances (keratitis, uveitis, scleritis, optic neuritis, angle-closure glaucoma, endophthalmitis) but often as an acute and transient symptom of the disease. Finally, pain is not a serious complication of modern ocular surgery. As a consequence, the number of experimental studies devoted to clarify the properties and neural basis of ocular sensations is scarce.

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However, in recent years, symptoms of ocular discomfort often described as ‘eye dryness’ have been reported with increasing frequency, as the result of repeated exposure to contaminated or air-conditioned environments, contact lens wear and the extended use of new surgical techniques for the correction of refractive defects, such as photorefractive keratectomy (PRK) or laser-assisted in situ keratomileusis (LASIK). This has prompted a renewed interest in the neural mechanisms involved in ocular sensations, looking for a relation between the unpleasant sensations experienced in these clinical conditions and the morphological and functional disturbances in the sensory supply to the anterior segment of the eye.

2. Sensory nerves of the cornea are functionally heterogeneous

The innervation of the cornea and bulbar conjunctiva is provided by a relatively small number of primary sensory neurons located in the ipsilateral trigeminal ganglion (about 1.5% of the total number of neurons of the ganglion, [de Felipe et al., 1999](#)). Nevertheless, the small size of the cornea and the extensive branching of the peripheral axons of corneal neurons makes this structure the most densely innervated tissue of the body ([Rózsa and Beuerman, 1982](#); [de Castro et al., 1998](#); [de Felipe et al., 1999](#); [Müller et al., 2003](#)). Corneal neurons can be classified as thin myelinated (A-delta type, 30% in the mouse) or unmyelinated (C type, 70% in the mouse), depending on the size and presence of a myelin sheath in the axon ([Belmonte et al., 1997](#); [de Felipe et al., 1999](#)). This feature is also reflected in the velocity at which they conduct nerve impulses to the central nervous system, that is much higher in the axons of A-delta neurons (mean 6 m s^{-1}) than in those of the C neurons (below 2 m s^{-1}) ([Belmonte and Giraldez, 1981](#); [Belmonte et al., 1991](#); [MacIver and Tanelian, 1993a,b](#); [Gallar et al., 1993](#)). A-delta and C neurons also differ in some of the passive and active electrophysiological properties of their neuronal membrane, that in turn determine the impulse firing characteristics of the neuron ([Lopez de Armentia et al., 2000](#)).

All peripheral axons of corneal neurons lose the myelin sheath when they enter the corneal stroma, mainly grouped in a variable number of radially oriented nerve bundles. They then branch extensively, forming a subepithelial plexus from which thin branches ascend up traversing the Bowman’s layer and enter into the basal layer of the epithelium. There, they run parallel to the corneal surface forming the leashes and terminate in the superficial layers of the corneal epithelium ([Zander and Weddell, 1951](#); [Chan-Ling, 1989](#); [Müller et al., 1997](#); for review see [Müller et al., 2003](#)). Corneal axons appear morphologically homogeneous when they are visualized using classical histology or electron microscopy techniques. However, immunocytochemical staining indicates the presence of

different neuropeptides within the cell soma and peripheral axonal fibres of corneal neurons, suggesting that they are functionally heterogeneous. About 58% of corneal neurons are immunoreactive to CGRP; 20% of them also contain Substance P (SP) ([Tervo et al., 1981](#); [Stone et al., 1986](#); [de Felipe et al., 1999](#); for review see [Müller et al., 2003](#)).

Indeed, electrophysiological studies confirmed that there are different functional types of corneal sensory fibres ([Giraldez et al., 1979](#); [Belmonte and Giraldez, 1981](#); [Tanelian and Beuerman, 1984](#); [Belmonte et al., 1991](#); [Gallar et al., 1993](#); [MacIver and Tanelian, 1993a,b](#); [Brock et al., 1998, 2001](#); for review see [Belmonte et al., 1997](#)). About 20% of them, all thin myelinated, respond only to mechanical forces of a magnitude close to that required to damage corneal epithelial cells. They are called mechano-nociceptors and fire one or at most a few nerve impulses in response to brief or sustained indentations of the corneal surface. This response pattern classifies them as ‘phasic sensory receptors’ that signal the presence of the stimulus and, in a very limited degree, the intensity, but do not encode its duration. The threshold force required to activate these receptors is apparently low (about 0.6 mN) well below that activating mechano-nociceptor fibres of the skin ([Bessou and Perl, 1969](#)). It should be remembered, however, that this intensity might be sufficient to damage the non-keratinized corneal epithelium. Mechano-nociceptors of the cornea are possibly responsible for the acute, sharp sensation of pain produced by a mechanical contact with the corneal surface.

The majority of corneal sensory fibres (about 70%), named polymodal nociceptors, are equally activated by near-noxious mechanical energy but they also respond to heat, to exogenous chemical irritants and to a large variety of endogenous chemical mediators released by damaged corneal tissue, by resident inflammatory cells or originating from the plasma leaking from limbal vessels (protons, potassium ions, ATP, prostaglandins and other arachidonic acid metabolites, amino acids, amines, cytokines, kynins, growth factors ([Belmonte et al., 1994](#); [Chen et al., 1997a](#))). Some of the polymodal nociceptor fibres belong to the thin myelinated group but most of them are of the C type. Polymodal nociceptors respond to their natural stimuli with a continuous, irregular discharge of nerve impulses that persists as long as the stimulus is maintained, and have a firing frequency roughly proportional to the intensity of the stimulating force. Therefore, the impulse discharge of polymodal nociceptors not only signals the presence of a noxious stimulus, but also encodes its intensity and duration. Polymodal nociceptors have a mechanical threshold slightly lower than mechano-nociceptors and when stimulated with heat, they begin to fire at temperatures over 39–40°C. A fraction of polymodal fibres (around 50%) also increase their firing rate when corneal temperature is reduced below 29°C ([Belmonte and Giraldez, 1981](#); [Acosta et al., 2001a](#)). As mentioned above, many chemical agents excite polymodal nociceptors. Using solutions of controlled

pH, or gas jets containing increasing concentrations of CO₂ (that forms carbonic acid in the corneal surface and produce immediately a local pH drop), a ‘chemical’ threshold of about one pH unit has been suggested (Belmonte et al., 1991; Gallar et al., 1993; Chen et al., 1995; Acosta et al., 2001a). pH reductions of this or larger magnitude often occur in inflamed tissues (Steen and Reeh, 1993). Polymodal nociceptors possibly contribute, together with mechano-nociceptors, to the sharp mechanical pain that arises when the cornea is acutely exposed to mechanical force, but they are also the principal source of nerve impulse activity caused by chemical irritation, heat or noxious cold (Belmonte and Giraldez, 1981; Beuerman et al., 1985). During inflammation, locally released mediators stimulate polymodal nociceptors, leading to a continuous firing that produces sustained sensations of pain (Handwerker and Reeh, 1991).

