An Investigation of the Role of Ocular Surface Conditions in Blinking

Anna M Ntola

School of Optometry and Vision Sciences

Cardiff University

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Summary

This thesis considers the question: What roles do the tear film and corneal nerves play in the normal blink mechanism? The hypothesis proposed is that tear film thinning, which occurs prior to full break-up, allows increased evaporation of the tear film. The evaporation produces a localised reduction in the tear film temperature, which is then detected by the temperature sensitive nerves in the corneal epithelium.

To test this hypothesis, a series of experiments was completed. The first study, which investigated the pattern of diurnal change in corneal sensitivity, revealed that corneal sensitivity increases during the day following post-sleep eyelid opening to reach a plateau approximately five hours after eye opening (Kruskall-Wallis, $p<0.05$). The second study assessed corneal nerve function under anaesthesia produced by 0.5% Proxymetacaine Hydrochloride. Onset of anaesthesia was observed within 2 minutes (Wilcoxon matched pairs test, $p<0.05$), with a maximum of anaesthesia reached at 15 minutes post-instillation (Wilcoxon matched pairs test, $p<0.05$). Corneal sensitivity did not return to pre-instillation levels at 60 minutes post-instillation (Wilcoxon matched pairs test, $p<0.05$). The third study assessed the effect of iris colour and ethnic origin on corneal sensitivity, skin sensitivity, tear film stability and blink rate. The study showed that corneal sensitivity for a cooling stimulus was affected by iris colour and ethnic origin (Kruskall-Wallis, $p<0.05$): as iris pigmentation increases, corneal sensitivity decreases. Although statistically tear film stability was found to be influenced by iris colour, no clear pattern of change was associated with iris colour (Kruskall-Wallis, $p>0.05$). Tear film stability was not affected by ethnic origin

(Kruskall-Wallis, $p>0.05$). Blink rate was significantly correlated to tear film stability (Spearman, $r = -0.536$, $p<0.05$).

The fourth study considered the relationship between blink rate, corneal sensitivity, tear film stability, anaesthesia, ocular surface temperature and ocular surface evaporation. Tear film stability was strongly correlated to blink rate, with the blink rate increasing as tear film stability decreases (Spearman, $r = 0.926$, $p<0.05$). Corneal sensitivity was significantly correlated to corneal sensitivity, but only at low corneal sensitivity levels. Blocking corneal sensitivity by anaesthesia, the blink rate was significantly reduced, suggesting that corneal sensitivity is involved in the mechanism controlling normal involuntary blinking (Wilcoxon matched pairs test, $p<0.05$). Tear film evaporation from the ocular surface was not correlated to the blink frequency (Spearman, $r = -0.381$, $p>0.05$). The amount of temperature cooling at the inter-blink interval was not correlated to blink rate (Spearman, $r = 0.241$, $p>0.05$).

The final experimental study examined involuntary blinking with contact lens wear discomfort. There was a significant increase in the blink rate with increasing discomfort (Kruskall-Wallis, $p<0.05$). Subjects experiencing discomfort had a less stable tear-film, both with (Mann Whitney test, $p<0.05$) and without (Mann Whitney test, $p<0.05$) contact lens wear, and had an elevated blink-rate compared to subjects experiencing comfort (Mann Whitney test, $p<0.05$). Ocular surface discomfort was not related to an elevated corneal sensitivity (Mann-Whitney test, $p>0.05$).

These series of studies showed that tear film stability and corneal sensitivity are involved in the blink mechanism, providing strong evidence that normal involuntary blinking is affected by sensory stimuli arising from the exposed ocular surface.

Acknowledgements

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Finally there is one that without his constant support I could not have achieved anything- Jesus Christ

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1. General Introduction

Normal blinking plays an important role in maintaining a healthy ocular surface. Blinking stimulates tear production and spreads the tear film to prevent drying of the ocular surface and to produce a smooth refractive surface of high optical quality. If blinking is deliberately prevented, the tear film will thin and break-up in a random manner. The factors involved in the control of blinking are not well understood, but there is evidence that the central nervous system and sensory stimuli arising from the exposed ocular surface play a role in the control of spontaneous blinking.

Based on the proposed evidence that sensory stimuli arising from the ocular surface may control blinking, the aim of this thesis was to focus on the ocular surface conditions and find out the role of the tear film and corneal nerves in the normal involuntary blinking.

The hypothesis proposed by this thesis is that tear film thinning, which occurs prior to full tear film break-up, allows increased evaporation of the tear film. The evaporation produces a localised reduction in the tear film temperature, which is then detected by the temperature sensitive nerves in the corneal epithelium.

To investigate the proposed hypothesis several ocular surface conditions were correlated with blink frequency: tear film stability, corneal sensitivity, temperature of the anterior ocular surface, and evaporation of the anterior ocular surface. Preliminary studies were also made in order create a background for the later studies.

The literature review that follows provides the background knowledge on the anatomy and innervation of the cornea, anatomy of the tear film, corneal sensitivity, ocular surface evaporation, ocular surface temperature, blinking, necessary for the understanding and continuation of this work.

2. Literature Review

2.1 Anatomy of the Human Cornea

The cornea is a transparent, avascular tissue that is continuous with the opaque sclera and semi-transparent conjunctiva. It covers one sixth of the circumference of the eyeball, and is the major refractive component of the eye, contributing approximately +48 DS.

The cornea has three primary functions. First, it serves the dual optical functions of maintaining a transparent "clear window" to the eye to allow the transmission of light. Second, it refracts light to help focus the object being viewed on the retina. Third, it protects the delicate intraocular structures from trauma (Nishida, 1977).

The corneal thickness varies across its surface. It is approximately 0.5 mm thick in the centre, but this increases gradually towards the periphery of the cornea where it is about 0.7mm thick. The radius of curvature is also not constant over the entire surface. It is steepest in the centre and becomes flatter in the periphery, creating an aspheric surface (Pepose and Ubels, 1992).

The cornea is described as a five-layered structure: 1) Epithelium (outermost layer), 2) Bowman's Layer (anterior limiting lamina), 3) Stroma, 4) Descemet's Membrane (posterior limiting lamina), and 5) Endothelium (innermost layer).

2.2 Corneal Structure

2.2.1 Epithelium

The epithelium forms the outermost layer of the cornea and is a non-keratinized, stratified, non-secretory, squamous epithelium. Its anterior surface is bordered by the tear-film, while the posterior surface is anchored, through its basement membrane, to Bowman's Layer (Klyce and Beuerman, 1988; Pepose and Ubels, 1992).

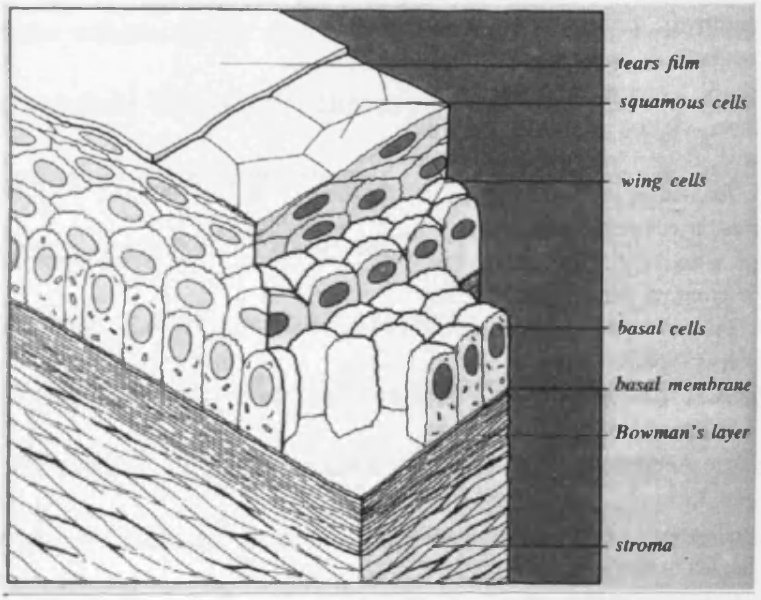


Fig 2.1: The layers of the corneal epithelium (From Saude T: Ocular Anatomy and Physiology, Blackwell Scientific Publications, Oxford, 1993).

The corneal epithelium consists of five to seven layers of three different types of epithelial cell: two to three layers of superficial cells, two to three layers of "wing" cells, and a monolayer of columnar basal cells. No spaces are evident between the cells. The corneal epithelium shows continuity with the conjunctival epithelium, which itself is continuous with the skin of the eyelid (Bergamanson, 2001). The epithelial layer measures approximately 50.6±3.9μm in thickness (Li et al., 1997).

The anterior surface consists of two to three differentiated layers of **superficial cells** (or squamous cells). They are extremely flat, polygonal cells with a diameter of 40-60μm and a thickness of 2–6 μm. The surface of these superficial cells is covered with microvilli that form microplicae. These microvilli are important in maintaining the adhension of the tear mucus layer to the epithelium {Hazlett, 1980 #2(Hazlett et al., 1980; Nichols et al., 1983; Pfister, 1973). Adhering to these microvilli is a glycocalyx that interacts with the mucin layer of the tears to promote the formation of a stable, smooth tear film on the corneal surface. These structures enlarge the total surface area, thus enhancing the stability of the tear film, and allowing the exchange of oxygen and nutrients between the cornea and the tears. When viewed by scanning electron microscopy, two different types of superficial cells can be observed: large, dark cells and small, light cells (Pfister, 1973). The large, dark cells have a dense coat of microvilli and are mature cells that are about to desquamated into the tear film. The smaller, light cells have fewer microvilli and are believed to be younger cells. The apparent difference is due to the dark cells having fewer microvilli than the light cells. The superficial cells are joined together by tight junctional complexes near the apical surface. These junctional complexes, called zonula occludens, provide a barrier to the intercellular movement of substances from the tear layer, and prevent the uptake of excess fluid from the tear film. Any breakdown in this barrier function leads to epithelial oedema, and is often referred to as "Sattler's veil" because of the significant visual effects of these conditions (Krutsinger and Bergamanson, 1985). These anatomic characteristics represent the mechanical barrier function of the corneal epithelium. Additional adhesions between the cells are provided by numerous desmosomes, which provide lateral mechanical stability (Khodadoust et al., 1968; Nishida, 1977; Pepose and Ubels, 1992).

Beneath the superficial cells are two to three layers of **wing cells**, so called because of their characteristic wing-like shape. These cells are in an intermediate state of differentiation between basal and superficial cells and are rich in intracellular tonofilaments called keratins (Klyce and Beuerman, 1988; Nishida, 1977; Pepose and Ubels, 1992). The wing cells are joined by desmosomes to superficial cells and to one another. Numerous large gap junctions are also present between the wing cells, allowing a high degree of intercellular communication in this layer.

The basal cells form a single layer of cuboidal, columnar cells, which rest on the basement membrane. Of the various types of corneal epithelial cells, only basal cells have mitotic activity (Klyce and Beuerman, 1988; Pepose and Ubels, 1992). Basal cells are the source of wing cells and superficial cells. The daughter cells push anteriorly and change their shape, becoming wing cells. As the cells continue to move anteriorly, they become superficial cells, before being shed into the tear film in a process known as desquamation. The epithelium turns over approximately once every seven days, resulting in an entirely new epithelium anteriorly to the basal mitotic cells (Hanna et al., 1961; Hanna and O'Bien, 1960). The basal cells are joined together by desmosomes and gap junctions, and by hemi-desmosomes to Bowman's Membrane (Gipson et al., 1987; Khodadoust et al., 1968).

The basement membrane (or basal lamina) is an extracellular product of the basal cells that lies anterior to Bowman's Layer and is about 40–60nm thick (Klyce and Beuerman, 1988; Pepose and Ubels, 1992). Posterior to the epithelial basal cells, the continuous, thin basement membrane is composed of the lamina lucida (adjacent to the cell membrane) and the lamina densa (adjacent to the Bowman's Layer). The

presence of the basement membrane between the basal epithelium and the underlying stroma fixes the polarity of the epithelial cells and creates a boundary that separates the epithelium from the stroma (Klyce and Beuerman, 1988; Pepose and Ubels, 1992). Furthermore it provides a matrix on which cells can migrate, helping to maintain a stratified and well-organized corneal epithelium. It also may play a role in epithelial wound healing (Nishida, 1997).

2.2.2 Bowman's Layer

An acellular membrane-like zone known as Bowman's Layer (or Bowman's membrane) is observed by light microscopy at the interface between the corneal epithelium and the corneal stroma in humans (Fig 2.1). Bowman's Layer is not a membrane but a simple condensation of collagen fibres and proteoglycans. It consists of randomly arranged, but tightly connected, collagen fibrils, which are finer in cross-section than the underlying stromal collagen, and proteoglycans. It is a modified stromal tissue and measures 16.6±1.1μm in thickness (Li et al., 1997). Bowman's Layer is considered to be the anterior portion of the stroma since the collagen fibers in the membrane are secreted by the stromal keratocytes and there is continuity between the fibers in the membrane and those in the stroma (Nishida, 1977; Pepose and Ubels, 1992; Assil and Quantock, 1993). Bowman's Layer plays an important role in the maintenance of the epithelial structure, but is not regenerated after injury. Recent clinical experience with excimer laser photoablation demonstrates that a normal epithelium is formed and maintained even when Bowman's layer is absent. Also, many other mammals do not have a Bowman's Layer, but still have a well-organised epithelial structure. Therefore the physiologic function of Bowman's Layer is not fully understood (Nishida, 1997).

2.2 3 Stroma

The stroma (or substania propia) is the middle layer of the cornea and is approximately 500μm thick, constituting about 90% of the corneal thickness. The characteristics of the cornea, such as its physical strength, constancy of shape, and transparency are principally based on the anatomic and biochemical characteristics of the corneal stroma.

The corneal stroma is formed by collagen fibrils, keratocytes (corneal fibroblasts) and matrix. The **collagen fibrils** have a high tensile strength and low extensibility. They are extremely uniform in diameter (22.5-35nm) (Giraud et al., 1975; Komai and Ushiki, 1991) and the distance between collagen fibers is also uniform and constant (41.4±0.5 nm) (Hamada, 1976). This regular arrangement of collagen fibers in the stroma contributes to corneal transparency. The collagen fibrils run parallel to one another, forming bundles that are called **lamellae**.

In the corneal stroma, the collagen fibers form about 300 lamellae (Hamada, 1976), which are distributed throughout the stroma. The lamellae run parallel to the surface of the eye and cross each other at various angles. Each lamella contains straight collagen fibrils that extend across the entire cornea, from limbus to limbus. They are stacked on top of each other, gradually becoming more orthogonal in orientation towards the posterior stroma (Meek et al., 1987). In the anterior one-third of the stroma, the lamellae are thin, and branch and interweave more than in the deeper layers (Goldman et al., 1968; Komai and Ushiki, 1991). In the posterior two-thirds of the stroma, the arrangement is more regular and the lamellae become larger (Komai and Ushiki, 1991). In the innermost layer, adjacent to Descemet's membrane, the

fibrils interlace to form a thin collagenous sheet that contributes to the binding between the stroma and Descemet's membrane. This anatomical variation between anterior and posterior lamellae may partly explain the tendency of the cornea to swell from a posterior to an anterior direction (Nishida, 1997).

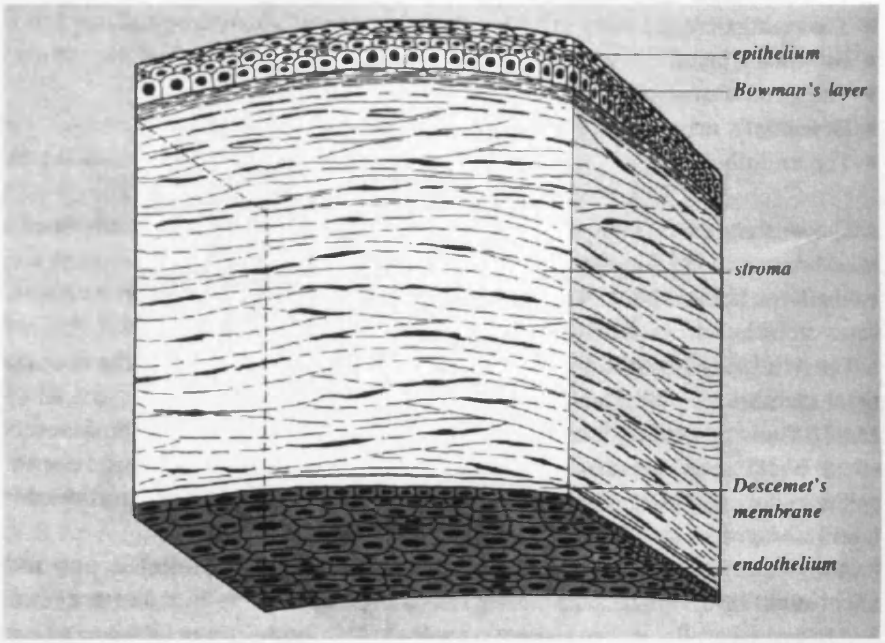


Fig 2.2: Cross-section of the cornea (From Saude, T: Ocular Anatomy and Physiology, Blackwell Scientific Publications, Oxford, 1993).

Fibroblasts or keratocytes are the principal cell of the stroma and occupy 3-5% of the stromal volume (Klyce and Beuerman, 1988). They are flattened cells, with scant cytoplasm, lying between the collagen lamellae (Poole et al., 1993). They have a spindle shape with long processes that extend horizontally. Recently, a number of researchers have reported the existence of gap junctions between keratocytes (Muller et al., 1995; Watsky, 1995; Doughty et al., 2001). Through the gap junctions the keratocytes can communicate across the cornea in both the horizontal direction and vertically. The highest density of keratocytes is found anteriorly and this declines

posteriorly by as much as 30% (Moller-Pedersen and Ehlers, 1995; Petroll et al., 1995).

2.2.4 Descemet's Membrane

Descemet's Membrane is the basement membrane of the corneal endothelium, and is 3-20μm thick. It is continuously synthesized at a high rate throughout life by the endothelial cells. At birth, the membrane is approximately 3μm thick, and it grows at a rate of 1μm per decade (Johnson et al., 1982). The most anterior portion is the oldest and is also the least uniform.

Although no elastic fibers are present, the collagen fibrils are arranged in such a way that they give an elastic property to the membrane. It consists of laminim and fibronectin, and it has been suggested that fibronectin has a role in the adhesion of the endothelial cells to the membrane (Gospodarowicz et al., 1979; Waring et al., 1982).

Descemet's membrane is tightly adherent to the posterior surface of the corneal stroma and reflects any change in the shape of the stroma. If the corneal stroma swells, folding of Descemet's membrane can be observed clinically. When Descemet's membrane is ruptured by physical stress, such as compression birth injury, aqueous humour penetrates into the corneal stroma, resulting in stromal oedema. Descemet's membrane does not regenerate, but if endothelial cells migrate over the denuded stroma at the site of a tear, Descemet's membrane covers the ruptured area, and the stromal oedema subsides clinically (Nishida, 1997).

2.2.5 Endothelium

A single layer of corneal squamous epithelial cells forms the **Endothelium**, which cover the posterior surface of Descemet's Membrane in a well-arranged mosaic pattern (Pepose and Ubels, 1992). The endothelial cells are polygonal, mostly hexagonal in shape, and are about 20μm in diameter and 5μm thick. In the normal cornea, the dimensions of endothelial cells are quite uniform. The anterior surface of the endothelial cells lies flat against Descemet's Membrane and the posterior surface bulges into the anterior chamber, and forms microvilli and marginal folds, thereby exposing greater cell surface area to the aqueous humour (Pepose and Ubels, 1992; Nishida, 1977; Bergamanson, 2001).

At birth, endothelial cell density is about 4000 cells/mm^2, but because the cells do not undergo mitosis, the density decreases throughout life to about 2000 cells/mm^2 in the eighth or ninth decades of life (Bourne and Kaufman, 1976). However, the cells have the ability to enlarge and to maintain tight apposition with neighbouring cells. Transmission electron microscopy has shown that several junctional complex structures are present (zonula occludens, macula occludens, macula adherens), but no desmosomes (Pepose and Ubels, 1992). Along the lateral sides, but near the apical side, junctional complexes are found. These include tight junctions (zonula occludents), gap junctions, and intermediate junctions. The gap junctions are concerned with intercellular communication, allowing the penetration of small molecules and electrolytes between endothelial cells, while the intermediate junctions (zonula adherents) provide cell-to-cell adhesion. This inter-connection between the endothelial cells provides a barrier to the aqueous humour entering the stroma (Waring et al., 1982). Clinically, any loss or damage to the corneal endothelial cells

leads to increased imbibition of water by the corneal stroma. Each endothelial cell contains a large numbers of mitochondria and golgi bodies, as well as a large ribosomal content and a pair of centrioles (Marshall and Grindle, 1978).

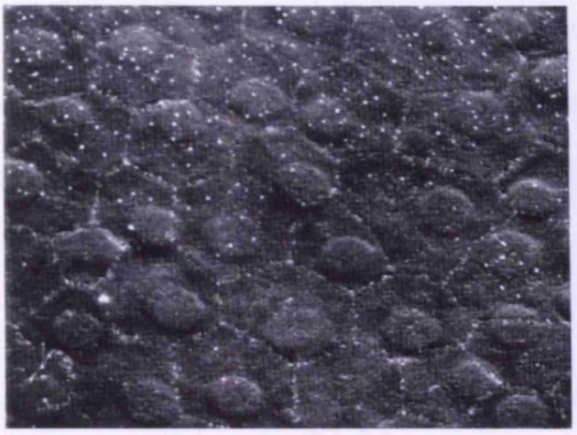


Fig 2.3: Scanning electron micrograph of the human corneal endothelium (Magnification: x1000) (From Hart WM: Adler's Physiology of the Eye, Mosby-Year Book, St Louis, 1992).

The most important physiologic function of the corneal endothelium is to regulate the water content of the corneal stroma, which is normally 78%, and so preserve corneal transparency (Pepose and Ubels, 1992).

2.3 Innervation of the Cornea

The human cornea is a densely innervated structure, richly supplied by sensory nerves, characterized as the most sensitive tissue in the body: 300-600 times that of the skin and 20-40 times that of tooth pulp. It is endowed with very sensitive nerves, derived from the trigeminal nerve, which respond to mechanical, thermal, and chemical stimulation, and serve a protective function (Rozsa and Beuerman, 1982).

In addition to these sensory fibres, the cornea contains autonomic sympathetic nerve fibres.