Another category of corneal nerve fibres, that represent 10–15% of the total population, are the cold-sensitive receptors, corresponding to A-delta and C fibres that discharge spontaneously at rest and increase their firing rate when the normal temperature of the corneal surface (around 33°C) decreases, while they are transiently silenced upon warming (Tanelian and Beuerman, 1984; Gallar et al., 1993). They also increase their firing rate as soon as the temperature of the cornea drops because of evaporation at the corneal surface, application of cold solutions or blowing of cold air on the cornea. Cold receptor fibres are able to detect and encode as a change in impulse frequency, small temperature variations of 0.1°C or less (Gallar et al., 1993; Carr et al., 2003), thus allowing the perception of corneal temperature reductions of that magnitude as a conscious sensation of cooling (Murphy et al., 2001; Acosta et al., 2001a).

Finally, it has been suggested (MacIver and Tanelian, 1993b) that the cornea possesses mechanically insensitive, ‘silent’ nociceptors, i.e. nerve terminals that in the healthy

tissue are not activated by mechanical or thermal stimuli but when local inflammation occurs, become responsive to these stimuli as well as to a variety of endogenous chemicals (Schaible and Schmidt, 1983; Davis et al., 1993). Although the experimental evidence for the presence of this class of nociceptors in the cornea is only indirect, they have been identified in virtually all somatic tissues and thus it appears likely that they also exist in the cornea.

The parent axons of trigeminal ganglion neurons that enter the cornea branch extensively and cover an area of the tissue surface known as the receptive field (Belmonte and Giraldez, 1981). Stimulation within the receptive field leads to depolarization of nerve endings and to impulse firing at the axon. Functional receptive fields of mechano-nociceptors and polymodal nociceptors are large and often include a portion of the adjacent episclera. Cold receptors have small receptive fields in the cornea and in the perilimbal area, where they are abundant and exhibit even smaller, spot like receptive fields (for review see Belmonte and Gallar, 1996). A small number of thick, fast-conducting nerve fibres also innervate the perilimbal episclera possibly with morphological specializations around their nerve endings (Krause-like corpuscles) (Lawrenson and Ruskell, 1991). These fibres respond to gentle mechanical stimulation and presumably contribute to non-noxious touch sensations evoked by gentle mechanical stimulation of the ocular surface, as those produced by blinking (Gallar, 1991).

3. Different functional types of sensory fibres also innervate various structures of the anterior segment of the eye

Electrophysiological studies dedicated to identify sensory receptor types in ocular structures other than the cornea are scarce. Nevertheless, they have shown that the same main functional classes of sensory afferents identified in

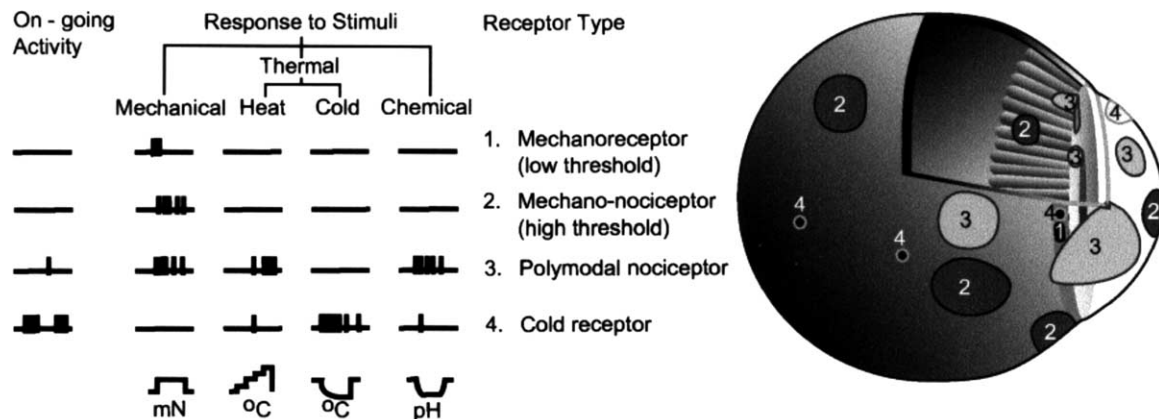


Fig. 1. Schematic representation of the types of sensory receptors found in the eye. In the upper part of the figure, the presence of ongoing activity at rest and the discharge of nerve impulses to the different modalities of stimuli have been displayed for each functional type of ocular sensory receptor. The shape and time course of the stimulus applied is represented in the lower line. In the right part of the figure, a scheme of the eyeball shows the location and receptor field size of the different types of receptors presented in the upper part of the figure (modified from Belmonte et al., 1997).

the cornea and the episclera, i.e. mechano-nociceptors, polymodal nociceptors and cold receptors, also innervate the bulbar conjunctiva (Aracil et al., 2001), the scleral surface (Zuazo et al., 1986; Gallar, 1991) and the iris and ciliary body (Zuazo et al., 1986; Mintenig et al., 1995). Cold fibres with response properties similar to thermal receptors found in the cornea and limbus are also present in unexposed areas of the iris and posterior sclera. It has been hypothesized that such scleral cold receptors contribute to the detection of choroidal and retinal blood flow changes for reflex blood flow regulation rather than to the production of conscious thermal sensations (Gallar et al., 2003a,b). Fig. 1 represents schematically the functional types of sensory endings identified electrophysiologically in the cat's eye.

4. Tissue injury and inflammation modify the activity of corneal sensory fibres

Electrophysiological recordings of corneal polymodal nociceptor fibres showed that when stimuli such as mechanical forces, temperature changes or chemical irritants approach injurious levels, the fibre started to fire nerve impulses at a frequency that increased rapidly with the amplitude of the stimulus and attained a maximum when overt cell damage was produced (Fig. 2). Removal of the noxious stimulus interrupted this activity transiently, but it reappears a few seconds afterwards as an irregular, low frequency firing of nerve impulses that persisted unabated for the duration of the experiment (Belmonte and Giraldez, 1981; Beuerman et al., 1985). Other manipulations, like experimental drying of the corneal receptive field of polymodal nociceptor fibres also determined the appearance of a low frequency, impulse activity in previously silent fibres, that increases in frequency with time and stops transiently when the corneal surface is re-moistened with saline. In these circumstances, cold-sensitive fibres that usually discharge rhythmically at rest also increase their firing frequency, presumably due to the reduction of temperature of the corneal surface elicited by evaporation during drying. Also, sliding of the lid over an abnormally dry anterior eye surface is expected to stimulate mechano-nociceptive endings and to enhance polymodal fibre activity.

A prominent feature of corneal polymodal nociceptor activity is that when a noxious stimulus (for instance, heating of the corneal surface) is repeated within an interval of few minutes, the impulse firing threshold decreases and the mean firing frequency in response to a given noxious stimuli increases. Also, the fibre often develops a low frequency, ongoing activity (Belmonte and Giraldez, 1981; Gallar et al., 1993). The decrease in threshold, enhanced responsiveness and spontaneous activity caused by repeated noxious stimuli is called 'sensitization' (Bessou and Perl, 1969; Handwerker, 1976), a specific property that is present in polymodal nociceptor neurons of all superficial and deep

somatic tissues including corneal polymodal neurons (Belmonte and Gallar, 1996). Sensitization develops because tissue injury releases endogenous inflammatory mediators from damaged cells, as well as in glial cells and neighbouring resident inflammatory cells that are activated by injury (Handwerker and Reeh, 1991). These endogenous chemical agents mediate the numerous cellular and molecular changes that characterize the local inflammatory reaction, including short- and long-term effects on membrane proteins located at the nociceptive nerve endings. Some of these receptor proteins are ion channels. Inflammatory mediators interact with them and increase or decrease their opening probability, which in turn changes the membrane potential and the overall excitability of the nerve terminal. Mediators also act on other receptor proteins in the nerve membrane, which in turn may trigger a variety of second and third messenger pathways leading ultimately to the opening of ion channels and to changes in nerve excitability and impulse propagation (see Bevan, 1996; Belmonte, 2003).

Only a few of the large number of inflammatory mediators that have been shown to produce sensitization of nociceptors in other territories (protons, K^+ , cytokines, amines, kinins, arachidonic acid metabolites, neurotrophins, peptides, for review see Handwerker and Reeh, 1991) were directly tested in the cornea, to explore their potential excitatory/sensitising effects on corneal nerve fibres. Prostaglandins, locally produced by the breakdown of arachidonic acid by cyclooxygenases, and bradykinin formed from a plasma protein precursor or liberated by resident inflammatory cells, are two important inflammatory substances that excite and sensitize corneal polymodal nociceptors (Belmonte et al., 1994; Fig. 3).

Sensitization is the cause of the sustained sensations of pain that arise from acutely injured tissues and of their hypersensitivity to new noxious stimuli (primary hyperalgesia) (Handwerker et al., 1984). Drugs such as the non-steroidal anti-inflammatory drugs (NSAIDs), that inhibit the action of cyclooxygenases and prevent the formation of lipid derivatives of arachidonic acid, not only reduce local inflammation, but also decrease sensitization of corneal nociceptors (Chen et al., 1997b; Fig. 4). This decrease of the enhanced sensitivity of nociceptors would explain in part the analgesic effect on post-surgical corneal pain treatment with these compounds (Stein et al., 1994).

Long-lasting local inflammation also produces more permanent changes in nociceptive terminals, including modified expression of the existing receptor molecules and expression of new ones. This is in part caused by growth factors; in particular nerve growth factor (NGF) that is produced in larger amounts by fibroblasts during inflammation. NGF phosphorylates a specific tyrosine-kinase receptor molecule (trkA), that is present in the peripheral terminals of the subpopulation of peptidergic nociceptor neurons (Woolf, 1996). Phosphorylation of trkA leads to activation of Raf and Ras proteins and of mitogen-activated