Corneal innervation is important for the maintenance of corneal structure and function, and provides protective mechanisms against factors that might be potentially damaging to the cornea (Muller et al., 2003). Innervation also plays an important trophic function in corneal repair in relation to disease, trauma or surgery (Ishikawa et al., 1994; Linna et al., 1998; Murphy et al., 1999a). Denervation and decreased corneal sensitivity are associated with impairment of epithelial and endothelial cell function, increased epithelial and endothelial permeability, decreased cell migration and cell mitosis (Auran et al., 1955). In addition, denervated corneas are predisposed to epithelial or stromal abnormalities, recurrent erosion, impaired wound healing, and infection (Rosenberg et al., 2000).

2.3.1 Corneal Nerve Supply

Most corneal nerve fibres are sensory in origin and are derived from the **Nasociliary Nerves,** which originate from the **Ophthalmic Branch** of the Vth Cranial Nerve, the **Trigeminal Nerve**, so called because it has three peripheral divisions – Ophthalmic, Maxillary, and Mandibular. However, in some cases, the inferior cornea receives some of its innervation from the Maxillary Branch of the trigeminal nerve. The Ophthalmic Nerve divides into three major branches: Frontal, Lacrimal and Nasociliary. Each of these nerves further sub-divides into terminal branches that innervate the eye and surroundings tissues.

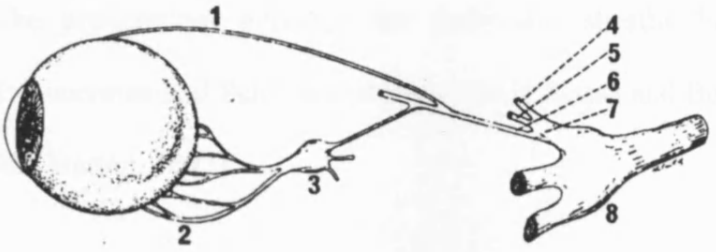


Fig 2.4: Sensory innervation of the eyeball: 1 long ciliary nerves, 2 short ciliary nerves, 3 ciliary ganglion, 4 lacrimal nerve, 5 frontal nerve, 6 nasociliary nerve, 7 ophthalmic nerve, 8 gasserian ganglion (from Draeger J: Corneal Sensitivity, Measurement and Clinical Importance, Springer-Verlag, Vienna, 1984)

The nerves from the nasocilliary branch pass through the **long ciliary nerve** which penetrate the posterior of the eye and pass between the sclera and choroid, coursing anteriorly to supply sensory fibres the cornea, iris, ciliary body, trabecular meshwork and sclera (Burton, 1992; Klyce and Beuerman, 1988).

2.3.2 Corneal Innervation

Nerve bundles enter the cornea from the limbus, in a radial fashion, and run parallel to the corneal surface, alongside the collagen bundles (Muller et al., 2003; Muller et al., 1996). Most of the stromal nerves fibres in humans are located in the anterior third of the stroma (Muller et al., 2001, 2003; Radner and Mallinger, 2002).

The bundles contain around 900-1200 **myelinated** and **unmyelinated** axons of diameter 0.5-5μm. The myelinated nerve fibres lose their myelin sheath within approximately 1mm of the limbus, and continue into the cornea surrounded only by Schwann cells sheaths (Muller et al., 2003). As the axons pass towards the epithelium, they ramify and divide to form a poorly characterized nerve plexus beneath Bowman's Layer, in the superficial stroma. At this level, the nerves are still

considered to be pre-terminal although the perineural sheaths have been lost (Matsuda, 1968; Beuerman and Schimmelpfennig, 1980; Rozsa and Beuerman, 1982; Rozsa et al., 1982; Burton, 1992).

As they course through the stroma, the unmyelinated nerve bundles in the central cornea contain a variable number of axons and are embedded in an electron dense amorphous extracellular matrix. Keratocytes are often located in close proximity to the nerve fibres and will occasionally enwrap adjacent nerve fibres in cytoplasmic extensions (Muller et al., 2003). Before penetration of Bowman's Layer, at the anterior stroma, just beneath Bowman's layer, the stromal nerves fibres form the sub-epithelial nerve plexus.

From the stromal plexus, the innervation of the corneal epithelium is demonstrated by four main structures (Schimmelpfennig, 1982):

1) Stromal nerves penetrating Bowman's Layer.
2) Sub-basal epithelial nerve plexus.
3) Dendritic cells interspersed among the basal cell plexus.
4) Fine nerve endings, originating from the basal epithelial plexus, and dividing dichotomously in the superficial epithelial layer.

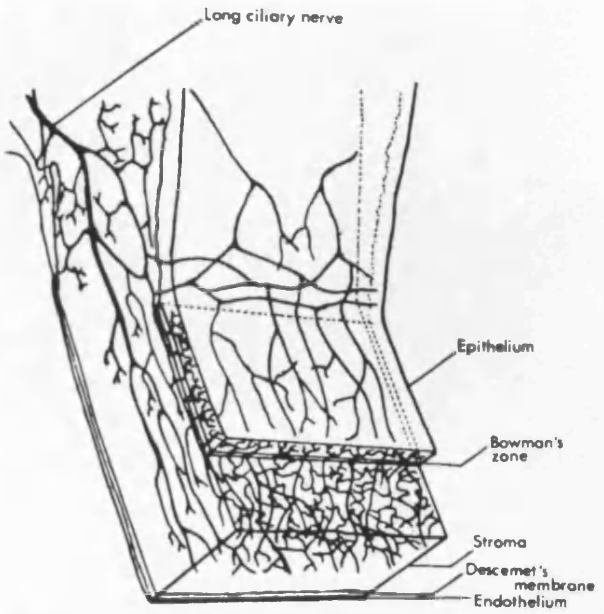


Fig 2.5: Innervation of the limbus and cornea (From Hogan MJ, Alvarado JA, Weddell JE: Histology of the Human Eye, WB Saunders, Philadelphia, 1971).

2.3.2.1 Stromal Nerves Penetrating Bowman's Layer

From the sub-epithelial stromal nerve plexus, the nerves turn abruptly 90° (along temporal to medial axis), and proceed towards the corneal surface. In human corneas, nerves penetrate Bowman's layer throughout the peripheral and central cornea (Muller et al., 1996). After penetrating Bowman's layer, the large nerve bundles divide into several smaller ones. Each small nerve bundle again turns abruptly at 90° (inferior to superior axis), and continues parallel to the corneal surface, between the basal cell layer and Bowman's layer, as an epithelial leash (Muller et al., 2003).

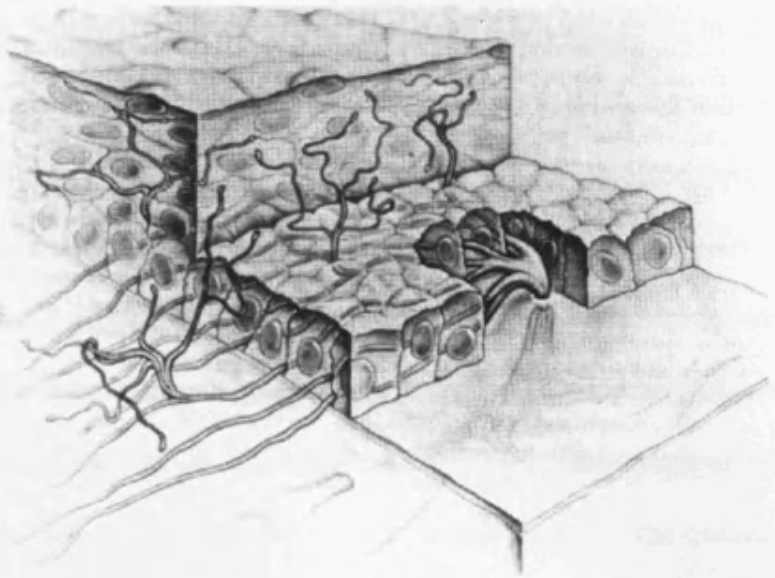


Fig 2.6: Graphical illustration of the three dimensional design of corneal epithelial innervation (From Rozsa AJ, Beuerman RW: Pain 14:105-120, 1982).

2.3.2.2 Sub-Basal Epithelial Layer Nerve Plexus

Long fibres, originating from the penetrating stromal nerves, are the main constituents of the sub-basal epithelial layer plexus which merges above and below Bowman's layer (Muller et al., 1996, 2003; Grupcheva et al., 2002). The fibres are mostly arranged in a parallel fashion and extend for a considerable distance across the cornea. During their course, they are connected by multiple thin, beaded fibres or just bridged by a short branch (Schimmelpfennig, 1982).

The sub-basal epithelial layer nerve plexus contains a mixed population of beaded and straight fibers. Only the beaded fibers bifurcate from the bundle and turn upwards through the epithelium (Muller et al., 2003).

Using *in vivo* corfocal microscopy of the human corneal apex, most studies have found the sub-basal layer nerve fibres to be orientated along the superior to inferior axis (90°) (Masters and Thaer, 1994; Linna et al., 2000; Rosenberg et al., 2000; Vesaluoma et al., 2000a; 2000b; 2000c; Oliveira-Soto and Efron, 2001). However, a few studies show nerve fibres orientated along the temporal to medial axis (180°). In an effort to provide a better understanding of nerve architecture in this sensitive area of the human cornea, Muller et al (2003) examined tissue sections away from the apex, in the nasal, temporal, superior and inferior areas of the cornea. This study revealed that leashes extend across the corneal apex preferentially in the superior/inferior direction. Other leashes approach the apex in the 30°, 60°, 120°, and 150° axes, but they do not reach the corneal apex nor enter the epithelium (Muller at al., 2003).

A recent study of Muller and Pels (ARVO, 2005), looking at the distribution of stromal and sub-basal nerves, established a scheme for the sub-basal nerve plexus in human corneas. At 6 and 12 o' clock large deep stromal nerves have essentially a vertical orientation, whereas nasal and temporal (2, 3, 4 and 8, 9, 10 hours) large nerves have essentially a horizontal orientation. The latter keep their orientation in the mid-anterior stroma and their number seems to exceed that of the vertical orientated nerves. The large nerves run obliquely towards the stromal surface and bifurcate into medium and subsequently into small nerves. In the apex only few small stromal nerves are present, indicating that most of the stromal nerves pass Bowman's layer in the mid-periphery to form the plexus of sub-basal nerves. These sub-basal nerves run parallel to Bowman's layer over 1-4 mm in a vertical direction in the apex, in a vertical or a horizontal direction in the mid-periphery and are present near the

limbus. However, below the limbal epithelium there is a plexus of curved nerves, which found only at the nasal side.

The orientation of the sub-basal nerves in the apex, nasal, temporal, superior and inferior periphery of intact human corneas was also established (Jacobi et al, Arvo 2005). In the apex, thick sub-basal nerves of most pairs of eyes had a preferred 6-12 orientation. In addition eyes had a second preferred orientation, which was for right eyes in the 5-11 and 6-11 direction and for left eyes in the 7-1, 8-2, 9-3 direction, indicating a second preference towards the temporal side. In the periphery screening was performed in the 12, 3, 6, and 9 o'clock position and thin sub-basal nerves ran in the 6-12 direction at the superior and inferior location and in the 3-9 direction at the nasal and temporal location. Because such findings had not been observed in previous publications, one person was scanned along every clock hour and these thin sub-basal nerve appeared to be radially organised along the circumference. In the mid-periphery close to the radial fibres many passages of stromal nerves through Bowman's layer as well as small curved stromal nerves below the epithelium (sub-epithelial nerves) were frequently observed.

The diameter of individual nerve fibres in the sub-basal plexus varies between 0.05 and 2.5 μm and most are in the range of 0.1-0.5 μm. These small sizes are consistent with A-delta and C-fibres, as described by electrophysiological methods (Giraldez et al., 1979; MacIver and Tanelian, 1993a; Belmonte et al., 1997).

2.3.2.3 Dendritic Cells Interspersed among the Sub-basal Epithelial Cell Plexus

Dendritic cells are interspersed within the basal cell layer and are possibly connected with the nerve fibres. The individual cells are dendritic in shape and seem to be interconnected by long processes, some of which are approached by thin, beaded nerve fibres (Segawa, 1964;Schimmelpfennig, 1982).

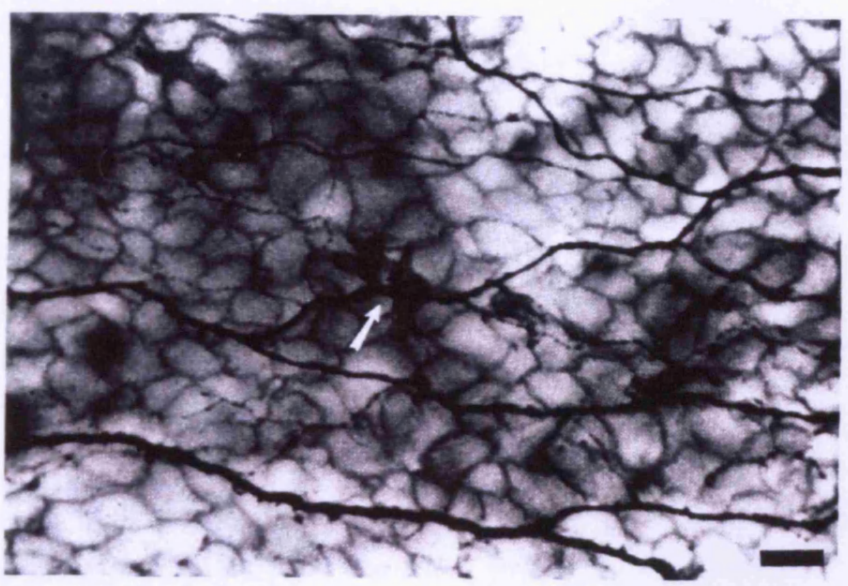


Fig 2.7: Parallel axon terminals are seen within the basal cell layer. Cell outlines of the basal cells are seen, as well as dendritic cells (arrow) (From Kaufman HE, Barron BA, McDonald MB, Waltman SR: The Cornea, Churchill-Livingstone, New-York, 1988).

2.3.2.4 Fine Nerve Endings

Epithelial leashes, arising from the sub-basal epithelial layer nerve plexus, consist of a mixture of straight and beaded nerve fibres. Occasionally, fibres in the leash bundle branch at right angles to the main bundle. Both single nerves and small nerve bundles

protrude between adjacent basal cells, from which they remain separated by unit membranes. Individual beaded fibers, but not straight fibers, separate from the sub-basal bundles and course obliquely through the wing cell layer into the more superficial epithelial cell layers, where they eventually terminate (Muller et al., 2003). Some of the fine nerve endings can extend up to the last desmosomal junction between two superficial cells, and are separated from the external environment only by this junction (Matsuda, 1968; Rozsa and Beuerman, 1982; Schimmelpfennig, 1982; Burton, 1992).

Two different types of fibres are observed and then arranged within the corneal epithelium according to their type. *Myelinated Aδ fibres* are long, straight slender processes of small diameter that respond to mechanical stimuli. They run parallel to the corneal surface at a depth of 10-20μm, for distances of 0.1-1.2mm within the basal cell layer. These endings could originate from the terminal region of single fibres, but more often are seen emerging along the length of fibres running just above the basal epithelial cell layer. *Unmyelinated C fibres* are beaded nerves, and appear as a cluster of bright spots in flat mount, running upwards from the epithelial plexus towards the corneal surface (MacIver and Tanelian, 1993a). They are large in diameter and respond to thermal and chemical stimuli (Tanelian and Beuerman, 1984; Belmonte et al., 1991; Gallar et al., 1993; Muller et al., 1996).

As the nerve fibres pass from the sub-basal epithelial layer nerve plexus towards the corneal surface, they branch extensively producing large receptive fields for each axon. These receptive fields can cover 50-200mm^2 of the corneal surface and may lie as far as three/fourths of the distance across the cornea from the axon's entry point at

the limbus (Zander and Weddell, 1951). Recordings from single nerve fibres in rabbits, has confirmed this innervation pattern by finding fibres that are activated from receptives fields that cover 25-33% of the corneal surface (Mark and Maurice, 1977). However, the receptive fields are typically much smaller in size and range from 5-20% (10.7-45.2mm^2) of the corneal surface (Tanelian and Beuerman, 1984). If we assume that each of the 400 nerve axons that penetrate Bowman's Layer has only one receptive field and that each receptive field covers 5% of the corneal surface, then that receptive field will overlap with 19 others. If each axon's receptive field covers 20%, it will overlap with 80 others. This produces a large level of overlap in the receptive fields which maximises sensitivity, at the expense of localisation.

2.3.3 Corneal Nerve Density

In vivo confocal microscopy studies of corneal nerves in control individuals, has shown an average of 6-8 nerve bundles per image (Rosenberg et al., 2000; Vesaluoma et al., 2000a; 2000b; Oliveira-Soto and Efron, 2001). If we consider that a confocal microscope image is about 0.1mm^2 in size, and that the total surface area of the human cornea is approximately 90mm^2, it can be calculated that there are approximately 5400-7200 nerve bundles in the human sub-basal plexus. Since each sub-basal nerve fibre bundle gives rise to many side branches, each containing 3-7 individual axons (Muller et al., 1997), it can easily be estimated that the total number of axons in the sub-basal epithelial plexus is between 19000-44000. If we assume that one fibre gives rise to 10-20 nerve terminals, then it can be extrapolated that there are between 315000 and 630000, or approximately 7000 nociceptors/mm^2. A homogenous distribution of nerve endings across the cornea guarantees an efficient detection of external stimuli (Muller at al, 2003). In conclusion we have to note that

the nerve bundles found in the sub-basal plexus of the human cornea form a regular dense meshwork over a large central and central-peripheral area. Because of their size the majority of nerves innervating the epithelium are C-fibres (Muller et al, 1997).

2.3.4 Corneal Neurons

Based on the sensory neurons responsiveness to various forms of stimulating energy, several sub-classes of corneal neurons can be distinguished (Belmonte and Gallar, 1996).

2.3.4.1 Mechano-Sensory Neurons

Mechano-sensory neurons that respond only to mechanical forces have been reported in cat and rabbit corneas (Lele and Weddell, 1959; Tanelian and Beuerman, 1984; Belmonte et al., 1991; MacIver and Tanelian, 1993a). These units belong to the highest conduction velocity group of corneal neurons (A-δ) and give large amplitude, fast action potentials. Pure mechano-sensory units represent about 30% of the population of the thin, myelinated fibres innervating the cat's cornea (Lele and Weddell, 1959; Belmonte et al, 1991) and about two-thirds of corneal sensory fibres in the rabbit (Tanelian and Beuerman, 1984).

Mechano-sensory units are more easily excited by a moving stimulus than by a sustained indentation (Mosso and Kruger, 1973; Belmonte and Giraldez, 1981). Sustained indentations evoke a burst of nerve impulses, whose duration, latency and instantaneous frequency are roughly proportional to the amplitude of the stimulus and

velocity of corneal indentation. In most units, long lasting mechanical pulses cause a complete adaptation of the response (Belmonte et al., 1997).

Within the receptive field of a mechano-sensory neuron, there are differences in thresholds between the center and the periphery. In corneo-scleral units of the cat, the threshold is usually lowest in the limbus, but increases by two to three times as the stimulus is moved into the sclera (Belmonte et al., 1991). MacIver and Tanelian (1993a) showed that the receptive fields of mechano-sensory units have an elongated shape, corresponding to the trajectory of the fibre observed with fluorescence microscopy. Electrical stimuli moving parallel to the long axis of the receptive area produced maximum activation, while perpendicular stimuli were less effective. This organization may provide a certain degree of directional sensitivity to this type of fibre.

2.3.4.2 Polymodal Neurons

The existence of a separate population of Aδ and C corneal neurons, each of which can respond to mechanical forces, temperature changes and chemical agents, was established through the application of controlled mechanical, thermal and chemical stimuli to the cat's cornea, while recording single unit activity of Aδ and C ciliary nerve afferents (Giraldez et al., 1979; Belmonte and Giraldez, 1981; Belmonte et al., 1991; Chen et al., 1995; Gallar et al., 1993). These polymodal neurons are the most abundant class of corneal sensory unit found in the cat. Corneal polymodal neurons have large receptive fields (about $25mm^2$), that often cover the adjacent episclera. They are usually silent at rest but may fire occasional spikes at very low frequency

(0.06/sec in A-δ, 0.1/sec in C fibres) in the absence of intended stimulation or corneal damage.

- *Response to mechanical forces*

Polymodal units respond to mechanical stimulation of the cornea with an irregular discharge of impulses like the pure mechano-sensory neurons. However polymodal afferents more often show spontaneous activity and have a slightly lower mechanical threshold. In response to a sustained mechanical indentation, they give a tonic, irregular discharge that persists throughout the stimulus with a variable degree of adaptation. This adaptation is roughly proportional to the intensity of the applied force. They also show a post-discharge after high intensity stimuli and fatigue when these are repeated at short intervals. All these response characteristics – tonic discharge, fatigue and long lasting post-discharge, are more prominent in unmyelinated C fibres, than in thin myelinated Aδ polymodal units (Gallar et al., 1993).

- *Response to temperature changes*

Heating the corneal surface excites both Aδ and C polymodal neurons when temperatures over 38-39°C are attained. The response to a sudden, suprathreshold temperature elevation consists of an accelerating train of impulses, whose frequency reaches a peak and then decays gradually to a lower, maintained level. During sustained heating of the cornea, this impulse discharge is irregular. Temperature increases between threshold and noxious levels are encoded by proportional elevations of the mean firing frequency of the impulse discharge. Returning to basal temperature stops firing transiently. Nevertheless when noxious thermal levels have

been exceeded, causing tissues damage, activity resumes after a few seconds later as an irregular, low-frequency, background impulse discharge (Giraldez et al., 1979; Belmonte and Giraldez, 1981; Belmonte et al., 1991; Gallar et al., 1993).

Cold is usually ineffective in activating corneal polymodal units. Only a small proportion of Aδ afferents were weakly excited in the cat by temperature decreases within the noxious range (Belmonte and Giraldez, 1981). In fact, temperature decreases below 20°C tend to diminish or silence background activity of polymodal neurons (Belmonte et al., 1997).

- *Response to chemicals*

In the cat's cornea, afferent units exhibiting sensitivity to mechanical stimuli (and to heat, when this stimulus was tested), where classified as polymodals if they also responded to acid and/or hyperosmolar NaCl (Belmonte et al, 1991; Gallar et al, 1993; Chen et al, 1995).

Local decreases in pH have been produced on the cornea by applying solutions of increasing acetic acid concentrations (down to pH 3.0), or using gas jet of CO_2, which combines with water to produce carbonic acid locally (Chen et al, 1995). About 60% of corneal fibres exhibiting mechano-sensitivity also respond, after a short latency, to acidic stimulation with acetic acid or CO_2 producing a discharge of impulses. In 15% of the fibers that were sensitive to CO_2, the impulse discharge appeared after a long latency (10-20 sec) (Chen et al, 1995).

Acetylcholine (ACh) and the enzymes required for its synthesis (AChE) are present at high concentrations in the corneal epithelium of various mammalian species (Alphen and H.M., 1957; Gnadinger et al., 1967; Fitzgerald and Cooper, 1971). The possibility that ACh released by injured corneal cells acts as a sensory mediator in the activation of corneal nociceptive endings was suggested by Fitzgerald and Cooper (1971), based on the observation that hemicholinium, which blocks Ach synthesis, apparently reduced the mechanical sensitivity of the rabbit cornea. Other roles for corneal ACh, such as modulation of ion transport or regulation of the phosphatidylinositol cycle (thus regulating the production of inflammatory mediators from arachidonic acid), were also proposed to explain the high concentration of this substance in corneal tissue (Pesin and Candia, 1982; Proia et al., 1986). However, the functions of ACh in the cornea remain largely mysterious.

2.3.4.3 Mechano-Heat Neurons

The term "mechano-heat nociceptor" was employed to describe cutaneous nociceptors that were presumably polymodal, but in which chemo-sensitivity was not systematically explored (Meyer et al., 1994). In the cornea of the cat, a small proportion of Aδ neurons presented mechanical and thermal sensitivity, but failed to respond initially to acetic or carbonic acids. These units exhibited a high mechanical threshold and developed sensitivity to acid after repeated noxious heating (Belmonte et al., 1991). Likewise, Aδ "bimodal afferents" responding to high intensity mechanical forces and to heat, but not to ACh, have been reported in the rabbit cornea (MacIver and Tanelian, 1993a). The comparatively high mechanical threshold of mechano-heat fibres of the cornea may indicate that they are, in reality, polymodal

nociceptors with the highest chemical threshold, rather than a specific sub-population of nociceptive fibres (Adriaensen et al., 1983; Belmonte et al., 1991).

2.3.4.4 Cold Neurons

Changes in multi-unit impulse discharges of corneal nerves induced by temperature reductions were described in early reports (Lele and Weddell, 1959; Mark and Maurice, 1977). Tanelian and Beuerman (1984) using a saline jet at controlled temperature, detected the existence in the rabbit cornea of sensory fibres conducting in the C range that responded to decreases in temperature. Similar units have been identified in the cat's cornea, where their functional properties have been studied in detail (Gallar et al., 1993).

Cold sensory neurons are unmyelinated and fire spontaneously at the resting temperature of the cornea (around 33°C), giving an irregular discharge of impulses (0.75/sec in the cat). They respond to cooling steps with a vigorous impulse discharge during the temperature drop, whose frequency is roughly proportional to the magnitude of the corneal temperature reduction. However, sustained low temperatures gave similar low frequency impulse discharges irrespectively of their value, indicating that these fibres do not encode steady state corneal temperatures. In accordance with these response characteristics, cold units fire repeatedly when cold air is blown to the cornea, or when a drop of saline is applied (Belmonte et al., 1997).

The receptive fields of corneal cold-sensitive neurons are smaller than those of polymodal units (about $10mm^2$), and are preferentially found in the periphery of the

cornea. Corneal cold fibres do not respond consistently to mechanical stimulation, but have a weak response to acid and hypertonic NaCl.

2.4 Innervation of the Conjunctiva

The conjunctiva is innervated by a plexiform network of sensory fibres originating from branches of the Ophthalmic and Maxillary nerves of the Trigeminal nerve (Oduntan and Ruskell, 1992). This plexus is denser in the palpebral conjunctiva than in the bulbar conjunctiva, especially at the lid margin. The nerve plexus is more concentrated around the blood vessels, the muscles, and Meibomian glands (Luhtala et al., 1991; Luhtala and Uusitalo, 1991 Elsas et al., 1994).

The morphology of the sensory nerves in the conjunctiva is different from that of the cornea. Except for free nerve terminals, the sensory nerves are encapsulated nerve endings in the conjunctiva, with a higher density in the limbal conjunctiva (Oppenheimer et al., 1958; Wolter and Mich, 1964; Lawrenson and Ruskell, 1991, 1993).

The function of the encapsulated nerves is still obscure. von Frey believed that Krause corpuscles were the specific receptors for cold (Oppenheimer et al, 1958). Other studies suggest that the corpuscles in the limbal conjunctiva are the receptors for touch (Lawrenson and Ruskell, 1991; 1993).

2.4.1 Sensitivity of the Conjunctiva

There are a limited number of studies measuring conjunctival sensitivity. The conjunctival mechanical sensitivity is lower than that of the cornea (Boberg-Ans, 1955; Draeger, 1984). The limbal conjunctiva is more sensitive than the bulbar conjunctiva to touch stimuli (Lawrenson and Ruksell, 1993). The edge of the eyelid is more sensitive than the bulbar conjunctiva to mechanical stimulation (Norn, 1973; McGowan et al., 1994). A study of Murphy et al (2002) assessed the response of the corneal and conjunctival sensory nerves to a cooling stimulus. It was found that the temporal conjunctiva was less sensitive than the nasal conjunctiva, as well as the temporal conjunctival and superior corneal locations had similar sensitivities. A recent study of Stapleton et al (2004) showed that inferior conjunctival sensitivity, to cooling stimuli, was lower than that of the central and inferior corneal sensitivity. It was also shown that conjunctival sensitivity was increased after wear of highly oxygen permeable contact lenses but unaffected by wear of low oxygen permeable contact lenses.

2.4.2 Conjunctival Neurons

Most of the sensory nerve endings in the conjunctiva are mechano-sensory afferents (70% in guinea pig), which respond to mechanical stimulation (Aracil et al, 2001). They are Aδ fibres with high conductive speed, small receptive fields (round or oval in shape and about 1 to 3mm^2 in size) and are rapidly adapting. These nerves are denser in the limbus and may morphologically correlate to the low-threshold mechano-sensory units in the cornea (Belmonte et al., 1997; Aracil et al., 2001).

Cold sensitive fibres are also found in conjunctiva with a relatively low proportion (7% in guinea pig). They are Aδ and C fibres, are sensitive to decrease in temperature and have no response to heating (Aracil at al, 2001).

Approximately 23% of guinea pig conjunctival neurons are polymodal receptors, which respond to mechanical, chemical, and heat stimulation. For these receptors, the mechanical threshold is lowest in the limbal region. The thermal threshold of the conjunctival nerves is 2-3 °C higher than in the cornea (Belmonte et al, 1997; Aracil at al, 2001).

Sensations evoked from the human conjunctiva are either cool or warm, when stimulated with thermal stimuli, which seems to be similar to that of the skin (Nafe and Wagoner, 1936; Kenshalo, 1960; Acosta et al., 2001).

2.5 Corneal Sensitivity

2.5.1 What is Corneal Sensitivity?

Corneal sensitivity is the ability of the cornea to respond to a mechanical, electrical, chemical or thermal stimulus, and is maintained by the free nerve endings within the epithelium of the cornea. Mechanical stimulation causes a sensation of touch or pain, electrical stimulation of pain or irritation, chemical stimulation of irritation, hot thermal stimulation of irritation and cold stimulation of cooling (Beuerman and Tanelian, 1979; Tanelian and Beuerman, 1984; Belmomte and Gallar, 1996). The patient's response to these different stimuli can be measured to assess the corneal

nerve function. Corneal sensitivity is measured using an instrument, called an Aesthesiometer.

Measuring corneal sensitivity is important for a number of reasons:

- It assesses the integrity of the cornea.
- It assesses the recovery of the cornea from any condition that has affected it, by observing the return of corneal sensitivity to normal levels.
- It assesses the baseline corneal sensitivity of each eye before contact lens fitting, allowing a better management of the patient.
- It ensures that corneal anaesthesia with a given anaesthetic is of sufficient depth to perform the appropriate procedure.

2.5.2 Methods of Measuring Corneal Sensitivity: Aesthesiometry

The first approach for the assessment of corneal sensitivity was by von Frey in 1894 who used horse hairs of different lengths attached with wax to the tip of a glass rod. These hairs had different tip configurations, evoking both touch and pain sensation, and they were calibrated on a precision scale. Other aesthesiometers that used the same, or some other method, were devised over the following years. However, in 1955, Boberg-Ans introduced his aesthesiometer that used a single nylon monofilament of constant diameter which could be varied in length to produce different forces when applied against the cornea.

In 1960, Cochet and Bonnet constructed an improved aesthesiometer, based on the Boberg-Ans instrument. The principal advance was to increase the diameter of the thread from 0.112mm to 0.12mm. This improved the mechanical stability of the

thread and allowed the maximum length to be increased from 55mm to 60mm. This, in turn, increased the possible range of stimulus intensities to 11-200mg/mm^2. Their improvements led the Cochet-Bonnet aesthesiometer to be the most widely used clinical aesthesiometer (Cochet and Bonnet, 1960). Details of its design and method of use and measurement are given in section 3.1.2.

However, the disadvantages of the Cochet-Bonnet aesthesiometer were recognised early. One of the important limitations is that it has a restricted range of stimulus pressure intensities, and often presents stimuli of supra-threshold levels. Another deficiency is the invasive and visible nature of its stimulus, both of which can modify the corneal touch threshold either by producing a slight trauma to the corneal epithelium or by making subjects anxious when they see the thread approaching their eyes. It also limits its clinical use, as it is not suitable for patients with corneal injury or after surgery (Murphy et al., 1996).

The development of ocular surface aesthesiometry underwent a change with the introduction of pneumatic aesthesiometry, overcoming most of the drawbacks of Cochet-Bonnet Aesthesiometer (Bonnet and Millodot, 1966; Millodot and O'Leary, 1981; Murphy et al., 1998). Pneumatic aesthesiometry uses air to evaluate the corneal and conjunctival sensitivity (Weinstein et al., 1992; Chen et al., 1995; Murphy et al., 1996; Vega et al., 1999; Belmonte et al., 1999). The pneumatic aesthesiometers of Murphy et al (1996) and Vega et al (1999) use a controlled pulse of atmospheric air aimed at the cornea, whereas the Belmonte Aesthesiometer (1999) uses air and CO_2 which can be mixed together at different temperatures to deliver chemical, thermal, and mechanical stimuli.

The Non-Contact Corneal Aesthesiomer (NCCA) (Murphy et al., 1966) uses a controlled pulse of air, directed at the corneal surface, to stimulate the sensitive cold C-fibres of the corneal epithelium. Further explanation of its design and method of use and measurement are described in section 3.1.1.

When the Cochet-Bonnet aesthesiometer was compared with a pneumatic aesthesiometer, no correlation in the measured thresholds was found, suggesting that this difference in results was due to the different modes of stimulation exerted by the nylon thread and the pneumatic air (Murphy et al., 1998; Vega et al., 1999; Acosta et al., 2001).

2.5.3 Factors Affecting Corneal Sensitivity

2.5.3.1 Physiological Factors

2.5.3.1.1. Corneal Topography

As described by Millodot (1984) using the Cochet-Bonnet Aesthesiometer, corneal sensitivity varies from a maximum at the centre of the cornea (average CTT, 10-14mg/mm^2) to a minimum in the periphery (average CTT, 20-30mg/mm^2). The superior region of the cornea, which is covered frequently by the upper lid, has the lowest sensitivity (average CTT, 30-45mg/mm^2). The inferior region has a similar sensitivity to the nasal and temporal periphery (average CTT, 25-30mg/mm^2). The values correspond well with anatomical findings showing nerve density to be highest centrally (Rozsa and Beuerman, 1982; Millodot, 1984; Lawrenson and Ruskell, 1993; McGowan et al., 1994). Similar findings were found by Roskowska et al (2004) who

also used mechanical stimulation using the Cochet-Bonnet Aesthesiometer, with the central cornea being more sensitive, and the horizontal meridian being more sensitive compared with the vertical meridian.

2.5.3.1.2 Age

Corneal sensitivity was found to remain unchanged between the ages of 10-50 years, with only a slow gradual reduction, as assessed by mechanical stimulation. Beyond that age, it diminishes rapidly, reaching half of the previous level after 65 years, and then continues to decline as age increases (Millodot, 1977a, 1984). A recent study of Roskowska et al (2004) evaluating the central and peripheral corneal sensitivity in relation to age, using the Cochet-Bonnet aesthesiometer, found that corneal sensitivity remains stable in the central zone until the age of 60, when it begins to decrease. The decrease of peripheral sensitivity starts earlier and it progresses at a faster rate. They concluded that the age-related decrease in corneal sensitivity involves the periphery at first and successively extends towards the centre. Finally, a recent study of Murphy et al (2004), using the Non-Contact Corneal Aesthesiometer to assess the cooling sensation threshold, demonstrated a gradual reduction in corneal sensation between the ages of 20 and 50 years.

2.5.3.1.3 Iris Colour

A striking finding is the effect of iris colour on corneal sensitivity as assessed by mechanical stimulation (Millodot, 1975a, 1976a). People with blue eyes have more sensitive corneas than those with brown eyes, and non-Caucasians with dark brown

irides usually have less sensitive corneas than Caucasians. However, the mechanism for these differences is still unknown (Millodot, 1975a, 1976a; Tota and La Marka, 1982). This effect of iris colour on corneal sensitivity will be further investigated in this thesis (Chapter 6).

2.5.3.1.4 Diurnal Variation

Corneal sensitivity shows a variation in its threshold during the day, being lowest in the morning and highest in the evening (du Toit et al., 2003; Millodot, 1972). Millodot (1972), considering the sensitivity to a touch stimulus, found a variation of about 28%, whereas du Toit et al (2003) considering the sensitivity to a cooling stimulus, using the noncontact pneumatic aesthesiometer (Vega et al., 1999) found a variation of 35%. This reduction in the sensitivity can be attributed to the reduction in oxygen tension at the epithelial surface with eyelid closure (Efron and Carney, 1979; Fatt and Hill, 1970). The diurnal variation of corneal sensitivity is further investigated in this thesis.

2.5.3.1.5 Hormonal Influences

Males and females present the same corneal sensitivity as assessed by both mechanical and thermal stimulation (Roskowska et al, 2004; Murphy et al, 2004), except for several days during the pre-menstruation and the onset of menstruation where sensitivity is reduced in women (Millodot and Lamont, 1974; Riss et al, 1982). In women taking contraceptives, no hormonal changes occur and the sensitivity remains more or less equal (Millodot and Lamont, 1974; Guttridge, 1994). Changes

in the sensitivity threshold also occur during pregnancy, with significant reductions in the last few weeks before delivery (Martin and Safran, 1988; Millodot, 1977b, 1984; Riss and Riss, 1981).

2.5.3.1.6 Environmental Factors

Ambient temperature can cause an effect on corneal sensitivity. Kolstad (1970) using a mechanical stimulation, observed a nine-fold reduction in sensitivity when the outside temperature changed from 22 to -14°C. This may explain the relative comfort of contact lens wearers in cold weather conditions (Kolstad, 1970).

UV radiation exposure, between 280-310nm, produces a reduction of about 75% in the normal level of corneal sensitivity. This reduction lasts for two hours approximately, after which the subject will experience severe pain (Millodot, 1984; Millodot and Earlam, 1984; Bergmanson, 1990).

2.5.3.1.7 Pharmacological Factors

The topical administration of anaesthetics to the eye produces a partial or complete loss in corneal sensitivity depending on the concentration, volume and efficacy of the drug used (Polse et al., 1978; Weiss and Goren, 1991; Lawrenson et al., 1993; Murphy et al., 1997; Nomura et al., 2001; Peyman et al., 1994). The effect of anaesthetics on corneal sensitivity is discussed in greater detail in Chapter 5.

Timolol is a beta-blocker widely used in the treatment of glaucoma to lower intraocular pressure. However, it has some anaesthetic effect and can produce a reduction in corneal sensitivity in some subjects. The effect only lasts for a short period of time (Van Buskirk, 1979; Martin and Safran, 1988; Weissman and Asbell, 1990). Only Kitazawa and Tsuchisaka (1980) reported no change in corneal sensitivity during four weeks of timolol administration.

The effect of topical administration of non-steroidal anti-inflammatory drugs, used to reduce fever and alleviate pain that accompanies injury or inflammation, on corneal sensitivity was demonstrated in rabbit (Loya, 1993; Loya et al., 1994) and human corneas (Szerenyi et al., 1994; Perry et al., 1995; Tauber et al., 1995; Aragona et al., 2000). A significant reduction in sensitivity occurred with repeated applications, but once instillation stopped there was a complete recovery to previous levels within 60 minutes.

2.5.3.2 Contact Lens Wear

Contact lens wear can produce a reduction in corneal sensitivity, depending on the contact lens type, the oxygen permeability of the material, the extent of daily wear, the years of wear, or whether the lenses are daily or extended wear types (Millodot, 1976b, 1984; Larke and Hirji, 1979). This diminution of sensitivity is beneficial, as it helps the subject to adapt more easily to the lenses.

Most studies have shown this reduction in sensitivity, which has been attributed to mechanical adaptation (Bradley and Schoessler, 1979; Millodot, 1976b; Murphy et

al., 2001), and the suppression of sensory nerve function by metabolic change, especially from tissue acidosis resulting from hypoxia (Thoft and Friend, 1975; Brennan and Bruce, 1991;).

A fuller review of the effect of contact lenses on corneal sensation is given in the review paper in Appendix.

2.5.3.3 Ocular and Systematic Disease

Corneal aesthesiometry can play a useful role in the diagnosis and management of many ocular conditions (Boberg-Ans, 1956; Brennan and Bruce, 1991), since corneal sensitivity is affected.

In glaucoma, there is a direct relationship between a decreased corneal sensitivity and optic atrophy, but no relationship has been found between increased intraocular pressure and loss of corneal sensitivity (Boberg-Ans, 1955).

Patients with diabetes mellitus have shown a decreased sensitivity as assessed by mechanical stimulation, but a better correlation is found between corneal sensitivity and retinal status (Schwartz, 1974; Nielsen, 1978; Rogell, 1980; Martin and Safran, 1988; Ruben, 1994; Hosotani et al., 1995; McNamara et al., 1998; Rosenberg et al., 2000). Eyes with normal fundi have normal corneal sensitivity, while those with background retinopathy show a somewhat decreased sensitivity, and those with proliferative retinopathy have a greater loss of corneal sensitivity (Rogell, 1980). It has also been shown that there is a trend of reduction in sensitivity with the duration

of diabetes (Rosenberg et al., 2000). This reduction in sensitivity is believed to be due to diffuse neuropathy affecting the peripheral sensory nervous system.

Also, a reduction in corneal sensitivity has been observed in Herpetic Keratitis, and a relationship has been shown between the severity of the disease and the loss of corneal sensitivity indicating that the more severe the disease, the greater the loss of the sensitivity. Recovery of sensitivity after superficial keratitis takes more than two years (Norn, 1970; Martin and Safran, 1988; Kodama et al., 1992; Rioux and Brunette, 1995).

Corneal Dystrophies, like Lattice Corneal Dystrophy (Type I), Hereditary Fleck Dystrophy, and Reis-Buckler dystrophy, are known to produce a decrease in corneal sensitivity (Boberg-Ans, 1955; Birndorf and Ginsberg, 1972; Millodot, 1984).

In Keratoconus there is a reduced corneal sensitivity, especially in the central area. The amount of reduction is inversely proportional to the severity of the cone (Millodot and Owens, 1983).

In Scleritis and Episcleritis there is a reduction in corneal sensitivity, although the patients with scleritis are more affected than patients with episcleritis. Sensitivity returns to normal when the scleritis is resolved except if a large area of scleral ectasia is present (Boberg-Ans, 1955; Lyne, 1977).

In Leprosy, a significant loss of sensitivity can occur, leading to corneal injuries that may lead to ulceration. This loss of sensitivity can result without any clinically

detectable eye pathology (Martin and Safran, 1988; Karacorlu et al., 1991; Hieselaar et al., 1995). In Myasthenia Gravis a reduced corneal sensitivity has been found to occur (Nazarian and O'Leary, 1985), as well as in Adie's Syndrome (Purcell et al., 1977).

2.5.3.4 Ocular Surgery

Ocular surgery, and particularly corneal refractive surgery, has the potential to disrupt the normal organization of corneal innervation, thus damaging corneal sensitivity. The extent of nerve loss and nerve regeneration depends on the type, depth, and extent of incision made during surgery (Lyne, 1982; Macalister et al., 1993; Murphy et al., 1999a). The recovery of the sensitivity can be correlated to the regeneration of the nerves Wilson, 1999(Kohlhaas, 1998; Wilson, 1999; Murphy et al., 1999a; Patel et al., 2001; Kaminski et al., 2002; Kumano et al., 2003).

2.5.3.5 Therapeutic Ocular Surgery

In traditional, large incision **Cataract Surgery**, the incision made cuts both the limbal nerves plexus and the large centripetal nerve fibres, causing the corneal epithelium and the stroma supplied by these nerves to become denervated. The recovery of sensation shows a small improvement one year post-operatively, but even after two years it is still below baseline levels for most cases (Guillon and Morris, 1982; Holden et al., 1982; Lyne, 1982; Kohlhaas, 1998). With small incision cataract surgery, central corneal sensation was found to remain intact (John, 1995). It has also

been found that corneal sensitivity after corneal incision cataract operation was more decreased than by scleral incision (Kadonosono et al., 1995).

Corneal transplantation, or **Penetrating Keratoplasty (PK)**, produces a complete loss of sensitivity within the graft. Corneal sensitivity is not detectable until 18 months post-operatively at the earliest. There is a slow recovery initiated from the periphery and progressing towards the centre of the graft (Ruben and Colebrook, 1979; Rao et al., 1985; Mathers et al., 1988; Tugal Tutkun et al., 1993). Skriver (1978) reported a return of corneal sensitivity to normal levels within 12 months after PK. However, Rao et al (1985) found that even 32 years post-operative, the graft was still hypoaesthetic. Macalister et al (1993) also found that even 4 years post-operative two thirds of the subjects had no central sensitivity and only 9% had normal sensitivity. After seven years of the transplantation, 39% of the subjects had no measurable sensitivity.

In **Trabeculectomy** or **Iridectomy**, the small incision arc causes small nerve damage, causing a sensitivity loss. Recovery to pre-operative levels occurs within nine months (Lyne, 1982).

In **Retinal Detachment** surgery, there is a significant decrease in sensitivity in eyes that were treated with an encircling band, whereas in eyes that were treated with a localised radial or circumferential silicone sponge explants alone, no significant decrease in sensitivity was found. The effect of sensitivity seems to be long-term, without correlating with the post-operative time (Binder and Riss, 1981; Gibson, 1981).