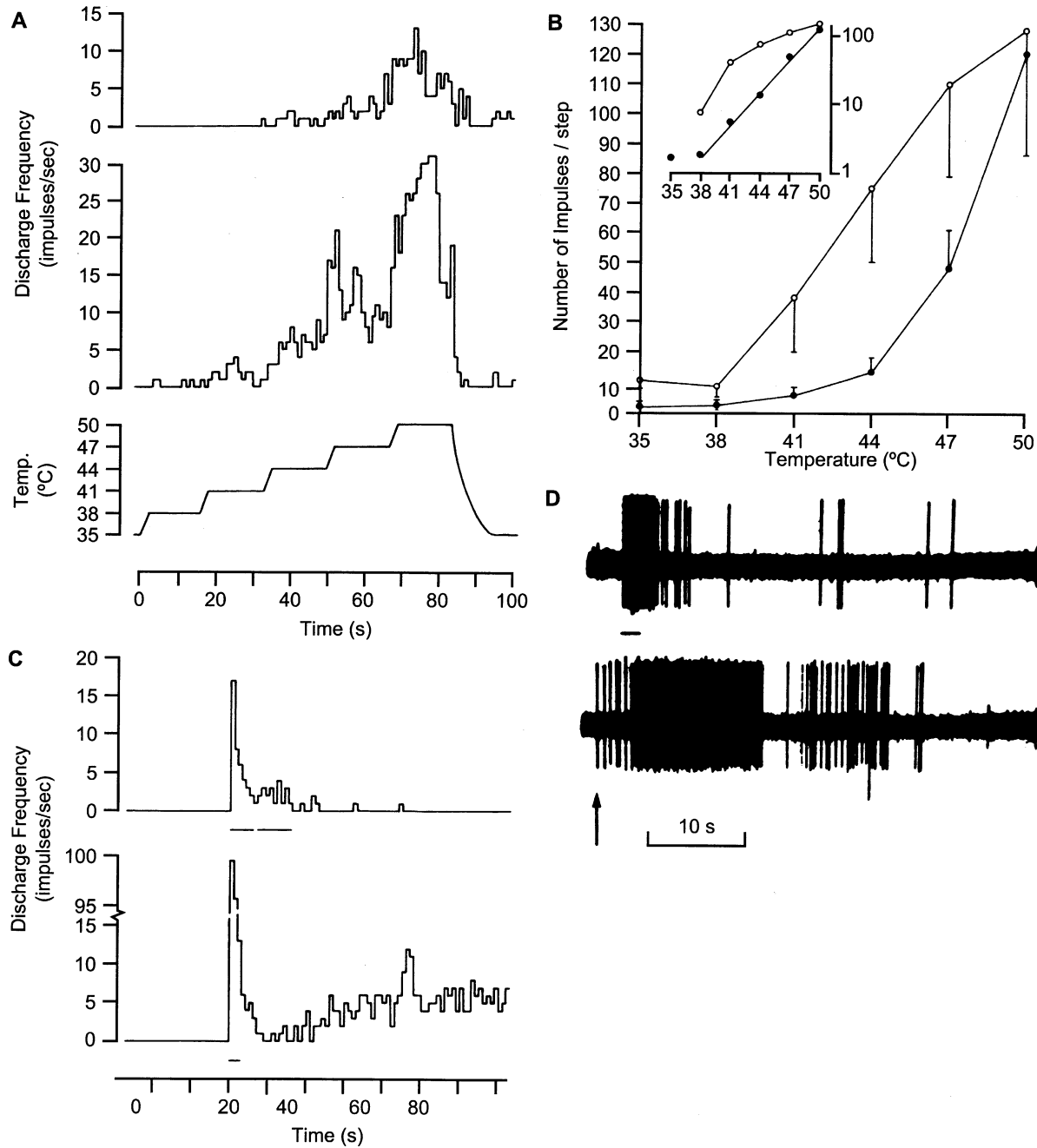


Fig. 2. Sensitization and response to injury of corneal polymodal nociceptors. (A) Stepwise increases of corneal temperature (lower trace) evoked an impulse discharge that during a second heating cycle repeated after a 3 min interval, started at a lower temperature value and reached comparatively higher frequency values. (B) Mean stimulus–response relation of eight corneal polymodal fibres in response to a first (filled circles) and a second (open circles) stepwise heating. (C) Response of a corneal polymodal fibre to sliding of a brush on the receptive field (upper trace) and to scratching with a needle (lower trace). (D) Impulse discharge evoked in another fibre by an injuring mechanical stimulus (upper trace, horizontal bar) and by application of a drop of 0.1N HCl (lower trace, arrow) to the receptive field (modified from Belmonte and Giraldez, 1981).

protein kinases (MAPK), which transduce the extracellular signal into diverse intracellular responses by transcriptional and translational regulation as well as by post-translational modification (Ji and Woolf, 2001, for review see Senba and Kashiba, 1996). During inflammation, augmented NGF levels induce the specific activation of some MAP kinases (p38 and ERK) at the nerve endings and cell somas of

nociceptor neurons. In the nerve endings, post-translational regulation by phosphorylation of key receptor and ion channel molecules determines their sensitization. After binding with NGF, trkA is also endocytosed and transported to the cell soma where it induces a longer-lasting activation of p38 (Ehlers et al., 1995). Another growth factor, the glial-derived neurotrophic factor (GDNF) that also modifies its

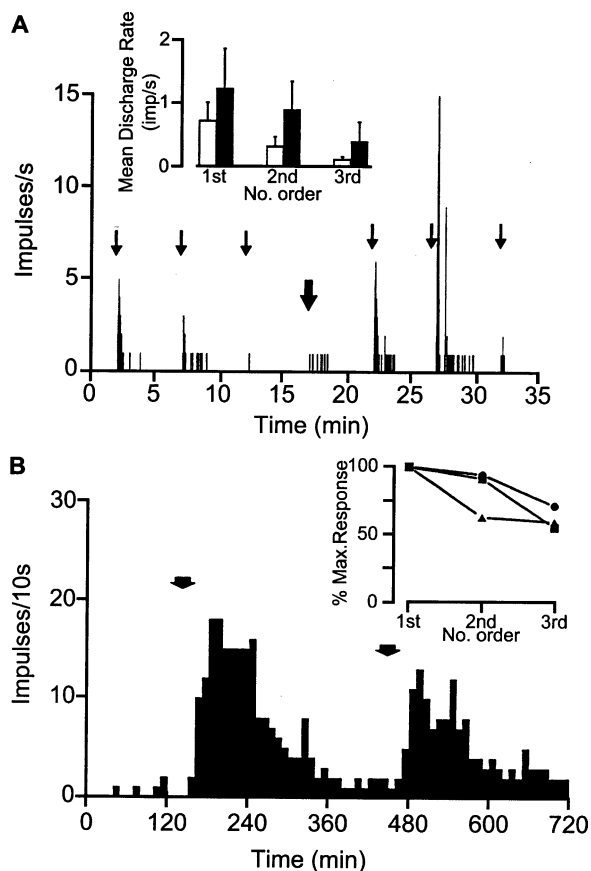


Fig. 3. Change of impulse activity in polymodal nociceptor fibres of the cat cornea induced by inflammatory mediators. (A) Frequency histograms of the impulse discharge evoked by repeated topical application of 10 mM acetic acid (small arrows). At the time indicated by the thick arrow, PGE₂ (10^{-6}) was applied to the receptive field. The inset depicts the values of mean impulse discharge in response to three successive stimulations with acid, before (open bars) and immediately after (filled bars) treatment with PGE₂. (B) Stimulus histograms of the impulse discharge evoked in a polymodal corneal fibre by two sequential application of bradykinin (10^{-5}) on its receptive field at the corneal surface. The inset shows the reduction of the response in three separate fibres with repeated stimuli (tachyphylaxis) (from Belmonte et al., 1994).

expression during inflammation, acts on the subpopulation of non-peptidergic primary nociceptive neurons where it also alters the expression of neuronal receptor and ion channel molecules (Senba and Kashiba, 1996).

An important characteristic of peptidergic nociceptor nerves of all territories including the cornea, is that their neuropeptide content is released by the nerve endings when calcium ions enters the cell as a consequence of membrane depolarization and impulse firing evoked by an injurious stimulus. CGRP and substance P, the principal neuropeptides found in trigeminal ganglion neurons produce vasodilatation, plasma extravasation and stimulation of cytokine release by local cells. They thus contribute to the early development of the local inflammatory reaction, the so-called neurogenic inflammation (Szolcsányi, 1996). Likewise, in the eye, direct stimulation of corneal sensory fibres either electrically or by application to the cornea of

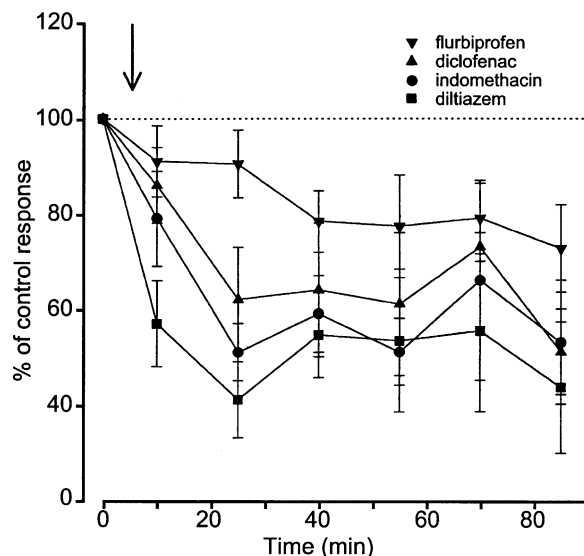


Fig. 4. Effect of non-steroidal anti-inflammatory drugs on the impulse response of corneal polymodal nociceptor fibres of the cat to a repeated pulse of CO₂ (chemical stimulation). The non-steroidal anti-inflammatory drugs sodium diclofenac (0.1%), flurbiprofen (0.03%), indomethacin (0.1%) and the calcium channel blocker diltiazem hydrochloride (0.045%) were applied topically at the arrow. Change in frequency is expressed as percent of the mean control frequency (from Chen et al., 1997b).

heat or chemical irritants such as capsaicin or detergents, releases CGRP. This effect was not obtained when cold stimuli were used, suggesting that corneal peptidergic neurons are mainly of the polymodal nociceptor class and that release of CGRP requires a vigorous stimulation of polymodal nerve endings (Belmonte et al., 2003).

When noxious stimuli are restricted to a limited area of the cornea, only a fraction of the nerve endings in the receptive field of the corneal polymodal neuron are stimulated. Excited endings produce nerve impulses that propagate centripetally, but that also invade antidromically other non-stimulated branches of the parent axon (Brock et al., 1998; Weidner et al., 2003). It has been shown that polymodal neurons of the cornea, unlike those of corneal cold-sensitive neurons, possess at their nerve endings sufficient concentration of sodium ion channels to sustain the propagation of these antidromic nerve impulses down to the most distal part of the nerve terminal (Brock et al., 1998, 2001). This property is possibly important to enable the release of neuropeptides contained in those corneal nerve endings that were not directly exposed to the stimulus but were depolarized antidromically. This antidromically-evoked depolarization seems to be the origin of the extension to non-injured areas of the cornea and conjunctiva of the neurogenic inflammation that follows a limited corneal lesion. The same mechanism could also be responsible for the local inflammation observed in the iris and ciliary body after to corneal injury. Conceivably, collateral branches of parent, corneal polymodal nociceptor axons that enter the root of the iris and

the ciliary body, when depolarized antidromically, release neuropeptides that mediate vasodilatation, plasma extravasation, migration of immune cells and nociceptor excitation/sensitization, typical of the neurogenic inflammatory response in these structures.

5. Short and long-term morphological and functional changes occur in corneal sensory neurons following injury of their peripheral axons

When the cornea is injured either accidentally or as a consequence of its surgical manipulation, the axons of corneal neurons that form the corneal nerves are severed to a variable degree. This may occur at their entrance in the cornea or at any level of their peripheral trajectory within the corneal stroma or the epithelium. The morphology and functional properties of corneal neurons suffering a peripheral axotomy change substantially. As a rule, the peripheral segment of the interrupted nerves exhibits Wallerian degeneration in the hours following injury (Wener et al., 1973). The area of the cornea that became denervated as a consequence of the lesion is invaded in the next days by outgrowths of neighbour non-injured nerve fibres. Also, the central stump of cut axons dilates and their surrounding glial and connective tissue cells proliferate, so that microneuromas are formed and the cut axon starts to regenerate, producing sprouts that grow and try to cross the scar tissue to penetrate the denervated area (Rózsa et al., 1983; Beuerman and Rózsa, 1984; Chan et al., 1990; Chan-Ling et al., 1990; Trabucchi et al., 1994; for review, see Müller et al., 2003).

Primary sensory neurons experience after axotomy a number of molecular changes that modify profoundly their functional properties. The absence after peripheral damage of the chemical signals that under normal conditions are transported along the axon centripetally to the soma such as NGF (Ehlers et al., 1995) modifies the expression of genes that encode ion channel and receptor proteins. In the injured neuron, these molecules are transported distally by axonal flow and accumulate at the neuroma endings. They include various classes of Na⁺ and K⁺ channels. Nociceptor neurons normally express three subtypes of sodium channels named Na_v1-7, Na_v1-8, Na_v1-9 that are largely responsible for the different currents that sustain the initiation and propagation of nerve impulses at the axon and cell soma (Baker and Wood, 2001; Baker et al., 2003). Axonal damage induces a significant up-regulation of the previously silent sodium channel gene Na_v1-3 and down-regulation of the Na_v1-8 and Na_v1-9 genes (Waxman et al., 1999). This determines the modification of sodium currents present in neuroma endings and favours the development of ectopic discharges typical of neuroma endings (Matzner and Devor, 1994). Also, responsiveness to mechanical and chemical stimuli becomes abnormal (Rivera et al., 2000).