2.5.3.5 Refractive Corneal Surgery

In **Epikeratophakia**, a technique that involves the grafting of a lenticule of donor tissue to the anterior surface of the cornea, there is a damaging of the radial nerve fibres located at the mid-stromal level (Koenig et al., 1983). The new anterior surface has no nerve supply and a new innervation must develop. The sensitivity of the central corneal epithelium overlying the donor lenticule is totally depressed three years after surgery.

In **Radial Keratotomy (RK)**, one of the two principal techniques for altering the curvature of the cornea, radial incisions are made to flatten the cornea. The degree of flattening depends on the type, depth, and number of incisions made, and the extent of sensitivity loss is also defined by these factors (Waring et al., 1983). The normal radial incisions cause a small damage on the corneal nerves as they are made along the axis of the radiating stromal nerve fibres. For the correction of astigmatism, a transverse incision will cut across the nerve fibres in a similar manner to cataract surgery and with a similar effect on sensitivity. A deeper incision will cause a greater damage to the nerves. Linnik (1984) reported a complete recovery of corneal sensitivity six months after radial keratotomy. Shivitz and Arrowsmith (1988) found that with an incision of less than 80% corneal thickness, 72.8% of patients had a normal sensitivity after one year, whereas with 90% corneal thickness incisions, no recovery of sensation occurred after one year. Kohlhaas et al (1994) showed that corneal sensitivity returned to normal levels one month after surgery.

Photorefractive Keratectomy (PRK) is the second principal surgical technique for refractive correction. This method utilises excimer laser technology that affects the

corneal nerve supply in a totally different way to incision surgery (Marshall et al., 1985). Rather than cutting the nerves by incision, the entire neural architecture within the zone of ablation is obliterated. The ablation removes the corneal epithelium, Bowman's membrane and the anterior stroma in the ablated area, causing damage to the epithelial nerve endings, epithelial and sub-epithelial nerve plexi, and in stromal nerves. Thus the loss of corneal sensitivity is more complete, and the extent and pattern of nerve degeneration is altered significantly (Tervo et al., 1994; Murphy et al., 1999a).

Many studies have been conducted on the effect of PRK on corneal sensitivity. Campos et al (1992) measured corneal sensitivity in 14 patients who underwent PRK for either compound astigmatism or severe myopia. They reported a mild decrease in sensitivity that did not persist for longer than three post-operative months. Ishikawa et al (1994) studied the changes in corneal sensation after PRK in 17 myopic eyes, dividing the subjects into two groups of shallow (0-30μm) and deep (31-70 μm) ablation depths. It was found that the recovery was dependent on the depth of ablation. For the shallow group the sensitivity returned to pre-operative levels within one month, while the deep ablation group had sub-normal sensitivity even six months post-operative. Kohlhaas et al (1994), using the Draeger aesthesiometer to measure corneal sensitivity in 156 eyes that had PRK surgery for correction of myopia, found that corneal sensitivity for patients with a pre-operative myopia up to 15 dioptres had a mildly reduced sensitivity one year after surgery. In contrast, for patients with pre-operative myopia up to 25 dioptres, there was a reduced sensitivity even two years after surgery. Lawerenson et al (1995, 1997) found corneal sensitivity to have recovered by six months after PRK surgery and for ablation depths up to 78 μm to not

affect the result. Murphy et al (1999a) investigated the effect of corneal sensitivity after PRK surgery using the Non-Contact Corneal Aesthesiometer and found that corneal sensitivity did not recover to pre-operative normal levels until 12 months. Additionally, for all the PRK procedures used in this study, which removed tissue over a range of depths from 26 to 78 μm, no depth effect on the recovery of corneal sensitivity was detectable at one year post-operatively. Perez-Santonja et al (1999) found a recovery after one month for nasal, inferior, temporal, and superior cornea, but recovery of the central cornea occurred three months post-operatively. Matsui et al (2001) reported that corneal sensitivity began to recover at 1 week, returning to pre-operative values at three months. Kumano et al (2003) reported no decrease in central corneal sensitivity at any post-operative interval after PRK, using a 6.5mm diameter laser and a beam of 6mm diameter to perform the ablation.

Laser in Situ Keratomileusis (LASIK) introduced by Pallikaris and Siganos (1990), offers good results for the correction of moderate and severe myopia, astigmatism and, more recently, hyperopia (Pallikaris et al., 1991; Pallikaris and Siganos, 1994; Guell and Muller, 1996; Salah et al., 1996; Perez-Santonja et al., 1997a, 1997b, 1997c; Ibrahim, 1998; Nassaralla et al., 2000, 2003). This technique combines the use of an excimer laser, for the refractive ablation, with a lamellar corneal flap technique. In LASIK, a corneal flap allows the excimer laser to be directed at a deeper region of the stroma, sparing the epithelium and Bowman's layer. The micro-keratome, used to create the flap, cuts the sub-basal nerve fibre bundles and the superficial stromal nerves in the flap margin, but spares the nerves within the hinge of the flap. The higher the refractive error correction, the deeper the ablation and the greater the amount of corneal tissue removed (Pallikaris et al, 1991). However, the

flap hinge preserves some of the corneal epithelial innervation and so the magnitude of corneal sensitivity loss after LASIK may be less than the loss after PRK (Kanellopoulos et al., 1997; Perez-Santonja et al., 1999; Patel et al., 2001).

The majority of studies using a mechanical stimulus to assess the recovery of corneal sensitivity after LASIK have shown that corneal sensitivity returns to normal levels by six months after surgery (Kanellopoulos et al, 1997; Perez-Santonja et al, 1999). Although Kim and Kim (1999) showed that corneal sensitivity did not return to pre-operative levels by 6 months after LASIK, in their study the laser treatment was not only for myopia, but also for compound myopic astigmatism that requires a different laser treatment pattern. Linna et al (2000) investigated the effect of LASIK on corneal sensitivity, and considered whether the morphology of the sub-basal nerves corresponds to corneal sensitivity after LASIK (Linna et al., 2000). Corneal sensitivity returned to pre-operative levels after six months of surgery. In *vivo* confocal microscopy reveals that LASIK induces alterations in sub-basal nerve morphology, thus enabling a direct comparison of corneal sensory innervation and sensitivity. Chuck et al (2000) reported that corneal sensation returned to near pre-operative levels by 3 weeks after LASIK. This observed time is substantially shorter than previously reported works (Chuck et al., 2000). Patel et al (2001), using a cool stimulus, found that corneal sensitivity was below normal levels at 14 weeks post-operatively. Nassaralla et al (2003), in an effort to evaluate the changes in corneal sensitivity after LASIK for the correction of different degrees of myopia, used a mechanical stimulus and found a full recovery of corneal sensitivity after 6 months for myopia ranging between 0.75-7.75 Dioptres, and after 9 months for myopia ranging between 8-16 Dioptres. The depth of ablation seemed to be an important factor in the

temporary decrease of corneal sensitivity and its recovery. Kumano et al (2003) found that the recovery of corneal sensation began 3 months after LASIK, with a complete recovery after 12 months. A significantly greater decrease in sensitivity was apparent in the LASIK patients with a nasal hinge than in those with a superior hinge. Stapleton et al (2003) showed that that the central corneal mechanical sensitivity was significantly reduced in subjects following LASIK, however inferior mechanical sensitivity and chemical sensitivity at both sites was unaffected. Recovery was seen three months after surgery, with a similar recovery trend to be observed for nerve morphology. A recent study of Donnenfeld et al (2004), investigating the effect of hinge width on corneal sensation after LASIK, found that corneal sensation was significantly reduced from preoperative levels through six months in the narrow-hinge group and through three months in the wider-hinge group, indicating a further loss in corneal sensation in eyes with a narrow hinge flap than in eyes with a wider hinge flap.

Laser Sub-Epithelial Keratomileusis (LASEK) is a modified PRK technique that is based on the detachment of an epithelial flap after the application of an alcohol solution, and then the repositioning of this flap following laser application (Lee et al., 2002b; Camellin, 2003; Herrmann et al., 2005).

A recent study by Herrmann et al (2004) investigated the recovery time of corneal sensation after LASEK for the correction of mild to moderate myopia (range -2.5 D to -8 D). Corneal sensation was significantly reduced at 3 days and 14 days after surgery. It increased during the first month after surgery, reaching baseline levels and staying stable at 3 and 6 months after surgery.

2.6 The Tear Film

2.6.1 Tear Film Structure and Function

A complex liquid known as the pre-ocular tear film covers the bulbar and palpebral conjunctiva and the cornea. The pre-ocular tear film has traditionally been described as a tri-laminar structure, consisting of three distinct layers: a superficial lipid layer, a predominantly watery aqueous phase beneath the lipid layer, and an underlying mucous layer (Wolff, 1946; Holly and Lemp, 1977).

The tear film is vital for normal corneal function and has a number of important roles. It fills in small surface irregularities in the corneal epithelium, thereby providing a smooth optical surface, which allows a sharp image to be focused on the retina. Additionally, since the corneal surface is avascular, it is highly dependent on the tear film for its nutrition. Oxygen from the ambient air dissolves in the tear fluid and is transferred to the corneal epithelium. Furthermore, it provides a slight amount of nourishment to the corneal epithelium, although the glucose concentration is extremely low. Finally, the tear film is the first line of defence against ocular surface infection. This is achieved by the anti-bacterial activity of certain constituent proteins and enzymes, the principal one being lysozyme (Milder, 1987).

The thickness of the human tear film is controversial, with widely different published results. Early estimates of tear film thickness were based on invasive tests, such as placing glass fibres against the cornea (Mishima, 1965), measuring fluorescence after instilling fluorescein (Mishima, 1965; Benedetto et al., 1975), or applying absorbent paper to the ocular surface (Ehlers, 1965). These methods produced thickness

estimates of between 4μm (Benedetto et al., 1975) and 8μm (Ehlers, 1965). Later, using confocal microscopy and interferometry, greater values were reported of 34-35μm (Prydal et al., 1992; Prydal et al., 1993) and 40μm (Prydal et al., 1992) in humans. It was proposed that this extra thickness was the result of previous under-estimation of the deeper, denser layers of mucous (Prydal et al., 1993). Using non-contact optical interferometry, (Danzo et al., 1994) gave values of 10.3 and 12.3μm . Slit-lamp measurements (Creech et al., 1998) gave a value of 10 μm, and examination of reflectance spectra (King-Smith et al., 2000) and optical coherence tomography (Wang et al., 2003) suggest that the human tear film is approximately 3μm thick. It seems quite clear that an agreed measurement of the tear film thickness is still lacking.

Although there is evidence that the tear film is uniform (Prydal et al., 1992, 1993; Dilly, 1994; Chen et al., 1997; Tran et al., 2003), this has not replaced the traditional concept of the three-layer structure of the tear film (Wolff, 1946; Holly and Lemp, 1977). There is an assumption that tears have a substantial free-fluid layer, but this concept needs to be revised, following the observation of a homogenous, fine network-like structure throughout the tear film in rats (Chen et al, 1997) and mice (Tran et al, 2003) with electron microscopy, following in vivo cryofixation with freeze substitution. Additionally, Tran et al (2003) reported a uniform electrical potential throughout the thickness of the murine tear film when sampled at a spatial resolution of 1μm, suggestive of a single tear phase. In this study, the time from death to the end of recording was less than 15min, before the tear film destabilised, and in less time than the typical inter-blink period for the mouse observed in life. Similar results were found when the path of the micro-electrode was retracted, so it is

likely that the tear film was minimally disturbed from its natural state during the procedure.

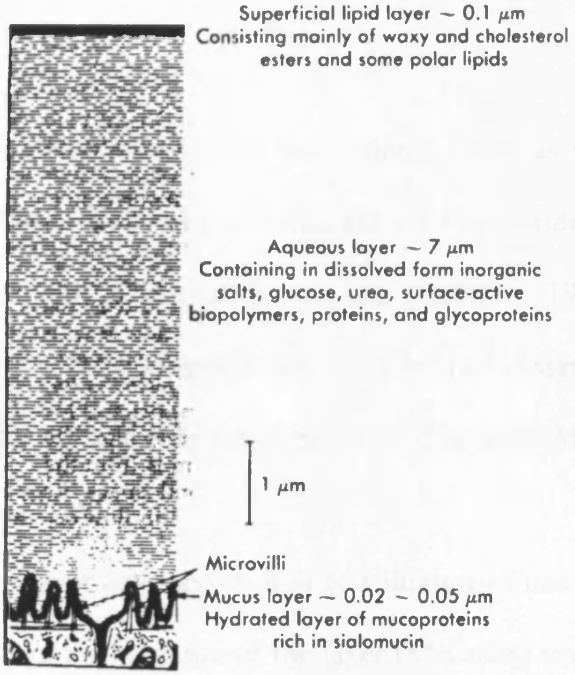


Fig 2.8: Composition and structure of the tear film. (From Holly FJ, Lemp MA: Surv Ophthalmol 22:69-87, 1977)

2.6.1.1 Superficial Lipid Layer

The outermost lipid layer was demonstrated in human tears in 1972 (Brauninger et al., 1972). It is produced by the Meibomian glands located in the tarsal plates of the upper and lower lids. A small portion may also be produced by the glands of Zeiss and Moll, located at the palpebral margin of the tarsus and at the roots of each eyelash respectively (Hurwitz and Stein, 1996; Holly and Lemp, 1977). The thickness of this layer can be estimated from its optical properties and it is usually no more than 0.1μm thick (McDonald, 1968). Its thickness depends on the palpebral aperture width and

this is demonstrated when the lipid layer is observed under a slit lamp. Any narrowing of the palpebral opening causes a thickening and compression of the oily layer, whilst when the eye is opened wider there is an appearance of a stretching event (Mishima, 1965).

The layer mainly consists of lipids of low polarity, such as waxy and cholesteryl esters (Andrews, 1970). High polarity lipids, such as triglicerides, free fatty acids and phospholipids are present in negligible amounts (Andrews, 1970; Nicolaides et al., 1981). This layer has been demonstrated both by the observation of interference patterns and by direct testing of the tear film for lipid activity (McDonald, 1968).

Many studies on this layer have shown it to be a mixture of non-polar and polar lipids that each play a role in the structure of the layer (McCulley and Shine, 1997). They suggest that this layer should be considered as two phases. A relatively thick outer layer, containing non-polar lipids such as wax esters, sterol esters, hydrocarbons, and triglycerides; and a thin polar inner layer, predominantly consisting of phospholipids.

The main role of the lipid layer is to retard evaporation of the aqueous phase in the open eye, since the thinness of the tear film would cause it to break-up if the lipid layer was absent especially under conditions of low humidity and turbulent airflow (Holly, 1981a). Mishima and Maurice (1961 a, b) and Mishima (1965) investigated the rate of evaporation of the tear film in the presence and absence of the lipid layer in rabbits, and found a 10-20 fold increase in evaporation in the absence of the lipid layer surface. Craig and Tomlinson (1997) showed a four-fold increase in evaporation in humans. Additionally, the lipid layer forms a barrier which prevents

tears from over-spilling onto the eyelid, prevents contamination of the tear film by the more polar lipids of the skin of the lids (McDonald, 1968), thickens and stabilizes the tear film through interaction with the underlying aqueous layer, and lubricates the action of the lids over the cornea and conjunctival surfaces (Lemp and Wolfrey, 1992). Other possible functions of tear lipids include anti-microbial activity and the production of pheromones (Tiffany, 1985).

The lipid layer is reformed by the actions of blinking, whereby lipid is spread over the ocular surface, which will lower the tension of the tears, allowing fluid to be drawn into the tear film and thickening the aqueous phase (Wolff, 1946). Bron and Tiffany (1998) inferred from their studies that blinking aids the delivery of meibomian oil onto the lid margin.

2.6.1.2 Aqueous Layer or Fluid Layer

The aqueous layer is believed to be the major intermediate liquid phase of the tear film, according to the model of Wolff (1946). The aqueous layer is produced by the orbital and palpebral portions of the main lacrimal glands and the accessory lacrimal glands of Krause, located in the upper conjunctival fornix, and Wolfring, located mainly in the supra-tarsal conjunctiva of the upper lid (Milder, 1987). The thickness of the aqueous fluid layer is said to range between 6 and 10μm (Mishima, 1965; Holly, 1987), and is claimed to account for over 90% of tear film thickness (Wolf, 1946).

The aqueous component of the tear film is not simply water that lubricates the ocular surface, but contains numerous electrolytes, proteins, peptide growth factors, vitamins, anti-microbials, cytokines, immunoglobulins, glucose, minerals, enzymes and hormones (Iwata, 1973; Franklin and Bang, 1980; Hugh et al., 1980; Prydal et al., 1992; King-Smith et al., 2000, 2004). Some of these proteins include albumin, lactoferrin, lysozyme and immunoglobulins. The immunoglobulins come mainly from the conjunctiva and are predominantly IgA and IgG (Hurwitz and Stein, 1996). These constituents serve to nourish and protect the ocular surface, and pass messages between the structures the aqueous contacts. In addition the aqueous provides the corneal and conjunctival epithelial nutrition such as oxygen and glucose. It also blocks physical invasion by forming a uniform barrier, and buffers or destroys chemical intrusion by its buffer system, lysozyme, and immunoglobulins and components (Kwan et al., 1972; Yamamoto and Allansmith, 1979; Sen and Sarin, 1980; Gillette et al., 1981).

95% of the aqueous layer is secreted by the orbital and palpebral portions of the main lacrimal gland, with the rest from the accessory glands of Krause and Wolfring. (Allansmith et al., 1976). The secretion is isotonic or slightly hypertonic and flows from the superior temporal fornix ductal openings of the main and accessory glands, across the exposed portions of the corneal and conjunctival surface. The flow is driven by the muscle action of the orbicularis muscle during blinking. When the tears reach the openings of the superior and inferior puncta, they are drained into the canaliculi during the relaxation phase of the blink (Doane, 1981; Lemp and Weiler, 1983). Some aqueous fluid is also lost by evaporation from the surface and by reabsorption through the conjuctival surface (Lutofsky and Maurice, 1986). Tear

volume ranges between 6-8μl, and the basal tear secretion rate is approximately 1.2μl/minute (Mishima et al., 1966). It has been suggested that all tear production is stimulus driven (Jordan and Baum, 1980), as tear production decreases during sleep and general anaesthesia (Cross and Krupin, 1977).

Normal human tears are slightly alkaline (pH 7.5±0.16) compared to serum (pH 7.35-7.45) (Abelson et al., 1981; Carney et al., 1989). The osmolarity ranges from 303.6±13.0 to 310-334 mOsm/Kg (Gilbard et al., 1978; Benjamin and Hill, 1983; Craig and Tomlinson, 1995).

2.6.1.3 Mucous Layer

The mucous layer is the innermost layer and rests on the underlying corneal and conjunctival epithelium. It is secreted by the goblet cells, of which there are approximately 1.5 million distributed over the conjunctival surface, and spreads directly over the microplicae of the corneal epithelial cells (Kessing, 1968). The mucous layer contains high molecular weight proteins with a high carbohydrate-to-protein ratio, known as glycoproteins (Mishima, 1965). There has been a variation in the mucin layer thickness with measurements ranging from 1μm over the cornea to 2-7μm over the conjunctiva (Nichols et al., 1985). These differences could be due to different types of mucins or that the processing for electron microscopy caused the loss of some unbound mucins at the surface.

Among the most important functions of the mucous layer is lubrication, allowing the eyelid margins and palpebral conjunctiva to slide smoothly over one another with

minimal friction during blinking and ocular rotational movements. The mucins are believed to serve as the foundations of the tear film and are the source of the lacrimal surfactant present in the dissolved state in the aqueous layer (Holly, 1987). Although the factors that control secretion of the tear mucins are not known, the tear mucins that coat the epithelial surface of the conjunctiva and cornea provide protection to the underlying epithelium from damage by helping it to heal rapidly and effectively (Bron et al., 1985). Tear mucins that are dissolved in aqueous tears provide high viscous characteristics to the mucous layer, and this assists in the protection against shear forces associated with blinking by providing lubrication on the front of the cornea (Dilly, 1994; Hodson and Earlam, 1994). The mucous layer also maintains the wettability of the ocular surface for a sufficient amount of time and, with the lipid layer, removes unwanted material from the surface of the eye. Another function is protection against noxious and pathogenic agents. The structure of the layer allows the slow release of immunoglobulins when the eye is open, lowering the susceptibility to airborne pathogens and antigens. Along with the immunoglobulins, the thick nature of the mucous is believed to reduce the ability of some bacteria to adhere to the surface of the eye and penetrate the mucous layer. It acts to decrease surface tension so that the aqueous component of the tears can spread over the epithelial surface. Abnormalities of the mucin layer or the epithelial surface will cause the tear film to break up rapidly into dry spots after a blink (Hurwitz and Stein, 1996).

The layer is complex and consists of mucous glycoproteins associated with a mixture of protein electrolytes and cellular material. Recent evidence suggests that the mucin component has a two-layer structure (Nichols et al., 1985). The innermost, tightly-bound component (glycocalyx), associated with the epithelial cell surface, is thought

to be a secretion of the cells themselves. Just above this is a thicker layer referred to as a "mucous blanket". This loosely attached outer mucin layer is thought to be the product of the goblet cells of the conjunctiva (Kessing, 1968). Nichols et al (1985) indicated that the glycocalyx layer has an overlapping distribution with the mucin layer, extending some 0.3 μm into the mucin layer. Dilly (1994) suggested that the function of the glycocalyx is to anchor the mucous layer to the cell surface and provide stability to the tear film. In disorders that affect the epithelial cells, the anchorage system is believed to be disrupted causing de-stabilisation of the tear film.

2.6.2 Tear Formation and Drainage

Blinking spreads the tear film over the cornea, and moves the tears toward the puncta with each blink. In addition, the temporal portion of the palpebral aperture closes more rapidly as the eyes close in blink. With each blink, the upper and lower eyelid approximate first in the lateral canthal area and then proceed toward the medial canthal area. These two physiological movements promote medial displacement of the tear film toward the lacrimal puncta (Lemp and Wolfrey, 1992). A schematic diagram of the drainage portions of the lacrimal system is shown in the figure below. Approximately 25% of the secreted tears are lost by the process of evaporation, and the remaining 75% are pumped into the nasal cavity through the lacrimal drainage system. The tears secreted into the upper temporal fornix move to the lacrimal puncta in three steps (Lemp and Wollfley, 1992). 1) At the lateral canthus, the tears move downward, by gravity, to form the lower marginal strip. 2) The lower canaliculus is believed to collect four times as much of the tear flow as the upper canaliculus. Capillary attraction helps to conduct tears into the puncta and the vertical section of

the canaliculus. 3) Finally, lid movement contributes to the transport of tears to the puncta by the act of blinking.