As a consequence, nerve impulses appear irregularly, both spontaneously and in response to stimuli that under normal conditions do not excite intact nerve terminals (Matzner and Devor, 1994). These changes are not limited to the nerve stump. Abnormal excitability also develops at the cell soma. Moreover, ‘cross-talk’ appears between injured neurons at the level of the axon and the cell body, further increasing peripheral and central excitability (Kajander et al., 1992).

In the case of the corneal innervation, the degree and magnitude of the morphological and functional disturbance of sensory neurons seem to depend on the extension of the lesion. In experimental wounds in animals, the time course of regeneration of injured axons appear to be faster after cryodamage or chemical injury of the corneal stroma than following a limbal keratotomy (Chan et al., 1990). In humans, the magnitude of stromal nerve injury and the temporal pattern of regeneration consecutive to the various refractive surgery procedures (radial keratotomy, photorefractive keratectomy, laser-assisted in situ keratomileusis) seem also to be variable. Nonetheless, in all circumstances in which stromal and epithelial nerves were destroyed, the injured area in the cornea was first entered by sprouts of intact neurons, some of them that had their receptive field limited originally to the conjunctiva (de Felipe and Belmonte, 1999), followed in the next weeks by the advance of regenerating axons of the severed nerves. Only a proportion of regenerated axons succeed in penetrating the denervated, injured corneal tissue. Microneuromas and nerves presenting a random and disorderly distribution pattern, with hyperregeneration and abnormal shapes, were often observed in the scar region and in the reinnervated territory long time after the lesion (Wolter, 1966; Chan et al., 1990; Trabucchi et al., 1994).

In parallel to morphological changes, functional alterations have been also reported in axotomized corneal neurons. These up-regulate the expression of the proto-oncogen c-jun although expression of neuropeptides CGRP and SP is not markedly altered (de Felipe and Belmonte, 1999). Several weeks after performing an experimental wound in the centre of the cornea with a trephine down to the upper one-third of the stroma, spontaneous impulse activity was observed in polymodal axons that had a receptive field ending abruptly at the border of the wound (Charco et al., 1996). These axons presumably had some of their peripheral branches cut at the wound margin. Moreover, mechanical stimulation of the receptive field of healing corneas of the cat with the Cochet–Bonnet esthesiometer or with a jet of gas containing 80% CO₂ 1 day to 3 weeks after mechanical or PRK wounding, evidenced an altered threshold and an abnormal responsiveness to these stimuli in the wounded area. In contrast, fibres of the same corneas innervating distant, non-injured areas remained silent at rest and

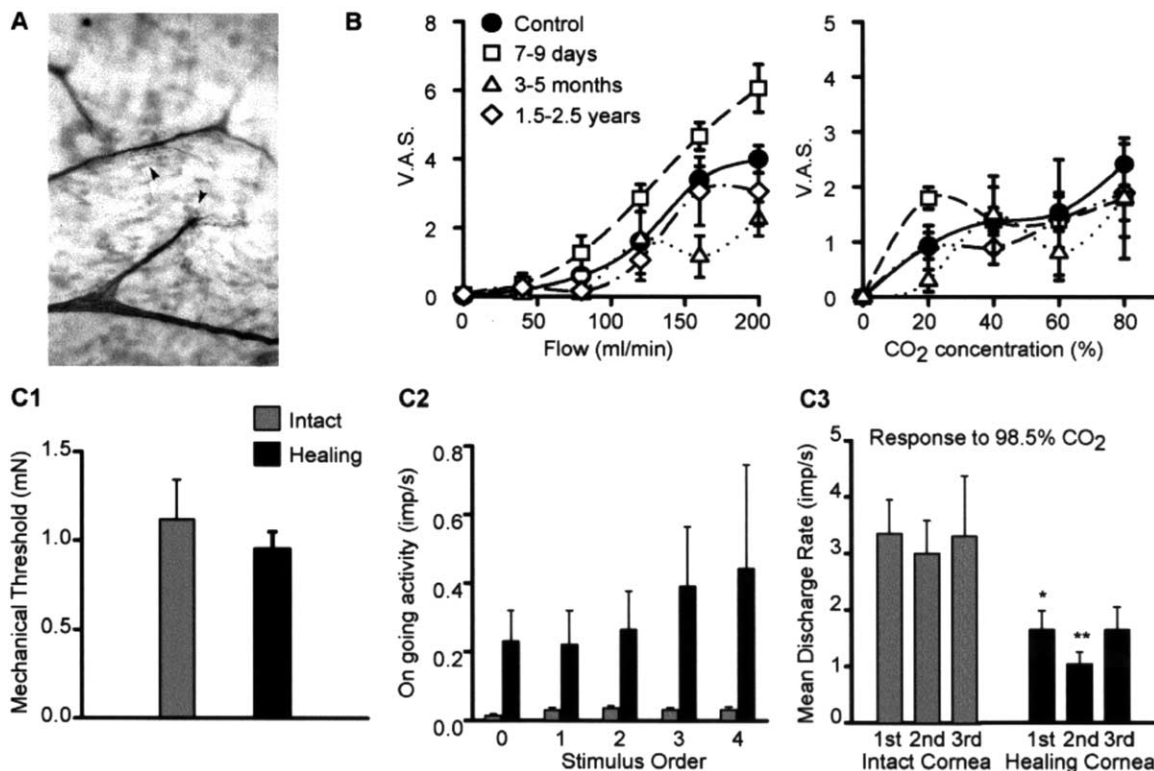


Fig. 5. (A) Image of deep stromal nerves of the rabbit in the center of a PRK wounded cornea 7 days after treatment, showing neuromas and sprouting of injured nerves (arrows) (from Trabucchi et al., 1994). (B) Changes in sensitivity of the corneal flap in human patients at different times after LASIK. Gas pulses of increasing flow (upper graph) and of increasing CO₂ concentration (lower graph) were applied with the gas esthesiometer. Intensity of the sensation, measured with a visual-analogue scale is represented in ordinates (from Gallar et al., 2003b). (C) Altered responsiveness of polymodal fibres innervating an area of the cornea that had suffered an epithelial wound 2 weeks earlier and was macroscopically healed at the moment of recording. (C1) Mechanical threshold of fibres innervating the intact and the healed area of the cornea. (C2) Mean impulse discharges evoked in the intact and the healed corneal areas by a 30 s CO₂ pulse repeated at 5 min intervals. (C3) Build-up of ongoing activity in the intervals between repeated CO₂ pulses, in the intact and the healed corneal areas (unpublished data from Gallar, Charco, Acosta and Belmonte).

responded normally to mechanical and chemical stimulation (Charco et al., 1996; Gallar et al., 1997; Fig. 5).

6. Different qualities of sensation are evoked by stimulation of the intact cornea and conjunctiva

Mechanical stimulation of the cornea using a calibrated hair or an air jet that applies a controlled force to the corneal surface has for years been the only method employed to evaluate corneal sensitivity in human subjects. Using this rather crude procedure, changes in corneal sensitivity induced by age, sex, pregnancy, iris colour, use of contact lenses, various types of ocular surgery or corneal diseases such as herpes virus infections, keratitis, iritis, uveitis, glaucoma, etc. have been evidenced in multiple and often repetitive clinical and experimental studies (see Draeger, 1984; Belmonte et al., 1997 for review). More recently though, the development of a gas esthesiometer (Belmonte et al., 1999) that permits the separate application on a limited area of the cornea or the conjunctiva, of controlled mechanical pulses, irritant chemical stimuli and hot or cold

air pulses has permitted measurement of the psychophysical characteristics of the sensation evoked by each type of stimulus, in more refined way. Such studies have shown that the quality of the sensations evoked upon stimulation of the ocular surface are different, depending on the modality of physical or chemical stimulus applied or of how these stimuli were combined. Electrophysiological recordings of nerve impulse activity evoked by stimulation with the gas esthesiometer in experimental animals showed that each stimulus type excited in a variable degree the various functional subpopulations of nerve fibres that innervate the cornea (Chen et al., 1995; Acosta et al., 2001a). In the human cornea, the sensations produced by suprathreshold mechanical or chemical stimulation and by heat always included a component of irritation, although each sensation possessed a different quality that allowed their identification as distinct from the others. In contrast, application of cold pulses that decreased the temperature of a point on the cornea moderately, (1–3°C), evoked a cooling sensation exclusively. This became irritating only when more pronounced temperature reductions were applied (Beuerman et al., 1977; Acosta et al., 2001a). The same was true

for the bulbar conjunctiva except that overall sensitivity was comparatively lower than in the cornea and light mechanical stimuli were felt as non-irritating (Acosta et al., 2001a,b; Feng and Simpson, 2003).

Thus, psychophysical studies indicate that in addition to the sharp, shooting pain evoked by acute mechanical stimulation of the cornea with the Cochet–Bonnet esthesiometer, attributable to the sudden excitation of A-delta mechano- and polymodal nociceptors, the slower activation of C-polymodal nociceptors with other modalities of noxious stimuli may elicit irritation feelings of different quality, closer to the sustained unpleasant sensations experienced after corneal injury. Moreover, simultaneous activation or inhibition of cold sensory fibres at the ocular surface contributes with a thermal component to define the subjective characteristics of the sensation, whose final quality is determined by the mixture of perceptual elements that results of the variable activation/inhibition of each separate class of corneal sensory fibres.

7. Damage of corneal sensory innervation causes hypoaesthesia and abnormal ocular sensations

The reduction of corneal sensitivity to mechanical stimulation, following penetrating keratotomy for cataract surgery, keratoplasty, or after keratectomy for epikeratophakia, is well documented. Threshold measurements with several types of esthesiometers, based on the force exerted by a calibrated filament pushed against the cornea (esthesiometers of Boberg-Ans, 1956; Cochet–Bonnet, 1960 or Draeger, 1984) evidenced a marked increase of threshold in the denervated areas that took months to recover and in general never returned to normal (Koenig et al., 1983; Kohlhaas et al., 1992). Actually, transplanted corneas or implanted lenticles in epikeratophakia remained totally anaesthetic for years or recovered at best a very limited mechanical sensitivity, usually restricted to the periphery of the transplant (Zorab, 1971; Rao et al., 1985; Mathers et al., 1988; Biermann et al., 1992).

Measurement of mechanical threshold with force esthesiometers has been additionally used to evaluate the disturbances of corneal sensitivity produced by the modern surgical techniques developed for the correction of refractive defects. These include radial keratotomy (RK), photorefractive keratectomy (PRK), laser-assisted in situ keratomileusis (LASIK), laser thermal keratoplasty (LTK) and intrastromal corneal rings (ICR), although PRK and more recently LASIK are the most favoured procedures for treatment of refractive disorders.

In PRK, the epithelium is debrided and then a surgical ablation of the superficial stroma is performed with the excimer laser. During LASIK, a hinged lamellar corneal flap is raised with a microkeratome. The stromal bed is then ablated with the laser and the flap repositioned. Measurement of corneal mechanical threshold in PRK patients

shows that after treatment, sensibility decreases in the centre of the cornea. It begins to recover after 1 week and progresses slowly to reach values not far from normal about 3 months later, although incomplete recovery 6 months and 1 year after surgery have been also reported (Kanellopoulos et al., 1997; Matsui et al., 2001). In LASIK patients, mechanical threshold measurement with the Cochet–Bonnet esthesiometer evidenced a marked reduction of corneal sensitivity in the central cornea and a less pronounced hypoesthesia in the nasal flap hinge and in the temporal flap edge in the first week post-surgery. In the following weeks, sensitivity recovered slowly but remained still low 1 month after surgery (Benitez-del-Castillo et al., 2001) with the central cornea always less sensitive than the flap borders and the hinge. In about 1–3 months mechanical threshold was partially recovered but most authors agree that preoperative values were never obtained before 6 months or longer (Kohlhaas et al., 1992; Kim and Kim, 1999; Linna et al., 2000; Matsui et al., 2001; Battat et al., 2001; Toda et al., 2001). Comparison of corneal sensitivity after PRK and LASIK has given conflicting results. Some authors (Yang et al., 1998; Matsui et al., 2001) claim that recovery of sensitivity is slower after PRK than after LASIK while others (Kanellopoulos et al., 1997) report the contrary.