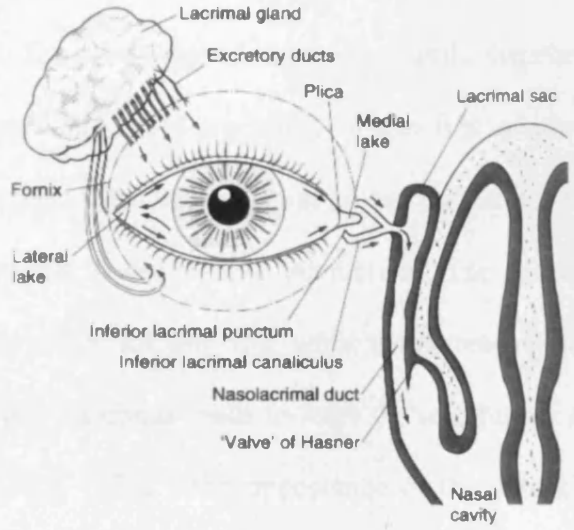


Figure 2.9: Schematic diagram of the secretory and drainage portions of the lacrimal system. The small arrows indicate the flow of tears (From Korb DR at al: The Tear Film: Structure, Function and Clinical Examination, Butterworth; Heinemann, Oxford, 2002).

2.6.3 Tear Film Formation and Rupture

The lid motion and spreading of the lipid layer of the tear film, together with the underlying hydrophilic mucous layer, control tear film formation following each blink.

When the eyelids close, they compress the superficial lipid layer and eliminate the tear film-air interface. Through the shear action across the thin aqueous layer between the moving lid and the ocular globe, the mucous layer is redistributed and the

lipid–contaminated mucous is dragged into the lower and the upper fornix (Norn, 1969; Wright, 1975).

When the eyelids open, they create a new tear-air interface of high surface tension, about 70 dyne/cm. The spreading of the excess lipids, together with the associated mucoproteins, follows this rapid spreading of the first single layer of lipid. The resulting superficial lipid layer is elastic and lowers the surface tension of the tear film to about 35 dyne/cm due to mucin-lipid interaction. The spreading of the lipids also thickens the aqueous part of the tear film, while the increasing negative pressure in the gradually thinning tear meniscus tends to limit the tear supply available for tear film formation (Holly, 1973, 1974). The appearance of the "black" line adjacent to the meniscus (McDonald and Brubaker, 1971)indicates that the tear fluid in the meniscus, as well as in the fornices, has become unavailable for tear film formation. In time, the tear film gradually deteriorates and becomes unstable, due to lipid contamination of the mucin layer coating the corneal epithelium. The mucous becomes incapable of maintaining its hydrophobic properties and breaks-up forming the "dry spots". This probably irritates the nerve endings in the epithelium and triggers the next blink.

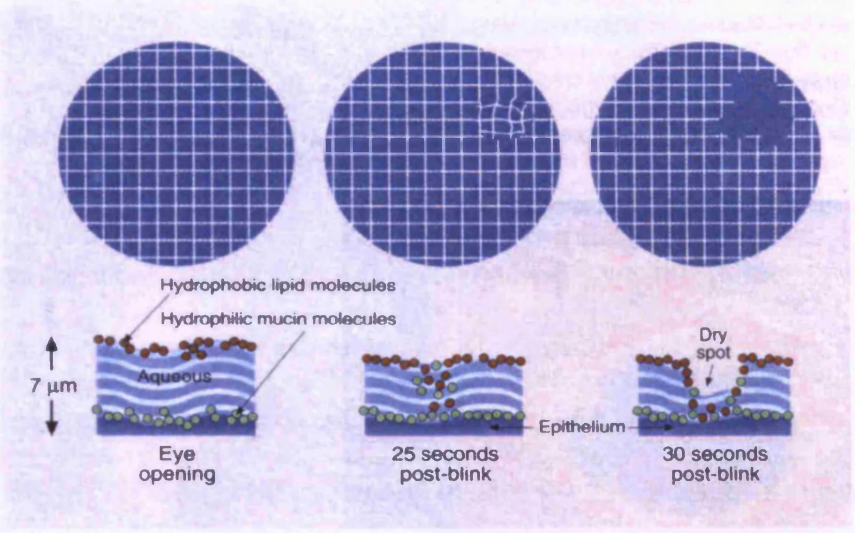


Fig 2.10: Schematic representation of the intact tear film observed at eye opening (left), followed by tear thinning at 25 seconds (middle) and tear break-up time at 30 seconds (right). The top row illustrates this sequence as observed using a protected grid (non-invasive break-up time measurement). The bottom row illustrates how a dry spot forms (From: Efron N: Contact Lens Complications, Oxford; Boston: Butterworth-Heinemann: Optician, 1999).

2.6.4 Measurement of Tear Film Stability

The pre-ocular tear film (POTF) in humans does not remain stable for long periods of time (Holly, 1981a, 1981b). When blinking is prevented, the tear film ruptures within 15-40 seconds and dry spots appear over the cornea (Lemp and Hamill, 1973). Observation of the rupture of the POTF before a subsequent blink is one of the most commonly used tests to examine tear film stability. This stability results from interactions between the three major components of the tear film: the mucous glycoproteins, the aqueous tears, and the superficial lipid layer.

2.6.4.1 Invasive Break-Up Time Measurement

Norn (1969) and Lemp and Holly (1970) suggested assessing tear film stability using sodium fluorescein (Norn, 1969; Lemp and Holly, 1970;). The fluorescein is instilled into the conjunctival sac and the cornea scanned with cobalt blue illumination, using the slit-lamp microscope, for the first sign of discontinuities in the film. These are seen as dark spots in the fluorescent film. The first appearance of the spots represents the rupture of the tear film. Further details of this method are given in the Instrumentation Chapter 3.3.1.

2.6.4.2 Non-Invasive Break-Up Time Measurement (NIBUT)

There are at least seven instruments used to measure the non-invasive break-up time which can be classified into two categories: Those with a small measurement field and those with a wide measurement field.

The narrow field techniques are of limited use, since they involve a very small part of the cornea. In general, the smaller the field, the lower the correlation with the full field of measurements. The instruments that belong to this category are: slit lamp specular reflection, the Baush &Lomb keratometer, the hand-held keratoscope (Guillon et al., 1992), and the HIRCAL modified keratometer (Hirji et al., 1989). The wide field instruments are: the modified bowl perimeter (Lamble et al., 1976; Mengher et al., 1985), the external illuminator (Young and Efron, 1991), and the Tearscope (Guillon, 1986).

The NIBUT instruments, both narrow and wide, also differ according to the nature of reflected mires. Some have a dark background with a bright grid (keratometer, hand-held karatoscope, HIRCAL modified keratometer, bowl perimeter), while others have a white background (slit lamp specular reflection, external illuminator, Tearscope).

The measurement techniques are divided into two categories, depending upon the type of target used. For the dark-field background instruments, the practitioner observes the appearance of any deformation of the target or grid. The time measured has been referred to as the non-invasive tear thinning time (TTT). This is the elapsed time recorded between a full blink and the appearance of any distortion of the target or grid. For the white background instrument and the slit lamp, the practitioner observes the appearance of any black spots within the tear pattern. The time measured in seconds between a full blink and the appearance of a black spot is the NIBUT.

The modified keratometer designed by Hirji and Callender, known as the HIRCAL modified keratometer, is the most useful of the narrow field instruments. It consists of a modified Bausch & Lomb keratometer where the mires have been replaced by a white grid on a black background. The image of the grid is reflected by the tear film and the appearance of distortion in the reflected pattern reveals a break.

The values obtained by any practitioner are technique-dependent, but even for a given technique, there may be variations due to differences in environmental conditions in the consulting room, such as humidity and airflow. For this reason it is suggested that, whichever technique is used, records of the first 50 sec should be kept and the

practitioner should stop measurements after 45 seconds of the eye opening, even if no break-up occurs, in order to avoid discomfort.

Mengher et al (1985), following an original design by Lamble et al (1976), developed a non-invasive method without the use of fluorescein. Their method is based on observing changes in the specular image of a grid pattern projected to the eye. A distortion of the grid line represents local thinning and discontinuity represents a break in the tear film.

The Tearscope allows the measurement of NIBUT by two techniques: a direct, non-invasive method of observing the break against the white background produced by the instrument; and indirectly by observing the deformation of rings or grid patterns inserted within the illuminated inner surface of the instrument. Further details of this instrument are given in the Instrumentation Chapter 3.3.2.

2.7 Tear Evaporation from the Ocular Surface

The tear film is part of the visual system and determines much of the well being and efficient performance of the eye (Milder, 1987). It is a dynamic system, consisting of tear production by lacrimal glands, secreting aqueous tears and washing over the epithelial and conjunctival cells, tear drainage through the lacrimal duct, and water evaporating to the air. Aqueous tears are covered by tear lipid secreted by the meibomian glands that spread to form an oily layer of the preocular tear film. Meibomian gland secretion limits evaporative tear loss, provides lubrication during blinking, and maintains an optically smooth surface (Mishima and Maurice, 1961a, 1961b; Holly, 1980; Tiffany, 1985; Rieger, 1992). The tear film is in a constant state

of flux, continuously thinning when the eye is open, and refreshed with blinking (Holly, 1980).

Tear evaporation has been studied as a major factor in tear dynamics (Rolando and Refojo, 1983; Tsubota and Yamada, 1992; Mathers et al., 1993; Shimazaki et al., 1995, 1998). Tear evaporation measurements in examinations for dry eye are recognised as an important technique for differentiating dry eye syndrome sub-categories, that is aqueous tear deficiency (ATD), lipid tear deficiency (LTD), and dry eye (also known as evaporative dry eye).

2.7.1 The Role of the Lipid Layer in Evaporation

The ocular surface is covered with a thin layer of lipids that functions like a monolayer to retard evaporation. The structure of the lipid layer and thus its anti-evaporative mechanisms remains poorly understood. A bipolar inner layer likely acts as a transition zone between the outside non-polar surface and the polar aqueous tear film. Phosphatidyl ethanolamines, sphingomyelin, and phosphatidyl choline are probably the key components of this polar lipid layer. A description of how polar lipids organise and create the extremely effective nature of the lipid barrier was presented by McCulley and Shine in 2000. The lipid surface is highly effective, dramatically demonstrated by the 95% reduction in evaporation from the ocular surface (McCulley and Shine, 2000; Mathers, 2004).

2.7.2 Measurement of Evaporation

Human tear evaporation rates have been reported, and differences between the results in normal subjects vary due to different measuring techniques. Hamano et al (1980) was the first who measured *in vivo* evaporation in the human. He found an evaporation rate of 26.9 x 10^{-7} g cm^{-2} sec^{-1}. Rolando and Refojo (1983) reported a device consisted of a modified, tight-fitting goggle, with dry air pumped into its chamber, to measure tear evaporation rate. After 1 minute of evaporation, the air in the chamber was mixed with the air in the system and measured for temperature and humidity. The air temperature of this system was 23°C. They found an evaporation rate of 4.07 x 10^{-7} g cm^{-2} sec^{-1}. This device had the drawback that it could not evaluate evaporation at a precise temperature or relative humidity. To minimize the contribution of skin evaporation the exposed area was covered with petroleum jelly. They established the concept of evaporation as a function of the inter-palpebral fissure, and created a chart describing the relationship between exposed area and inter-palpebral distance.

In 1990 Yamada and Tsubota described a device where they also used a chamber filled with dry air that was sealed and the relative humidity was measured over time. They reported a normal evaporation rate of 15.6 x 10^{-7} g cm^{-2} sec^{-1} at 40% relative humidity. In their next publication in 1992 (Tsubota and Yamada, 1992) similar results were reported. It was also shown that the instillation of artificial tear eye drops increased evaporation. Tomlinson et al (1991) reported the development of a device called the ServoMed Evaporimeter that was attached to a modified swimming goggle. The instrument measured the relative temperature and humidity of two sensors placed above the ocular surface (further details on this instrument are given in the

Instrumentation Chapter 3.5.1). The evaporation effect from the closed eye was subtracted and they found an evaporation rate of 20 x 10^{-7} g cm^{-2} sec^{-1} in normal subjects.

Mathers and co-workers (1993) reported the development of a device for measuring evaporation. Using a small, closed chamber filled with dry air, the rise in humidity was plotted and the evaporation was calculated. The evaporation rate of the closed eye was subtracted to remove the effect of evaporation from the skin. Evaporation rate was found to be 14.7 x 10^{-7} g cm^{-2} sec^{-1} at 30% relative humidity (Mathers et al., 1993).

A recent report on evaporation rate by Goto et al (2003) used a device that streamed air of known humidity across the ocular surface and the evaporation effect from the closed eye was subtracted. The rate was found to be 4.1 x 10^{-7} g cm^{-2} sec^{-1} at 30% relative humidity, and was much lower than had previously been found. The relative humidity of the incoming air, not reported for this result, was factored out of the final equation and these results may not be comparable to other reports.

2.7.3 Evaporation and Dry Eye

Hamano's early studies suggested that evaporation rates in dry eye subjects were much lower than in normal subjects, and so did the studies of Yamada and Tsubota who found similar low evaporation rates in dry eye patients (Hamano et al., 1980; Yamada and Tsubota, 1990). However, Rolando's results in dry eye patients or patients with ocular pathology, found evaporation rates to be twice than that found in normal subjects (Rolando et al, 1983).

Mathers et al (1993), following the development of their own device, showed that dry eye subjects had high evaporative rate, 47.6 x 10^{-7} g cm^{-2} sec^{-1}, compared with normal rate of 14.7 x 10^{-7} g cm^{-2} sec^{-1}. These results were confirmed with additional studies in 1996 that found a rate of 25 x 10^{-7} g cm^{-2} sec^{-1} in dry eye patients compared to normal patients of 13 x 10^{-7} g cm^{-2} sec^{-1} (Mathers and Daley, 1996). Craig and co-workers (2000), using a device based on steady state differential humidity within a chamber, examined both dry eye patients and healthy subjects, and found evaporation rates to be higher in the dry eye group than in the normal group.

2.7.4 Meibomian Gland Function and Evaporation

Meibomian gland dysfunction (MGD) appears to alter the composition of the lipid layer, causing an increase in the evaporation rate of the tear film. Mishima and Maurice (1961 a, b) described the protective effect and evaporative control of this layer. Rolando et al (1983) reported an increase in evaporation in patients with ocular surface disease, some of whom had MGD. In 1993, Mathers et al demonstrated that MGD caused a meaningful increase in evaporation rate and their results correlated closely with gland dropout. Schimazaki et al (1995) also found an increased evaporation in patients with meibomian gland dropout. Craig and Tomlinson (1997) found an increased evaporation in subjects with thin lipid layer and in subjects with abnormal interference patterns, indicating meibomian gland. A recent paper by Goto et al (2003) confirmed that patients with obstructive MGD have elevated evaporative rates that increased proportionally with the severity of the meibomian gland obstruction.

In conclusion, evaporation has been found to increase in patients with dry eye and with meibomian gland dysfunction. When dry eye disrupts the ocular surface it appears to alter the integrity of the lipid layer in some way that raises evaporation. In obstructive MGD the lipid layer is also compromised, but the cause of this is not known (Mathers, 2004). The inconsistencies found in the published results are more due to subject selection, measurement techniques, and instrumentation used.

2.7.5 Contact Lenses and Evaporation

The use of contact lenses has also been shown to increase tear evaporation rates, by disrupting the superficial lipid layer (Tomlinson and Cedarstaff, 1982, 1992; Cedarstaff and Tomlinson, 1983). The increase in evaporation noted with different types of contact lenses was not found to be consistently related to the type of material from which the contact lens was made. These contact lenses included 38% and 70% hydrogels, silicone elastomers, PMMA, and modified PMMA hard lenses (Tomlinson and Cedarstaff, 1982). A similar investigation by Cedarstaff and Tomlinson (1983) considered the effect of soft contact lenses, ranging in initial water content from 38% to 70%, on the tear evaporation rate. All the types of soft lenses caused an increase in the evaporation rate, and this increase was not found to be related to the initial water content of the soft lens. Water loss by dehydration of the lens made only a minor contribution to the total increase in evaporation.

2.7.6 Artificial Tear Solutions, Saline and Evaporation

Instilling artificial tear solutions and saline causes the rate of evaporation to increase (Trees and Tomlinson, 1990). This suggests that tear film instability is produced by

the instillation of these drops, which may be due to the increased fluid volume within the eye. Hence a disruption of the lipid layer of the tear film occurs, which is responsible for the inhibition of tear evaporation (Mishima and Maurice, 1961a, b). The results of Trees and Tomlinson (1990) suggest that the tear film evaporation rate after the instillation of both artificial tear solutions may return to baseline faster than after the instillation of saline. A similar increase in the rate of tear evaporation has been found after the instillation of a single drop 0.5% proparacaine (Rolando and Refojo, 1983).

2.7.7 Diurnal Variation of Tear Evaporation

Human tear evaporation rate has been found to vary through the day (Tomlinson and Cedarstaff, 1992), indicating that evaporation is at its lowest immediately on awaking, rising rapidly within the first two hours, and remaining constant for the next twelve hours. The initial low tear evaporation on awaking may explained by two reasons. Firstly, tear production is low on awaking, and secondly, tear film stability is high on awaking because of a thick lipid layer (Mishima and Maurice, 1961a, 1961b; Baum, 1986; Tomlinson and Cedarstaff, 1992).

2.7.8 Sex, Age and Tear Evaporation

Tear evaporation rate was correlated between the sexes and with increasing age (Rolando and Refojo, 1983; Tomlinson and Giesbrecht, 1993). Tomlinson and Giesbrecht (1993) reported a gender difference with a higher tear evaporation rate in females. This may be a factor in the greater predisposition to dry eye problems in females as they grow older (Lemp, 1980). This result is not supported by Rolando

and Refojo (1983) who found no significant difference between the sexes. However, they had a relatively narrow age band of subjects, 28-71 years, and one-third of their 52 subjects were females.

2.7.9 Conclusions

The evaporation from the ocular surface is dramatically reduced by the lipid layer covering it. With this layer intact, evaporation represents a small loss of water for which the lacrimal gland easily compensates. However the function of how the lipid layer accomplishes this reduction in evaporation is not well understood and is probably as complex as is the structure of the lipid layer itself (Mathers, 2004).

2.8 Ocular Thermography of the Anterior Eye

Temperature measurement of the anterior segment of the eye is of potential importance in a variety of research applications, such as quantifying the precise thermal profile of the ocular surface for physiological modelling (Alio and Padron, 1982b), studying environmental influences on the temperature of the eye (Schwartz, 1965; Freeman and Fatt, 1973), monitoring corneal wound healing (Coles et al., 1988), investigating injury (Kolstad and Opsahl, 1969; Mikesell, 1978) and disease process (Mapstone, 1968b; Horven, 1975), as well as modelling the effects of contact lens wear (Hill and Leighton, 1965a, 1965b; Fatt and Chaston, 1980; Martin and Fatt, 1986). This measurement is also of importance in research (Yang and Yang, 1992) and in clinical situations including ocular physiology (Raflo et al., 1982; Craig et al., 2000), pathology (Keeney and Guibor, 1970; Morgan et al., 1999), ocular

inflammation (Efron et al., 1988), tear film and photorefractive surgery (Mapstone, 1968c, 1970; Betney et al., 1997; Mori et al., 1997).

2.8.1 Development of Infrared Imaging in Medicine

One of the most important advances in temperature measurement over the past forty years has been in detecting radiated heat from the body surface. Radiated heat from the human body is not visible, but belongs to the infra-red region of the electromagnetic spectrum. It is radiation produced by motion of atoms and molecules. The hotter an object, the more the atoms move, and more radiation is produced. All objects with temperature above absolute zero emit infrared radiation from their surface (body).

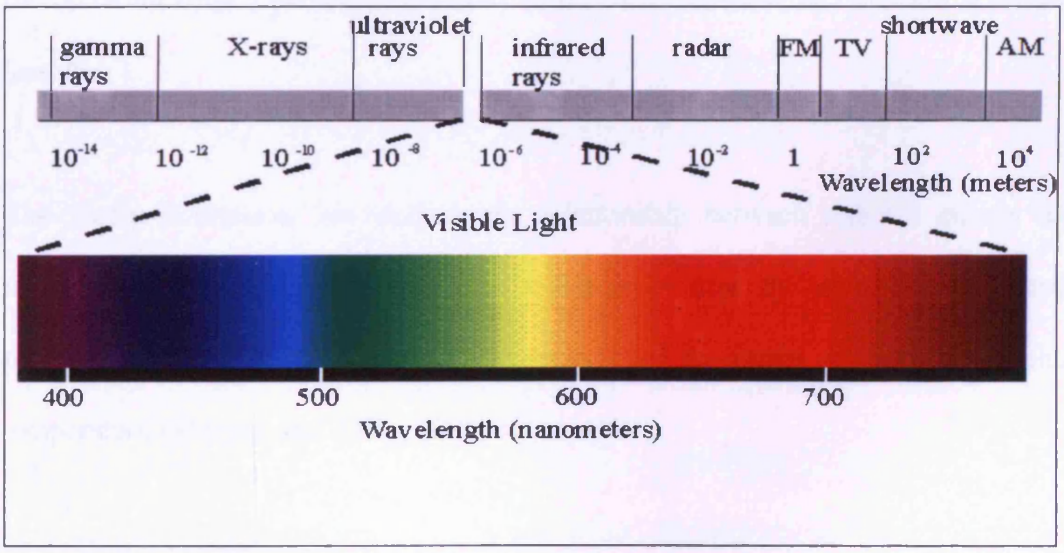


Fig 2.11: The electromagnetic spectrum.

(From:http://images.google.co.uk/images?q=The+electromagnetic+spectrum&hl=en&btnG=Search+Images).

Scientists, using "black body principles", tried to define the relationship between the temperature of an object and infrared radiation emitted by its surface: When a body at a given temperature radiates energy from its surface, the condition and the colour of the surface are of special importance. A black body radiates the maximum possible energy at a certain temperature – a perfect radiator (and also a perfect absorber due to thermal equilibrium). In practical terms it consists of a cavity, in a form of a hollow sphere, the inside of which is matt black. All other surfaces are compared to this black body.

All bodies, including skin and the ocular surface, follow this ideal rule, and a correction factor is applied called the "emissivity" of the object/body. The emissivity is defined as the ratio of the radiation emitted by the object concerned, compared to the radiation from a perfect black body at the same temperature, and will always be less than 1.

The Stefan-Boltzmann law defines the relationship between radiated energy and temperature by stating that the total radiation emitted by an object is directly proportional to the object's area and emissivity and the fourth power of its absolute temperature (Morgan and Tullo, 1996; Jones, 1998).

A typical infrared-measuring device consists of a system for collecting radiation from a well defined field of view and a detector that transducers the radiation into an electrical signal. The build-up of a thermal picture is produced by the use of an optical scanning system and image processing to display the image on a monitor. There are two categories of detectors: thermal detectors (such as thermocouples) that

are slower to respond, and photon detectors that respond more rapidly. Such materials can be cadmium mercury telluride and indium antomide, which detect by means of a photoconductive and photovoltaic effect (Jones, 1988; Morgan et al, 1993). The imaging system has optical components such as a germanium lens and a scanning system, to transmit and focus the infrared radiation. For an infra-red detector a chopper device is essential to study an object of constant or slowly changing temperature, like the eye (Jones, 1998; Morgan et al., 1993).

2.8.2 Thermometry and the Eye

The developments in ocular thermometry have reflected the general advances in temperature measurement. It is possible to measure ocular temperature using both contact and non-contact methods.

2.8.2.1 Contact Methods of Ocular Thermometry

Contact methods of assessing eye temperature, which can include measurement with a thermistor or thermocouple (Rosenbluth and Fatt, 1977; Dixon and Blackwood, 1991), have a number of disadvantages. If patient discomfort is to be avoided, topical anaesthetics are required, probably producing an alteration of the ocular surface temperature. The "fin cooling effect" of the probe increases the available surface area for heat conduction away from the eye, especially when the probe is used at minimal depth or when large gauge needles are used (Fatt and Forester, 1972; Rosenbluth and Fatt, 1977). Furthermore, the results taken with contact methods are not instantaneous, and only a small area is measured (Fatt and Forester, 1972; Morgan

and Tullo, 1996). Also, the contact measurements of temperature can vary with the pressure of application of the probe (Mapstone, 1968b).

2.8.2.2 Non-Contact Methods of Ocular Thermometry

Non-contact methods alleviate these difficulties of direct techniques. Mapstone pioneered the application of infrared thermometry to the determination of ocular surface temperature in the late 1960s. He used a hand-held detector, a bolometer, which measured infrared radiation from a small area to study ocular temperature in a number of different eye conditions (Mapstone, 1968a, 1968b, 1968c, 1968d). This technique involved the measurement of the infrared energy emitted by the cornea and equating this to the relationship between infra-red radiance and temperature for a black body. Infra-red thermometry has the major advantage of being non-contact and allowing virtually instantaneous measurement of temperature. There is no risk of trauma and contamination, topical anaesthesia is not necessary, and the possibility of variation in the temperature readings with pressure application is avoided (Mapstone, 1968b; Morgan et al., 1995).

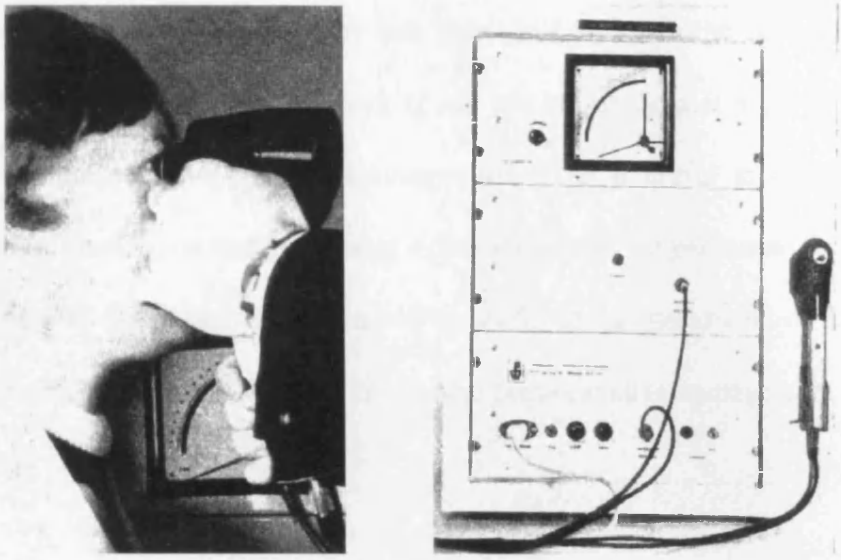


Fig 2.12: Mastpone's bolometer set-up (Mastpone, 1968b).

A more advanced non-contact measurement provides the examiner with a colour-coded image of the temperature of the eye, rather than a reading on a dial. This has the advantage of being entirely non-invasive and allowing the surface thermal pattern to be seen. Measurement and colour coded display of temperature is usually referred to as ocular thermography (Mapstone, 1970; Alio and Padron, 1982b; Efron et al., 1989; Morgan et al., 1993; Morgan and Tullo, 1996).

Thermographic instrumentation exploits the inherent relationship between the temperature of a body (T), its emissivity (ε), and the amount of electromagnetic energy which it emanates, expressed in terms of its radiant emittance (W). The equipment measures the radiant emmitance of an object by monitoring the conductivity of the semi-conductor detector onto which the radiation falls. If the emissivity of the object is known (it is assumed to be 0.97 for the ocular surface), then its temperature can be determined.

More recently, ocular thermography has been used to study the orbit and lacrimal system than the eye itself (Rosenstock et al., 1983; Cennamo et al., 1990). Results from these studies have shown thermography to be a useful method of gaining additional information rather than being a diagnostic tool. In particular, Rosenstock et al (1983) found that thermography could be useful in the assessment of dacrocystitis and canaliculitis, and that is helpful in pre and post-operative management of lacrimal conditions.

2.8.3 What is Actually Being Measured by Infrared Thermometry?

Mapstone recognised that the tear film of the ocular surface area must play an important role in the measured temperature. He regarded the cornea and the tear film as one continuous water phase, both behaving as black bodies. Water is an effective absorber of infrared radiation (Lerman, 1980), and it is reasonable that the high water content of tears, cornea and lens will ensure high absorption characteristics.

It appears that the measured temperature is actually the tear temperature, and only when the tears are absent it can be said that the spectrum detected is that of the cornea itself (Hamano et al., 1969; Fatt and Chaston, 1980; Morgan et al., 1993). The tear film must play a fundamental role in the measurement of temperature by infrared thermometry. Since it is a dynamic structure, changes in its thickness, composition, and evaporation rate may alter the thermographic patterns recorded.

2.8.4 Temperature Profile Across the Ocular Surface

There is a characteristic temperature profile across the cornea, noted by all studies in ocular thermography (Alio and Padron, 1982a; Efron et al., 1989) (Fig 2.12).

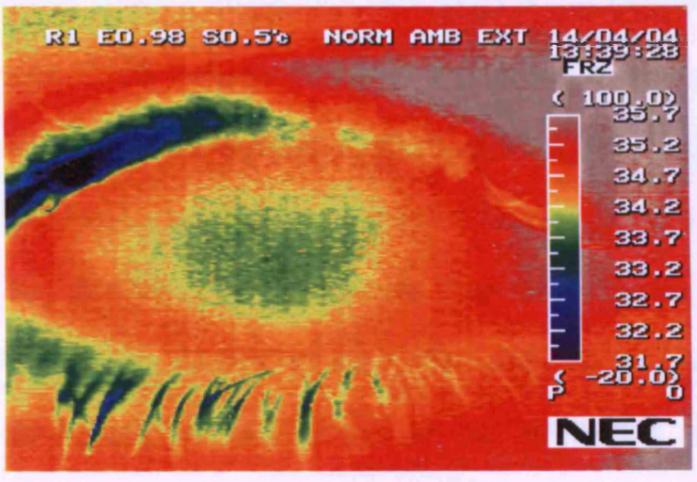


Fig 2.13: Typical ocular thermogram with latest infrared camera, NEC San-ei Thermo Tracer TH7102MX thermo-camera.

Efron et al (1989), used a wide-field, colour-coded, infrared imaging device to investigate the temperature profile across the ocular surface and the temporal stability of central corneal temperature. Temperature increased towards the periphery of the cornea with the limbus being 0.45°C warmer than the geometrical centre of the cornea (GCC). The temperature apex (the coldest point) of the cornea was slightly inferior to the assumed GCC, and the mean temperature of the GCC was 34.3°C is consistent with recent studies. A considerable decrease in cornea temperature was found following a blink, and a separate experiment was conducted to quantify this phenomenon, measuring the temperature at the GCC while the subject avoided blinking. The results suggests a decrease in temperature during the initial 15-20 sec

after the blink, in all subjects, followed by stabilization of temperature in those subjects who were able to maintain eye opening for longer periods.

Of particular interest is the presence of ellipsoidal isotherms, with the major axis horizontal, concentric about a temperature apex which is slightly inferior to the GCC. Such a distribution in the temperature is not unexpected in view of the combined heating effects of the warmer vascular surrounding tissues on the avascular cornea, which is continually losing heat to the atmosphere. Specifically, the concentric nature of the pattern may be attributed to the circular shape of the cornea and diffusion of heat from the richly vascularized limbus. The shape of the palpebral aperture accounts for the slightly elliptical nature of the distribution, and the inferior displacement of the temperature apex can be explained in terms of the inferior position of the mid-point of the palpebral aperture relative to the GCC (Efron et al., 1989).

Morgan et al (1993) investigated the ocular surface temperature in 95 healthy subjects and found a similar pattern to Efron et al (1989). Across the anterior eye, the isotherms were again elliptical in shape. Efron et al (1989) had postulated that this was due to the elliptical nature of the palpebral aperture. In order to confirm this, Morgan et al took thermograms on a subject before and after the application of a pair of eyelid contractors. With the eyelids open in this manner, the isotherm patterns appeared less elliptical, reinforcing the hypothesis of Efron et al (1989).

The variation in temperature across both eyes of a normal population found the conjunctival locations to be warmer than the limbal positions, which in turn were

warmer than the center of the cornea. The temperatures measured at these locations seemed to be related to the differences in vasculature across the anterior eye, whereby anterior ocular temperatures increased with the density of vasculature (Morgan et al., 1993).

2.9 Blinking

The maintenance of corneal integrity is dependent, in part, on the proper formation of the pre-corneal tear film. The formation and stability of this film is, in turn, partly dependent on the blinking action of the eyelids (Holly and Lemp, 1977; Doane, 1980). Proper blinking is essential for maintaining ocular surface health, by stimulating tears and spreading the three-layer tear film (Tsubota and Nakamori, 1995; Holly, 1985; Tiffany, 1985) (Holly, 1985; Tiffany, 1985; Tsubota and Nakamori, 1995). Improper blinking is known to result in a defective tear film (Abelson and Holly, 1977).

Blinking is a universal mammalian phenomenon which occurs spontaneously, voluntarily or as a reflex. Blinking may be induced by such stimuli as bright lights or eye irritation. It is a complex phenomenon involving multiple cranial nerves, and must therefore be controlled centrally. Blinking serves a number of functions:

1) Tear film production and lubrication of the ocular surface

2) Assisting tear film in removing foreign bodies from the tear film

3) Protection of the eye from noxious threats

4) Assist tear film in producing smooth optical surface for the eye

5) Pauses in the act of reading

6) Regulation of processing visual sensory input to the brain.

Published values of spontaneous blink rates in adults lie in the range of 12-25 blinks per minute (Zametkin et al., 1979; Carney and Hill, 1982a; Jancovic et al., 1982; Tsubota, 1998; Zaman et al., 1998). The normal spontaneous adult blink rate remains approximately constant. Each subject displays a characteristic pattern of blinking, mixing inter-blinking periods of shorter and longer durations in a regular fashion (Carney and Hill, 1982a; Ponder and Kennedy, 1928).

2.9.1 Blinking Action of the Eyelids

Eyelid movements can be classified as opening and closing movements. The levator palpebrae superioris (LPS) muscle, and the tarsal muscle of Muller (two smooth muscles, the superior and inferior tarsal muscles), are responsible for the opening of the eyelids, whereas the orbicularis oculi muscle (OO) is responsible for the eyelid closure (Esteban et al., 2004). The levator palpebrae superioris is supplied by cranial nerve III. It originates with and travel parallel to the superior rectus but continues forward to insert into the upper lid. The levator holds the eyelid up when the eyes are open, and it functions in concert with the superior rectus, increasing the elevation of the lids when the eyes look up. The orbicularis oculi, supplied by the cranial nerve VII, closes the eyes by depressing the upper lid and elevating the lower lid. The tarsal muscles are small smooth muscles at the edge of the bony orbit. They are supplied by postganglionic sympathetic fibres and help keep the eyes open.

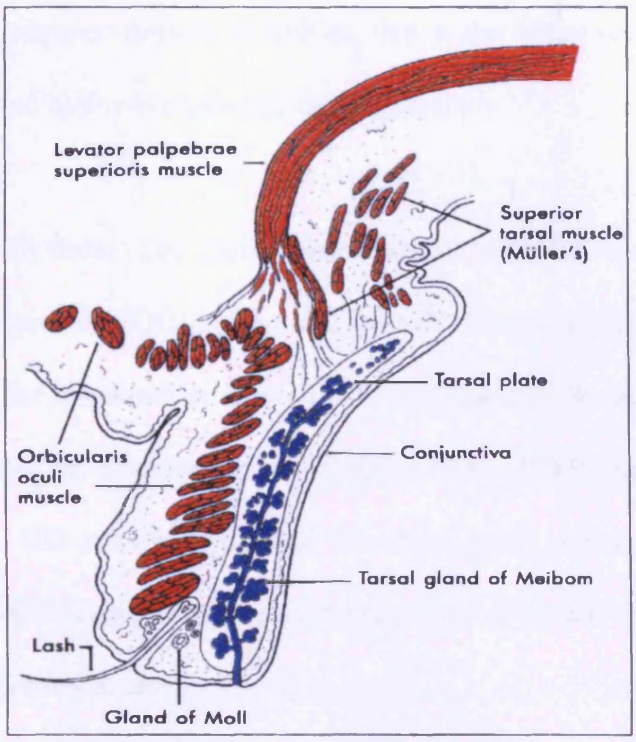


Fig 2.14: Muscles responsible for the opening and closure of the eyelids (From Haines DE: Fundamental Neurosciences, Haines DE, Churchill-Livingstone, 1997).

Opening of the Eyelids: The contraction of the levator palpebrae superioris muscle elevates the upper eyelid. This muscle is innervated by the superior division of the oculomotor nerve, which also supplies the superior rectus muscle.

Innervation to the levator palpebrae superioris muscles follows Hering's law (Becker and Fuchs, 1988; Schmidtke and Buttner-Ennever, 1992; Vander et al., 1997, 1998; Esteban et al., 2004). Synergistic extraocular muscles receive simultaneous and equal innervation. Motor neurons for the levator muscle arise from a single, unpaired, central, caudal nucleus of the oculomotor complex, and single motor neurons may innervate the levator muscle bilaterally. Hence any supranuclear input into the motor neuron nucleus influences both levator muscles.

The inferior and superior muscle of Muller, that is the minor retractor of the upper eyelid, is innervated by the sympathetic nervous system.

Closure of the Eyelids: The main muscle responsible for eyelid closure is the orbicularis oculi muscle (OO). The OO muscle fibres (myofibres) receive their innervation from the intermediate zone of the facial nucleus (Becker and Fuchs, 1988; Schmidtke and Buttner-Ennever, 1992; Vander et al., 1997; Vander et al., 1998). Anatomically the OO muscle is divided into three parts: pre-tarsal, pre-septal, and orbital. Deep and superficial heads of the pre-tarsal orbicularis muscle contribute to the lacrimal pump mechanism.

Variable LPS tonic activation, with the OO inactive, occurs in maintaining the ocular opening, the gentle closing and opening of the eyes, and the lid adjustment to the vertical globe positions. LPS inhibition, with OO activation produces all types of blinking and firm closure of the eyelids (Gordon, 1951; Bjork, 1954; Loeffler et al., 1966; Esteban and Salinero, 1979).

Normal blinking consists of two components. One is the inhibition of the sustained activity of the LPS muscles that keep the eyes open and the other is the brief, concurrent activation of the OO muscles. Once a blink stops, normal tonic activity of LPS is immediately resumed, while the OO returns to a resting position (Gordon, 1951; Bjork and Kugelberg, 1953; Esteban and Salinero, 1979). Reflex blinks show a duration of around 200ms (Bour et al., 2000) which is shorter than for voluntary or spontaneous ones (Evinger et al., 1991). The lowering of the eyelid occurs during the down-phase, and is directly related to a brief contraction of the OO. Their respective

durations show a linear relationship (Evinger at al, 1991). The closing tendon-aponeurotic forces, released immediately prior LPS inhibition, help promote ultimate closure. The following up-phase depends entirely on the resumption of LPS activity, which may exhibit an initial reinforcement over the level previous to the blink, a facilitation post-inhibition (Bjork and Kugelberg, 1953; Holder et al., 1987), or it may simply resume the same basal level of its tonic activity with the eyes open. The former pattern results in a shorter up-phase (Evinger at al, 1991).

Co-ordinated opening and closing movements of the eyelids make up the act of blinking. Blinking has a fundamental function in corneal wetting and eye protection (especially spontaneous and reflex blinking), but also is involved in visual information processing. A complete blink may be defined as a movement of both eyelids which begins in the normal open position, reaches a halfway point when the upper and lower lid ciliary margins oppose each other, and ends when the upper and lower lid return to the starting open position.

2.9.2 Blinking and Tear Drainage

As the eyelids close, tears enter the lacrimal puncta and are pumped through the canaliculi into the lacrimal sac by the blinking movements. Each canaliculus has a short 2mm vertical segment that joins a longer horizontal segment of 8 mm. At the junction of the horizontal and vertical segment, the canaliculus opens out into an ampulla. Orbicularis fibres are in close contact with the puncta and the canaliculus, so that when this muscle contracts in blinking, the puncta is drawn nasally, the ampulla is compressed, and the horizontal limb of the canaliculus is shortened, forcing tears into the lacrimal sac.

In blinking, the contraction of the orbicularis compresses the lateral wall of the sac. This creates a negative pressure within the lacrimal sac that draws the tears along the canaliculus, by the same orbicularis contraction, into the lacrimal sac. When the orbicularis relaxes after the blink, the sac collapses and this forces the accumulated tears into the naso-lacrimal duct. The figure below illustrates the sequences of events in the blink-driven tear drainage process.

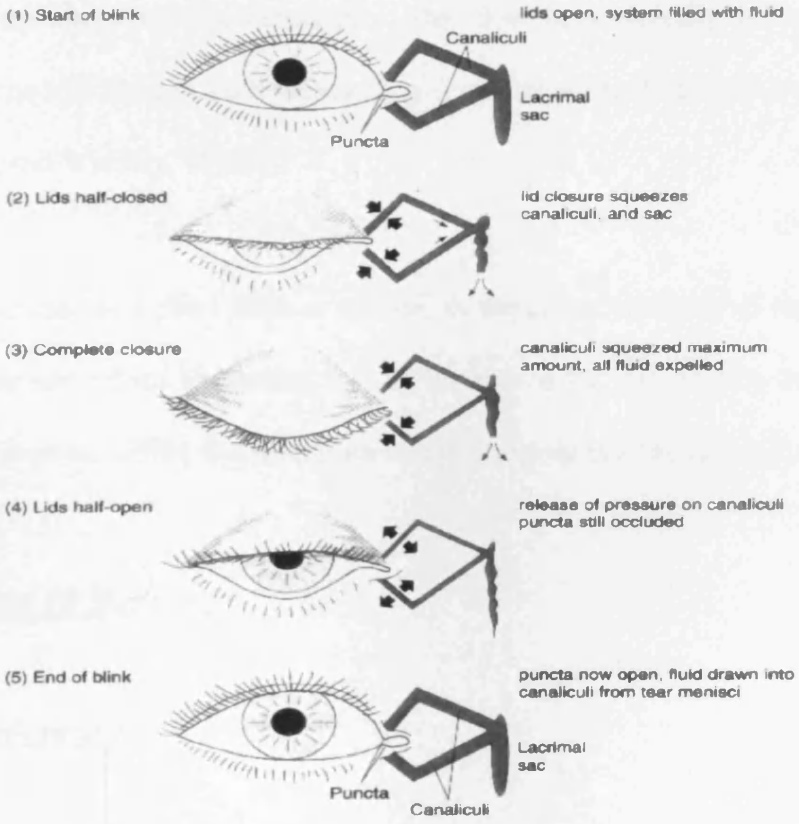


Fig 2.15: Doane's model relating blinking and tear drainage (From Korb DR at al: The Tear Film: Structure, Function and Clinical Examination, Butterworth-Heinemann, Oxford, 2002).

When the blink starts (1), the canaliculi contain tear fluid drawn in immediately following the previous blinking. When the lids are half-closed (2), the puncta is occluded by the abutting lid margins and the canaliculi and sac start to be compressed. When the lids have reached their maximum point of lid closure (3), the canaliculi are compressed forcing the contained fluid, into and through, the sac. When the lids are opened halfway (4), the pressure on the canaliculi and sac is reduced and the punctal openings are still occluded by the lid margins. Finally, when the lids are fully open, the canaliculi and sac expand to their normal configuration, and tear fluid is drawn via the punctal openings into the canaliculi. This flow lasts typically 1-2 seconds, by which time the tear fluid in each meniscus is drawn down to its normal level (Doane, 1981; Lemp and Wolfrey, 1992).

The naso-lacrimal duct plays little or no role in the active transport of tears, but the variable folds and valves (including that of Hasner) in the duct form a baffle which prevents air currents within the nose from being drawn up into the drainage system.

2.9.3 Types of Blinking

2.9.3.1 Voluntary

Voluntary blinking is the closure and reopening of each eyelid as a willed act, dependent on the individual. Cortical areas close to those of the frontal eye-fields probably mediate these movements (Bruce et al., 1985). Voluntary sustained closure of the eyes can be achieved either by inhibition of the steady tonic activity of LPS without OO activation, leading to soft eyelid closure, or by LPS inhibition plus sustained OO contraction, causing forced closing of the eyes. In both cases, LPS

inhibition is a relevant feature by being the unique factor in the gentle closing and by preceding the appearance of the OO activity in the forceful closing (Bjork and Kugelberg, 1953; Bjork, 1954; Esteban et al., 1978; Esteban and Salinero, 1979).

2.9.3.2 Involuntary

Involuntary blinking is divided into ***spontaneous*** and ***reflex*** blinking.