The differences in sensitivity disturbances as well as the variability found among individuals in the degree and time course of sensibility recovery after PRK or LASIK are not surprising considering the variability among studies in the type of lesion, excision depth, extension of nerve damage, etc. The reduced discrimination capacity of the Cochet–Bonnet esthesiometer is also a limiting factor. When the more sensitive gas esthesiometer (Belmonte et al., 1999) was employed and corneal mechanical and chemical sensitivity was measured at variable times after LASIK surgery (Gallar et al., 2003b) corneal sensitivity to mechanical and chemical stimulation was noted to be seriously reduced in the first week after surgery, appeared enhanced around the flap and in the hinge area 2 weeks later, and then dropped remarkably and remained significantly below control levels, 3 and 6 months post-LASIK. Sensitivity to both types of stimuli was close to normal only 2 years post-surgically (Fig. 5). Thus, gas esthesiometry revealed that hyperalgesia occurred transiently in the surroundings of the wounded area, presumably due to sensitization caused by the acute inflammation of sensory fibres at the hinge area. This procedure further evidenced that long-lasting, subtle disturbances of corneal sensation persist at times when coarse mechanical sensitivity has apparently returned to normal. The observed changes in corneal sensitivity following photorefractive surgery correlate well with the reduction in the innervation density assessed with conventional histological techniques or with ‘in vivo’ confocal microscopy, both in experimental animals and in human patients (Linna and Tervo, 1997; Linna et al., 2000; Lee et al., 2002; Moilanen et al., 2003). These studies also suggest a correlation between regeneration of subbasal

nerves after surgery and recuperation of mechanical sensitivity (Moilanen et al., 2003).

In parallel with the above-described hypoesthesia, spontaneous acute and chronic pain appear in a variable degree following refractive surgery procedures. In RK maximal pain peaked at 3 hr after surgery and persisted as a moderate or low discomfort for at least 1 week (Epstein and Laurence, 1994). Pain after PRK usually began 30–60 min after the procedure and became severe within 4–6 hr, remaining high during the first day and persisting for days. Immediate pain (during days 1–3) also appeared following LASIK although it was comparatively lower than when PRK was used (Atrata and Rehurek, 2003). It is worth noting that the incidence of long-lasting discomfort symptoms attributable to nerve damage that was reported by patients subjected to this type of surgery is surprisingly high. A study with 231 PRK patients and 550 LASIK patients performed by Hovanesian et al. (2001) revealed an incidence of dryness symptoms in 43 and 48%, respectively. Soreness of the eye to touch was reported by 26.8 and 6.7%, respectively. Sharp pains occurred in 20.4% of PRK patients and 8.0% of LASIK patients and complaints of the eyelid sticking to the eyeball in 14.7 and 5.6%, respectively. All symptoms occurred predominantly on waking and according to this study they were significantly more common, more severe, and more prolonged after PRK.

The high incidence of subjective reports of dry eye sensation in patients that underwent LASIK surgery suggested that lacrimal gland secretion might be reduced in these patients. Several authors have measured tear production and clearance rate after LASIK (Aras et al., 2000; Battat et al., 2001; Benitez-del-Castillo et al., 2001; Toda et al., 2001). In all cases a modest depression of the tear production was detected although tear film stability appeared unaltered. Ocular surface structures and the main lacrimal gland with their interconnecting neural reflex loops form a functional unit (Stern et al., 1998). In this context, the decrease in tear secretion can be attributed to the reduction of corneal sensitivity caused by LASIK denervation.

Both the reduction of corneal sensibility and the presence of pain and dysethesias consecutive to PRK and LASIK are in turn the consequence of the molecular and functional changes that follow neural injury. In LASIK surgery, the lamellar incision made with the microkeratome to create the flap, cuts the nerve fibre bundles of the superficial stroma and the subbasal nerve plexus at the flap. Nerve fibre bundles in the corneal bed located in the middle-third of the stroma are spared but suffer a variable degree of injury during photoablation. Confocal microscopy studies (Linna and Tervo, 1997; Linna et al., 2000; Lee et al., 2002; Moilanen et al., 2003) have additionally shown that morphological recovery of corneal innervation may take longer than 12 months after both PRK and LASIK.

It should be noted though that the sensations of irritation that develop consecutive to these procedures occur in

parallel with a low mechanical sensitivity of the cornea (Battat et al., 2001). Thus, they do not seem to originate from nerve endings that have their transduction apparatus intact but are most likely the final consequence of the abnormal activity developed by neuromatous and regenerating nerve endings, triggered by a variety of endogenous and exogenous stimuli. In that context, it is important to point out that discomfort sensations described as ‘dry eye’ sensations do not necessarily reflect a real dryness of the corneal surface but rather the subjective interpretation given by the patient to the sensations of discomfort by the abnormal impulse activity in severed corneal nociceptor fibres that would be actually triggered by other causes, such as presence of local inflammatory mediators, mechanical stimulation by blinking or light dryness that under normal conditions would have been insufficient to activate intact nerve endings. The patient refers the sensation evoked by this abnormal activity to ocular dryness, because in normal life, dryness of the cornea and conjunctiva would be the main cause of activation of the various populations of corneal sensory endings and that are now abnormally discharging, evoking that quality of sensation.

Thus, although it is conceivable that the reduction of tear production secondary to refractive surgery will contribute to the abnormal excitation of injured nerve fibres, it seems more accurate to attribute many of the subjective discomfort symptoms reported by refractive surgery patients to the neuropathic activity of injured nerves than to real ocular dryness. Accordingly, it would be wise to direct therapeutic strategies to attenuate this post-surgery symptom not only to attenuate ocular surface dryness but also to decrease the abnormal excitability of corneal microneuromas, favouring the functional recovery of injured corneal nerves.

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