2.9.3.2.1 Spontaneous Blinking

Spontaneous blinking is a continuous, almost periodic and symmetrical brief movement of closing and opening of the eyelids which occurs in the absence of an obvious external stimulus or voluntary effort. Its primarily purpose is to reform and distribute the precorneal tear film. It is the most common form of eyelid movements.

True blinking movements are undeveloped during the neonatal period and early infancy. The rate of periodic blinking is much lower than in adults. Zametkin and co-workers (1979) found a steady increase in blink rate from infancy to adolescence, stabilising in adulthood. In the adult, bilateral blinking movements occur periodically at regular intervals during waking hours. In elderly people, blink rates exhibit no substantial changes, while blink kinematics show a decrease in amplitude and velocity (Sun et al., 1997). In a recent study of Petrikovsky et al (2003), the blinking activity in healthy human fetuses was investigated and detected in 89% of the cases, with a mean frequency of 6.2 movements per 60 minute observation period. Vibro-acoustic stimulation was also used, which was associated with increased fetal blinking. They concluded that blinking is a normal fetal activity and the increased frequency of

blinking activity associated with vibro-acoustic stimulation may be considered a part of the normal startle reflex.

The relative importance of sensory input from the ocular surface, tear stability and centrally mediated factors in the determination of spontaneous blinking in neonates and infants is unclear. Although dopaminergic neurones appear early in human development, there are postnatal changes in the organisation of the basal ganglia which are associated with altered levels of endogenous dopamine (Meng et al., 1999; Herlenius and Lagercrantz, 2001). The low rate of spontaneous blinking at birth may therefore be explained by the relative immaturity of the dopaminergic system.

In a normal blink, no appreciable upward rotation of the globe is observed (Doane, 1980; Riggs et al., 1987), although this was reported by the clinical literature by Moses (1975). The confusion arises because of the differences between normal, unforced blinks, and those that are voluntary or where the eyelid is restrained from its normal motion. A forced blink or a restraint of motion of the upper eyelid results in a significant demonstration of Bell's phenomenon-upward gaze with lid closure. In a normal blink, the globe moves posteriorly by 1 to 6mm as the upper eyelid descends, probably caused by eyelid pressure during the closing phase of the blink (Doane, 1980). In recent findings, it has been found that each eye rotates with an amplitude and in a direction that is strongly influenced by initial conditions of direction of gaze and lid position (Riggs et al, 1987).

Although the exact mechanism of spontaneous blinking is debatable, studies have shown that the preliminary changes occurring in a spontaneous blink involve

contraction of the orbicularis rather than relaxation of the levator palpebrae superioris (Kessler et al., 1995). Lid closure occurs from the lateral canthus to the medial canthus, forming an integral part of the lacrimal pump mechanism.

The rate of spontaneous blinking is influenced by a variety of factors, physiological, psychological, pharmacological and environmental. Thus anxiety (King and Michels, 1957) and attention (Patel et al., 1991a; Acosta et al., 1999) reduce the rate of blinking. The rate of blinking may be taken as an index of the degree of mental tension or stress experienced by the subject and, to a certain extent, may be taken as an index to differentiate between phlegmatic and highly-strung individuals.

The factors involved in the control of spontaneous blinking are not well understood, although spontaneous blinks are likely to be initiated by both central and peripheral triggers. Recent evidence that the central nervous system plays a role in the control of blinking comes from several sources. Blink frequency is highly dependent on attention and cognitive states (Schmidtke and Buttner-Ennever, 1992) and spontaneous blink rates are significantly influenced by dopaminergic activity in the basal ganglia (Taylor et al., 1999). Furthermore, changes in the rate of blinking occur following the administration of dopamine receptor agonists and antagonists (Elsworth et al., 1991). Recent work has determined that the control of spontaneous blinking is also determined from sensory stimuli arising from the exposed ocular surface (Tsubota, 1998). Support of this hypothesis includes the observation that the blink rate is significantly lower following the instillation of a topical anaesthetic (Collins et al., 1989) and that blink rates are higher under conditions which favour tear evaporation (Tsubota, 1998).

2.9.3.2.2 Reflex Blinking

Reflex movement of eyelids occur when it is elicited by sensory stimulation, such as a cutaneous touch, auditory signals, bright visual stimuli, and corneal or ocular irritation. It is also associated synergistically with facial movements in yawning, sneezing, eating, and vomiting. Its most important role is as a protective mechanism for the eye on the stimulation of the ophthalmic division of the fifth Vth Cranial (Trigeminal) nerve. Reflex blinking operates at high speeds and is manifestated by simple neural circuits. The neural pathway of reflex blinking consists of the trigeminal nerve as the afferent nerve, and the facial nerve via the polysynaptic connection in the brainstem, as the efferent nerve (Esteban, 1999; Esteban et al, 2004).

Involuntary blinking is also sub-divided into: *a twitch blink*, consisting, of a small movement of the upper lid; *an incomplete blink*, where the descending upper lid covers less than two-thirds of the cornea; or a *complete blink*, where the descending upper lid covers more than two-thirds of the cornea (Abelson and Holly, 1977; Collins et al., 1989). *Reflex Blinking* is a rapid closure movement of short duration that is elicited by a variety of external stimuli.

2.9.4 Factors Affecting Blink Rate

2.9.4.1 Cortical Factors

The factors involved in the control of spontaneous blinking are not well understood, but it is believed to involve central and peripheral triggers. From studies of

spontaneous eye-blink activity, it has been concluded that its control is primarily determined by central (CNS) mechanisms and not on the state of the cornea and conjunctiva (Ponder and Kennedy, 1928; Stern et al., 1984; Karson, 1988). Blink activity is under cortical control and thus is correlated with certain mental activities and cognitive state variables (Ponder and Kennedy, 1928; Holland and Tarlow, 1972; Stern et al., 1984).

There is ample evidence that various mental activities including reading, memory use, or emotions modify blink rate, and this has been presented as a strong argument for central determinants of spontaneous eye-blink activity (Stern et al, 1984; Karson, 1988). The performance of simple behavioural tasks such as listening, talking, arithmetical exercises or silent rehearsal, significantly increase basal blink rate (Karson et al., 1981b; Tanaka and Yamaoka, 1993). Conversely, tasks that require visual information processing like reading reduce basal spontaneous blinking (Karson et al., 1981b; Goldstein et al., 1992). York et al (1971) found the blink rate declined from 15 blinks/ min to 4 blinks/ min when the visual task changed from watching a film to underlining every letter a on a page of text. The data suggest that blink frequency depends on the type and difficulty of the task and on the degree of attention and fatigue (Stern et al., 1994). In a recent study by Cho et al (2000) it was found that the blink rate was affected by the position of gaze and not by the level of task difficulty. The mean blink rates were significantly lower when performing the tasks at down gaze (reading normal English words and reading mirror-image English words) than when performing the tasks at primary gaze.

While working with computers, blink rate falls below resting conditions. Patel et al (1991) investigated the effect of visual display unit (VDU) on the blink rate and stability of the pre-corneal tear film in sixteen normal healthy subjects. They found an average 5-fold drop in blink rate (18.4 blinks/ min before VDU to 3.6 blinks/ min during VDU use) but tear film stability appeared to be unaffected. Acosta et al (1999) found a significantly reduced blink rate in about 40% of normal young subjects, during the performance of an attentive computer task that required strong visual attention. While working with computers, blink rates fall below the resting conditions, and other investigations showed a similar effect (Yaginuma et al., 1990; Tsubota and Nakamori, 1993; Dumery and Toi, 1997). This is usually combined with an increase of the exposed ocular surface, due to the opening of the eye to watch the video display unit. Both circumstances increase tear evaporation (Tsubota and Nakamori, 1995). The ensuing dryness of the ocular surface has been proposed as the origin of the ocular discomfort signs reported by computer users (Rolando et al., 1983; Tsubota and Nakamori, 1993). These include burning or itching, foreign body sensation, lacrimation, photophobia, ocular or orbital pain and headache. The influence of visual attention on blinking has been interpreted as the result of a cortical influence on the pontine reticular formation neurons that elicit spontaneous blinking (Karson, 1989).

A close relationship between blink rate and dopaminergic level transmission is supported, based on the variations observed in a series of diseases with impairment of this neurotransmitter. Variation of blinking rate has been observed in a number of neurological diseases, related to alteration of the dopaminergic activity. Patients with Schizophrenia and Parkinsonism tend to have increased and decreased blink rates,

respectively. The mean blink rate in patients with schizophrenia was significantly greater than that of the normal control subjects. During neuroleptic treatment, the mean blink rate was reduced only in drug-naive patients, but not in the previously neuroleptic treated schizophrenics (Stevens, 1978;; Karson et al., 1981a, 1984, 1986; Kleinman et al., 1984; Mackert et al., 1988, 1990; Mackert et al., 1991; Chen et al., 1996). Parkinson's disease is associated with reduced dopaminergic function and reduced blink rate. People who suffer from this disease experience low blink rates which increase after levodopa, a precursor of dopamine which is converted to dopamine in the brain and is currently the treatment for Parkinsonism (Karson et al., 1982, 1984; Deuschl and Goddemeier, 1998; Nakayama et al., 1998; Kimber and Thompson, 2000). Prolonged blinks are observed due to a delay in the levator resuming activity after the end of the orbicularis twitch (Loeffler et al., 1966).

Infrequent blinking in Parkinson's disease provides important evidence that one determinant that affects blinking is central dopamine activity. Since nigrostriatal cell loss (Forno, 1981) and reduction of striatal dopamine content (Hornykiewicz, 1981) are critical pathophysiologic changes in Parkinson's disease, the reduced blinking in this disorder probably indicates the influence of the nigrostriatal dopamine system on blink rate (Stevens, 1978).

In Progressive Supranuclear Palsy (PSP) (an under-recognized brain disorder, where gradual loss of certain brain cells causes slowing of movement and reduced control of walking, balance, swallowing, speaking and eye movement), blink rate is extremely reduced, around three blinks per minute and slow blinks have also been described (Golbe et al., 1989). In Blepharospasm (BSP) which is a disease where there are

repeated involuntary contractions of the orbicularis oculi muscles, ranging from a mere increase in the rate of spontaneous blinking to a state in which the eyelids are clamped tightly shut for long periods, the blink frequency is significantly increased, especially during the onset phase of the disease (Jancovic et al., 1982; Dening, 1987; Grandas et al., 1988; Elston et al., 1989).

Spontaneous blink rates are significant influenced by dopaminergic activity in the basal ganglia (Taylor et al., 1999). Altered blink rates are observed in several neuropsychiatric disorders that are known to affect dopaminergic neurotransmission (Taylor et al, 1999). Alterations in the rate of blinking occur following the administration of dopamine receptor agonists and antagonists (Elsworth et al., 1991).

One report (Helms and Godwin, 1985) describes a decreased blink rate in patients with major depression, while another found an increased blink rate (Mackintosh et al., 1983) which fell to normal levels during treatment. The effect of blink rate was found to be independent of medication, but was related to the degree of improvement in the patient's condition.

It has also been suggested that the blink rate reflects ease of seeing (Luckiesh and Moss, 1939; Bitterman, 1945; Tsubota et al., 1996), although others have found that the blink rate is independent of text readability or illumination (Tinker, 1948, 1949).

2.9.4.2 Ocular Surface Factors

Ocular surface factors also have been associated with blinking. Gilbard and Farris (1983) reported that ocular surface damage significantly increases the blink rate. For example, contact lenses, may disrupt the tear film (Sharma and Ruckenstein, 1985), thereby increasing the blink rate (Tada and Iwasaki, 1984). Contact lens wearers have been shown to blink less and to exhibit a higher proportion of incomplete blinks than non-contact lens wearers (Holly, 1981b). Blinking at frequent intervals is necessary for keeping moist the front surface of the lens, and the peripheral portions of the cornea not covered by the lens. Pointer et al (1985) noted that soft contact lens wearers adopt a sub-conscious blinking strategy to suppress blinking during critical tasks. On the other hand, it has been shown that the blink rate during contact lens wear increases significantly both with soft and hard lenses, with the subjects exhibiting a more regular blinking pattern and an increased number of complete blinks (Carney and Hill, 1984; Hill and Carney, 1984).

Blinking also appeared to be determined by local ocular surface conditions. Collins et al (1989), testing the hypothesis that corneal sensitivity influences the normal rate of involuntary blinks, anaesthetised the cornea using proxymetacaine hydrochloride. They found a significant reduction in the blink rate, with the blink rate before instillation at 24.8 blinks/ min and after instillation at 17.2 blinks/ min. This significant decrease in the blink rate supports the hypothesis that corneal sensitivity is at least partly involved in the mechanism controlling normal involuntary blinks.

The effect of corneal anaesthesia on blink rate was evaluated by Moore and Kardon (1997). Using proparacaine HCL 0.5% and anaesthetizing the cornea unilaterally and

bilaterally, they found a significant reduction in blink rate with either unilateral or bilateral corneal anaesthesia, and that the effect appeared to be additive between the two eyes. They hypothesised that blink rate is primarily determined by the total amount of trigeminal sensory input between the two eyes, but this is modulated by central processing.

Prause and Norn (1987) hypothesised that there might be a relationship between the tear film break up time (TBUT) and blink rate. Their investigation showed a significant but low negative correlation ($r = -0.33$), indicating that there is an association between TBUT and blink frequency. Thus the periodic blink is dependent not only on central stimuli, but also on the stability of the pre-corneal tear film.

A possible correlation between TBUT and blink frequency was investigated in a group of 41 Chinese subjects by Yap (1991). A stronger correlation ($r = -0.69$) was found, supporting the hypothesis of Prause and Norn that the break-up of the tear layer may be a stimulus for normal involuntary blinking.

Al-Abdulmunem (1999) made a similar investigation of the relationship between blink rate and TBUT using 159 healthy young females. A strong and significant correlation was found ($r = -0.74$) between the two parameters, which agrees with the study of Yap (1991).

Himebaugh et al (2001) investigated the relationship between blink rate and TBUT with four different visual tasks in both normal and dry eye subjects. Average blink rates were 12 blinks/ min while looking ahead, 10 blinks/ min while watching a

movie, 7 blinks/ min during a letter task (identifying rapidly changing letters) and 5 blinks/ min while playing a computer game, showing a gradual decrease in blink rate while difficulty of the visual task is increasing. Normal subjects showed areas of tear thinning or occasional inferior break up during the computer and letter tasks. Dry eye subjects showed tear break-up between blinks for all visual tasks, especially during the letter task and the computer game, where extensive tear break-up reflex tearing occurred.

Yolton et al (1994), in a study investigating the effects of gender and birth control pill on spontaneous blink rates, showed that a relationship might exist between blink rate and tear stability. However only an extremely weak correlation was found, showing that TBUT test is not a good predictor of blink rates.

Other investigations of blink rate and tear film stability showed either low or no correlation (Goldstein et al., 1985; Patel et al., 1991a).

2.9.4.3 External Environment

Hata et al (1994) reported the effect of corneal temperature change on blink rate. Recruiting eight normal subjects, they measured their central corneal temperature and inter blinking interval (IBI) in 20°C and -4°C rooms. As central corneal temperature decreased significantly from 34.72±1.45°C in the 20°C room to 31.9±1.6°C in the -4°C room, so too did the IBI, i.e. in cooling conditions the blink rate increased. Corneal temperature change may be the key factor for blinking, since subtle corneal

temperature change, due to tear evaporation in each blink, could be the initiator for blinking.

A second investigation by Hata et al (1995), reported that change of temperature and humidity has an influence on blinking. Central corneal temperature and inter blinking interval (IBI) were measured in four normal subjects in a 50°C (humidity 20% and 80%) room, 20°C (humidity 20% and 80%) room, and 0°C room (humidity 80%). When increasing the humidity of the room from 20% to 80% in the 20°C room, central corneal temperature increased from 34.8±0.8 to 35.9±1.1°C. IBI increased from 3.43±1.67 to 6.62±2.10 seconds in the 20°C room. When increasing the room temperature from 20°C to 50°C in 20% humidity, central corneal temperature increased from 34.8±0.8 to 36.2±0.6°C. They concluded that in cool conditions and low humidity, both corneal temperature and blink rate increased. They also found that as tear evaporation began on opening of the eye lids, the corneal temperature began to decrease after blinking.

Studies of inter-blink interval (IBI) in different humidity environments (Ponder and Kennedy, 1928) showed that the evaporation of tears from the ocular surface was not a major determinant of eye-blink rate. Increased evaporation might be expected in those individuals with a wider palpebral aperture, and thus with a greater exposure of the ocular surface area (Rolando and Refojo, 1983; Sotoyama et al., 1995). Changes in spontaneous eye-blink frequency have been noted when the palpebral aperture is deliberately changed by asking the individuals to maintain a normal straight-ahead, superiorly, or inferiorly directed gaze (Tsubota and Nakamori, 1995; Nakamori et al., 1997). Such tasks or alteration in eye positions have been noted to change the normal

blink rate (Ponder and Kennedy, 1928; Stern et al., 1984; Karson, 1988). However, most recent investigations have shown that as the exposed ocular surface increases, the eye-blink frequency increases (Nakamori et al., 1997).

Nakamori et al (1997) investigated the relation between blinking and ocular surface conditions, concentrating on the local control of blinking. This study demonstrated an important association among the blink rate, maximum blink interval, and ocular surface conditions. Significant changes in the blink rate and the maximum blink interval were induced by factors that directly or indirectly affect the ocular surface: exposed ocular surface, topical anaesthesia, wind and video display terminal use.

A similar attempt was made by Tsubota et al (1996) to confirm the theory that local conditions affect patterns of blinking. They found that the pattern of blinking in normal subjects was stable and regular, with relatively low variation. In contrast, the pattern of blinking in patients with dry eyes was more frequent and erratic. The fact that the dry group blinked more often under windy condition and less often when they were wearing protective spectacles or using artificial tears, indicated that their frequency of blinking was primarily determined by the need to maintain the moistness of the ocular surface. The relationship between the habitual palpebral aperture, the exposed ocular surface, and spontaneous eyeblink activity was assessed by Zaman et al (1998) in elderly individuals. No correlation was found between spontaneous eye blink frequency, inter-blink interval, maximum inter-blink interval, and the palpebral aperture or the exposed ocular surface. These results suggest that the exposed ocular surface itself does not appear to be an important determinant of spontaneous eyeblink activity in elderly individuals.

3. Instrumentation

3.1 Measurement of Corneal Sensitivity

3.1.1 The Non-Contact Corneal Aesthesiometer (NCCA)

The Non-Contact Corneal Aesthesiometer (NCCA) assesses corneal nerve function by using a controlled pulse of air, directed at the corneal surface. Technically, it is composed of:

1) A means of generating and maintaining a flow of air.

2) A reservoir for storing and pressurising that air-flow.

3) A valve for controlling the air-flow, and thus the stimulus intensity.

4) A pressure sensor and display to allow monitoring and setting of the stimulus intensity.

5) A mechanism for controlling the duration of the air-pulse stimulus.

6) A means of delivering the air-pulse stimulus to the eye.

Atmospheric air is pumped, using a simple air pump, into an air reservoir. This reservoir acts to dampen the pressure pulses created in the air flow by the pump itself and allows the air to be slightly pressurized to ensure a steady supply. The pressure of the air is monitored using an electronic pressure sensor, which displays its readings digitally in millibars (mbars) above atmospheric pressure. The air outflow, and thus pressure, of the reservoir is regulated using a manually controlled valve. Two electronically controlled two-way switch valves direct the flow of the air either to an exhaust jet or, when a stimulus is to be applied to the eye, via the stimulus jet. The stimulus jets are 35mm in length and 6mm in diameter. They each have a bore of

0.5mm diameter through the centre, lengthways, through which passes the air stimulus. The time duration of the stimulus can be varied by electronic control of the switch valves to deliver an air pulse of 0.5, 0.9, or 1.5 seconds duration. The various components of the device are connected using nylon tubing. The reservoir, valves, pressure sensor and its display, and the connecting tubing are all placed within a self-contained polycarbonate sealed box. The figure below illustrates the layout and connections (Murphy et al., 1996).

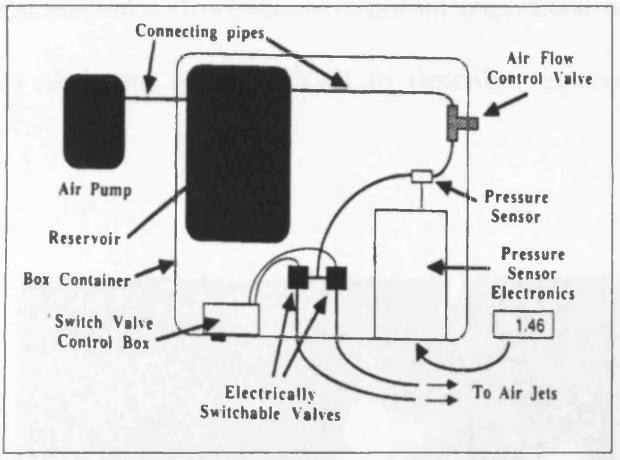


Figure 3.1: Diagram of the technical components of the Non Contact Corneal Aesthesiometer (NCCA) (From Murphy PJ et al: Ophthal Physiol Opt 16: 101-107, 1996).

3.1.1.1 Method of Use

The stimulus jet is positioned close to the eye by means of a slit-lamp attachment, which allows accurate alignment of the jet. This air-jet mounting attachment consists of a vertical metal bar, which is placed in the hole found at the top of the slit-lamp's rotational pivot. It is 17 cm tall, from the bottom of the plate to the air-jet, which is mounted in a holder. Holes bored in a plastic mount allow fibric-optic fixation targets to be positioned to aid in controlling a subject's fixation temporally, medially, or

superiorly, allowing repeatable stimulation of the medial, temporal and inferior corneal regions, respectively. The testing distance of 10mm is set by using a clear, plastic centimetre ruler attached to the side of the plastic mount, and extending towards the eye, similar to the scale on an exophthalmometer. Alignment of the air-jet with the centre of the cornea is carried out visually (Murphy et al., 1996).

The sensation felt by the subject is described in a variety of ways, the most common being as a "cold" sensation. It is also described as feeling like a breeze on the eye, or as a pressure type sensation. However, it is not an unpleasant sensation. Subjects, report the stimulus as being quite difficult to describe, especially when close to threshold.

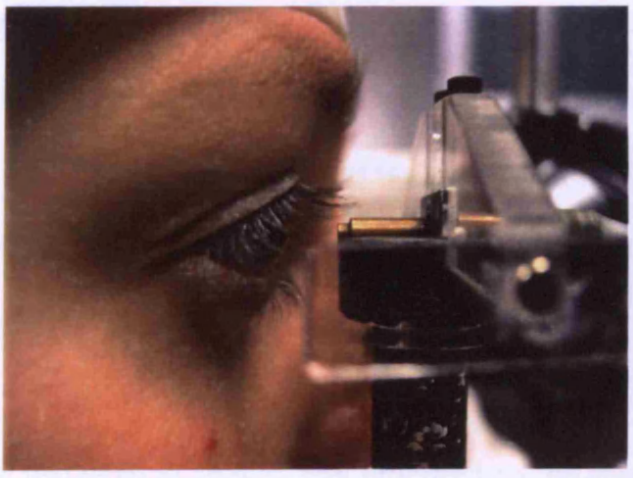


Fig 3.2: Positioning the stimulus air-jet close to the eye.

Each subject is positioned at the slit-lamp, so that the tip of the stimulus air-jet is positioned 10mm away from the corneal apex. At this distance the area of stimulation is known to be 0.8mm^2 and is considerably lower than the overall surface area of the cornea which is approximately 95 mm^2 (Murphy et al, 1996). The subject fixates on a distant target, to help keep the corneal apex or eyelid aligned with the air-jet. The

stimulus is presented using the method of limits, sometimes known as the double staircase technique. In this method, the stimuli are presented in either an ascending or descending order. The first stimulus is one of higher pressure than the threshold of the subject to demonstrate the sensation felt. Then, the stimulus pressure is gradually decreased until it is below the threshold of the subject, and the pressure at which the stimulus was last felt is noted. The pressure is then gradually raised again until the subject can detect the stimulus, and the pressure at which the stimulus was first felt is recorded. This process is repeated until the threshold is consistently located (Murphy et al., 1996).

3.1.1.2 Mode of Stimulation

The air-pulse stimulus of the NCCA has been described by subjects as a cool sensation or as a breeze against the eye. As the cornea is not touched by any type of stimulus probe, there is no direct mechanical stimulation, suggesting that it is the C fibres rather than the Aδ fibres that respond to the NCCA stimulation. Murphy et al (1999b) investigated whether corneal surface temperature change was the component in the mode of stimulation of the NCCA. Using a thermal camera to observe corneal surface changes for a stimulus pressure of 1.0 mbars over a stimulus duration of 0.9 seconds, a detectable temperature change was produced on the anterior surface of the eye. The cooling of the tear film was proposed to be related to the process of evaporation. As the air-flow strikes the cornea it increases the rate of evaporation from the tear film by altering the humidity equilibrium of the air above the cornea and by removing any evaporated water molecules from the vicinity. This evaporation removes energy from the tear film at the stimulus location and the temperature of the tear film drops. This temperature change is transferred to the cornea by conduction,

and the nerve endings in the corneal epithelium detect this change in the temperature (Murphy et al., 1999b).

3.1.2 Cochet-Bonnet Aesthesiometer

The Cochet-Bonnet Aesthesiometer (Cochet and Bonnet, 1960) is based on the instrument devised by Boberg-Ans (1955, 1956). The instrument uses a nylon thread of diameter 0.12mm to directly stimulate the mechanically sensitive corneal nerves. With this thread stimulus intensities of 11-200mg/mm^2 are possible. A second thread of 0.08mm diameter is also available that can give lower intensity stimuli (2-90 mg/mm^2). In practice the thinner thread is rarely used and is no longer commercially available.

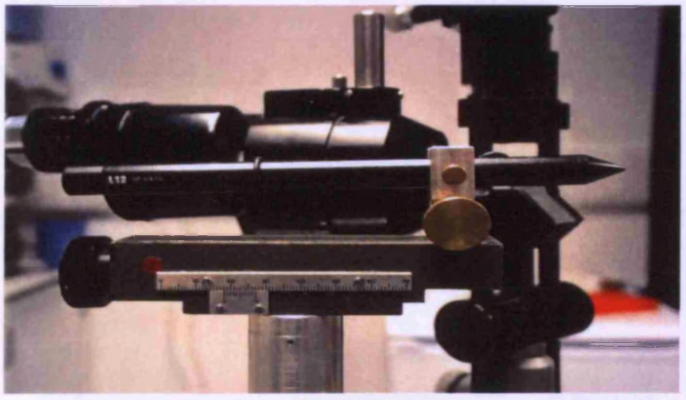


Fig 3.3: The Cochet-Bonnet Aesthesiometer attached to a slit-lamp holder.

The instrument consists of a small pen (the body of the instrument) containing the nylon monofilament thread. The constant diameter of the nylon monofilament enables a pressure to be exerted on the corneal surface that is dependent on its length. To measure corneal sensitivity, the tip of the nylon thread filament is gently pressed against the cornea. Sufficient pressure is put on the filament in order to bend it and a

response from the subject indicates whether the filament has been felt. As the filament can be extended to different lengths, various levels of pressure can be obtained: the longer the monofilament used, the lower the pressure required to bend it, and the shorter the monofilament used the higher the pressure required to bend it. So the pressure exerted is inversely proportional to the monofilament length. The length of the filament at which the subject detects the stimulus can be converted to pressure using a calibrated scale (Draeger, 1984).

3.1.2.1 Method of Use

The aesthesiometer is mounted in a holder attached to slit-lamp, allowing movement in the three cardinal directions. The nylon monofilament must touch the cornea at right angles to its surface to produce the correct stimulus pressure, and this point is reached when the thread is observed to just bend. The humidity in the room should not exceed 60%, because the nylon thread will be affected and give a false reading (Cochet and Bonnet, 1960).

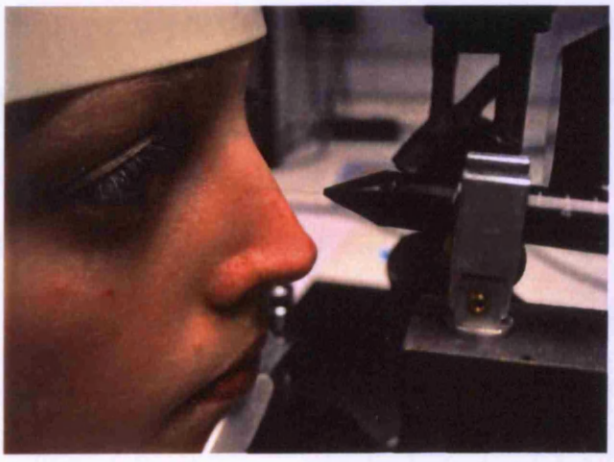


Fig 3.4: Nylon monofilament of the Cochet-Bonnet aesthesiometer moving towards to the eye.

The touch threshold of the cornea is determined using the method of constant stimuli. Beginning with the lowest pressure, four to eight presentations are made at each length of the nylon thread, and the patient is asked to indicate when the probe is felt by giving a hand signal. A few blank measurements are also made (the nylon thread is brought close to the cornea without touching it) to test the reliability of the patient. If the stimulus is not felt with the lowest intensity, the length of the monofilament is then decreased (in 5mm steps) until the threshold is obtained. The CTT is defined as the length of the nylon thread at which the subject responds to 50% of the number of stimulations. This length is converted into the stimulus pressure using the table given with the aesthesiometer. Corneal sensitivity is defined as the reciprocal of the CTT (Cochet and Bonnet, 1960).

3.1.2.2 Mode of Stimulation

The nylon thread of this aesthesiometer stimulates the corneal nerves directly by pressing against the eye to produce a corneal deformation (Cochet and Bonnet, 1960; Lawrenson and Ruskell, 1993). By varying the length of the thread, a range of stimulus pressures can be applied by producing different amounts of corneal deformation. It is this deformation that causes the sensation of touch. The Aδ fibres, running parallel to the corneal surface, respond exclusively to mechanical forces (MacIver and Tanelian, 1993; Belmonte and Gallar, 1996; Muller et al., 1996, 2003), and we can assume that the predominant nerve fibres types, stimulated by the Cochet-Bonnet aesthesiometer, are the mechanical stimulation-specific Aδ fibres.

3.2 Measurement of Corneal Thickness

3.2.1 The Haag-Streit Optical Pachometer

The corneal thickness was measured using the Haag-Streit Pachometer (Mishima and Hedbys, 1968) mounted on a slit-lamp. The pachometer is based on the optical principle, originally described by Jaeger (1952) and consists of two glass plates in front of the right eye microscope. The lower plate is fixed and the upper plate is rotatable around a vertical axis. The incident light comes through a vertical aperture in a thin metal diaphragm extending from the attachment, which maintains an angle of 40° between the incident light beam and the axis of the right microscope. The right eyepiece is replaced by a special slit that divides the visual field into lower and upper halves. The light passing through the upper rotatable and the lower fixed glass plates, is seen in the upper and the lower visual field, respsectively (Mishima and Hedbys, 1968).

3.2.1.1 Method of Use

The measurement assesses the apparent thickness of the corneal optical section, when the slit-beam passes through the tissue perpendicularly to its surface (Jaeger, 1952). The setting of the doubled device moves a scale relative to a pointer, which permits the apparent corneal thickness to be recorded. To get a good corneal section, the thinnest possible slit beam is used with a short bright slit. The slit beam should be accurately focused throughout the measurement, and, to assist with this, the maximum objective magnification is used. (Mishima and Hedbys, 1968).

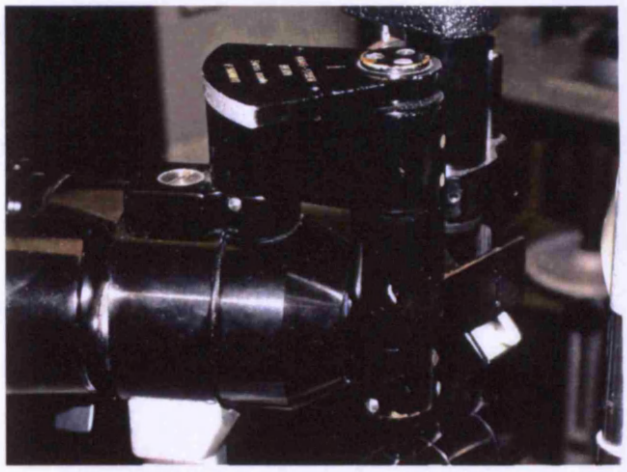


Fig 3.5: The Haag-Streit Pachometer attached to a slit-lamp for the measurement of corneal thickness.

The patient is asked to look at a target that can be adjusted by the observer to any position, depending on which part of the cornea the measurement will be taken. The optical section of the cornea is then placed at the centre of the visual field of the microscope and the slit positioned perpendicularly to the corneal surface. With the slit beam in focus, the sector-shaped disc at the top of the pachometer is rotated, so that the corneal endothelium of the upper field is in line with the corneal epithelium of the lower field. The scale reading on the disc gives the corneal thickness in changing millimetres.

The actual corneal thickness corresponding to the scale reading varies, depending upon the corneal curvature. For the same radius of corneal curvature, some correction is necessary for the scale reading, depending on the range of the thickness values. This is because the scale divided the angle into equal segments, but the relationship between the corneal thickness and the rotated angle of the glass plate is not linear. Within the normal range of the human central corneal thickness (0.45 to 0.57mm), practically no correction is necessary (Mishima and Hedbys, 1968).

3.3 Measurement of Tear Film Stability

3.3.1 Invasive Tear Break-Up Time (TBUT)- Fluorescein

Traditionally, tear break-up time has been measured by staining the tears with fluorescein. The fluorescein is instilled into the conjunctival sac and the cornea is scanned with cobalt blue illumination, using the slit-lamp microscope, for the first sign of any discontinuity. The dye is usually applied by wetting a fluorescein-impregnated strip with saline. In the experiments conducted in this thesis, 0.7 μl of 2% fluorescein was instilled using a P100 micropipette (Wolf Laboratories, UK) to ensure a measured doze was instilled in each subject. The fluorescein was expelled from a sterile, single-doze Minim (Chauvin Pharmaceuticals, Essex, UK) into a sterile Eppendorf dry tube (Fisher Scientific, Loughborough, UK, Ltd), from which it was extracted by the micropipette. A new sterile pipette tip was used on each instillation. Subjects were asked to tilt their head back and look down. The upper eyelid was then held open and the fluorescein was instilled into the conjunctival sac. A wide beam (full aperture) was used, so that the whole cornea was illuminated and viewed with 10X magnification, using moderate illumination. A yellow filter (Baush & Lomb) was used to improve contrast. Any discontinuities in the tear film were seen as dark spots in the fluorescent film. The first appearance of a spot represented rupture of the tear film. This "fluorescein" break up-time has been defined as: the time interval between a complete blink and the appearance of the first randomly distributed dry spot; and the period is usually greater than the time interval between two consecutive blinks. Normal values range from 15 to 35 seconds, and values less than 10 seconds are suggested to be abnormal (Norn, 1969; Lemp and Holly, 1970; Rengstorff, 1974).

Three measurements of the tear film break-up time were taken to provide an average TBUT.

3.3.2 Non Invasive Break-Up Time (NIBUT): Tearscope Plus

The Tearscope Plus (Keeler, UK, Ltd) is a hand-held instrument that it is used in conjunction with a slit-lamp biomicroscope. The Tearscope, developed by Guillon (1986) is specifically designed to prevent any artificial drying of the tear film during the examination. It is based on an illuminated, translucent, tubular design which acts as support for inserts and add-ons. With the patient's head positioned on the slit-lamp chin rest, the slit-lamp source was positioned nasally and switched off, since illumination is provided by the Tearscope Plus itself. The Tearscope was mounted on the slit-lamp between the microscope and the subject. This allowed the instrument to be held close to the eye and positioned to allow observation through the observation hole via one of the biomicroscope objectives. Close position of the Tearscope to the eye maximizes the area illuminated. A translucent grid pattern was inserted within the illuminated inner surface of the instrument to project a regular grid pattern on to the tear film surface. A deformation in the pattern reveals the imminent rupture of the tear film. Initially a low magnification was used although this was increased to 20-40x to examine the interference patterns in detail (Guillon and Guillon, 1988; Veys, 2002).

Picture 3.6: Set-up of the Tearscope for the measurement of the tear-film break-up time non-invasively.

3.4 Measurement of Ocular Surface Temperature

3.4.1 Themo-Camera for Ocular Thermography

To measure dynamic temperature measurements of the ocular surface area, an infrared radiation thermography instrument was used. This is the NEC San-ei Thermo Tracer TH7102MX thermo-camera. This instrument was provided on-loan by Dr James Wolfson, Department of Vision Sciences, Aston University.

Fig 3.7: The TH7102MX thermo camera for measuring ocular surface temperature.

Modern infrared temperature measurement usually employs a scanning system to provide information of the surface temperature across a large area. The data can be transformed into a colour-coded image which is displayed on a monitor and can be interpreted easily. Measurement and colour-coded display is referred to as thermography (Morgan et al., 1993).

The camera has the following features:

1) It uses an uncooled focal plane array for the detector (Silicone) which makes it a truly portable device; LCD colour view-finder.

2) Self calibrating: no need for black body device.

3) The detector is sensitive to 8-14μm, which is suitable for the emission spectrum of the anterior eye.

4) It has three frame speeds (7.5, 30, 60Hz), as well as a static facility.

5) Temperature resolution of 0.08°C at 30Hz, 0.16°C at 60Hz.

6) Accuracy of ±2% (over widest range).

7) Addition of a close-up lens allows close focus at 60mm, with a spatial resolution of 100μm.

8) Pixel size of image 320(H) x 240(V).

9) Colour/ monochrome facility.

10) Run/ freeze facility.

3.4.1.1 Collection and Analysis of Data

The processing within the camera allows up to ten point or five box settings for measuring temperature, but to permit greater flexibility, purpose-written software has been developed by Dr James Wolffsohn (Aston University) using Labview® (National Instruments, USA). This program run on a PC computer connected to the camera. Data is collected from the dynamic thermal profile and, with the image in monochrome, the software program enables interpretation of the tones of grey as temperature, given a set range for the 256 gradations. The sensitivity of the camera can be altered to extend detecting range as required. Emissivity is set at 0.98.

The program collects dynamic information from 21 points on the cornea, allowing the continuous monitoring of the temperature of the anterior eye and the development of dynamic thermal profiles. For these studies, a customised program was used to collect dynamic information from 5 points on the cornea (centre, superior cornea, inferior cornea, nasal sclera, temporal sclera).

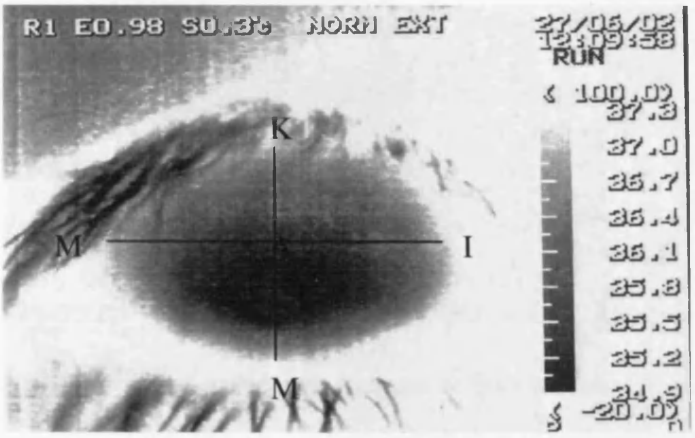


Fig 3.8: Output display screen of monochrome image analysis software.

At a viewing distance of 60mm, the camera's field of view is 34.5mm (horizontal) x 26.0mm (vertical), represented by 520x247 pixels on the computer screen. This means that for most subjects, the whole anterior eye surface is visible.

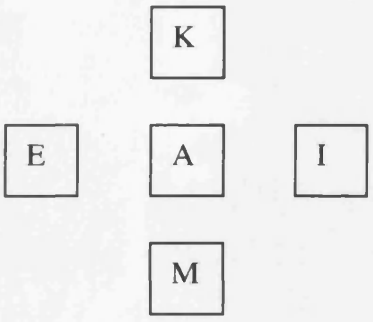


Fig 3.9: The five data points: A= Centre of the Cornea, K= superior cornea, M= inferior cornea, E= temporal sclera, and I= nasal sclera.

3.4.1.2 Method of Use: Set Up of the Camera

The camera was set-up in a dedicated area of the laboratory, which provided control over environmental temperature and humidity. Background radiation was reduced by the use of a black thick curtain, and by maintaining a short distance from the radiation source to the detector with a close-up lens. The lens cap (coated with blackbody

paint) was used to calibrate the camera before each experiment. This allows a correction factor to be calculated by the camera to take into account any reflection of the environmental temperature from the eye.

The camera has a special slit-lamp attachment which allows good control of camera alignment. The close-up lens focuses the camera at 60mm from the camera objective, and the slit-lamp is moved to bring the image of the eye into focus. The distance between eye and lens is checked by placing a rule of length 60mm between the lens and the closed lid.

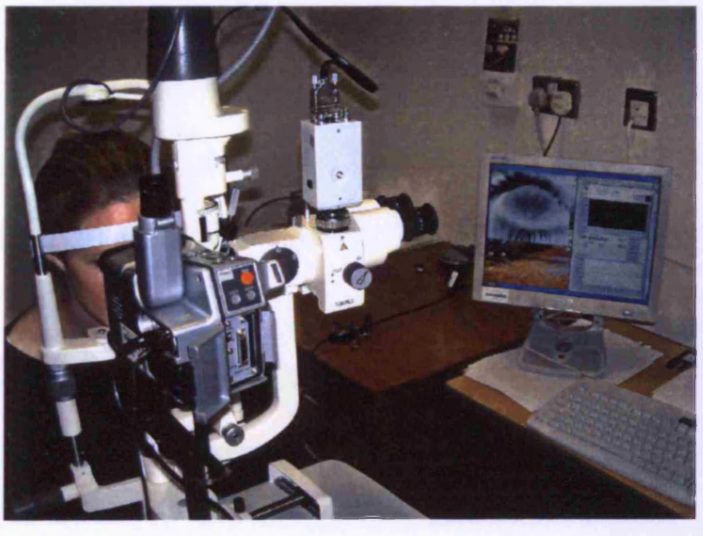


Fig 2.10: Camera set-up for the measurement of the ocular surface temperature.

Measuring the temperature of the right eye only, the patient was asked to stabilise their head on the slit lamp chin-rest and look straight ahead into the camera, blinking normally. Proper alignment was achieved, when the center of the cornea was positioned at the central point A, the superior and inferior cornea at the data points K and M, and the nasal and temporal sclera at the data points E and I. A clear image at the computer could then be seen as shown in Figure 11. The subject was asked to

make a good blink (eye-closure) for 2-3 seconds and then to avoid blinking for 8 seconds, always looking into the camera. During the 8 seconds without blinking, the camera recorded the dynamic temperature change. Five measurements were recorded for each subject and the average of these measurements was the dynamic thermal profile for each subject.

3.5 Measurement of Evaporation from the Ocular Surface Area

3.5.1 Evaporimeter

Tear film evaporation rate measurements were made with a modified ServoMed EP-3 Evaporimeter (ServoMed, Kinna, Sweden). This instrument was provided on-loan by Dr Ian Pearce, Department of Vision Sciences, Glasgow Caledonian University. The instrument is based on the theoretical principal that, in the absence of forced convection, a linear relation exists between the vapour pressure (product of relative humidity) and the distance from an evaporative surface (Nilsson, 1977). The ServoMed Evaporimeter calculates the difference in vapour pressure recorded by two sensors placed above the evaporative ocular surface (Trees and Tomlinson, 1990; Craig and Tomlinson, 1997). The sensors measure relative humidity and temperature at two heights and, from knowing the vapour pressure at these two points, and the distance between them, the evaporation rate can be calculated (Trees and Tomlinson, 1990).

The Evaporimeter is connected to a PC computer that allows control of data collection and analysis. The software program requires the ambient temperature and humidity

recorded at the time of the evaporation measurements during the conversion analysis, and these are measured for the Laboratory while taking measurements.

3.5.1.1. Method of Use

The ServoMed Evaporimeter is attached to a modified swimming goggle mounted on a slit-lamp. The swimming goggle is used to keep the probe from coming in contact with the subject's eye and to isolate the eye from the outside environment (Trees and Tomlinson, 1990). This goggle arrangement allows the subject to blink normally.

The evaporation rate measured by the goggle-mounted probe is a combination of the evaporation rates of the pre-ocular tear film and the facial and lid skin within the probe. The evaporation rate of the pre-ocular tear film is measured with the subject's eye open, whereas the skin evaporation rate is measured with the subject's eye closed.

Although the swimming goggle is usually mounted on the slit-lamp and the subject's head is supported by a chin and head rest while the modified goggle surrounds the eye, in our experiment the subject was asked to hold the goggle mount with their left hand, and to apply light pressure on the goggle to maintain a close fit with the skin around the eye.

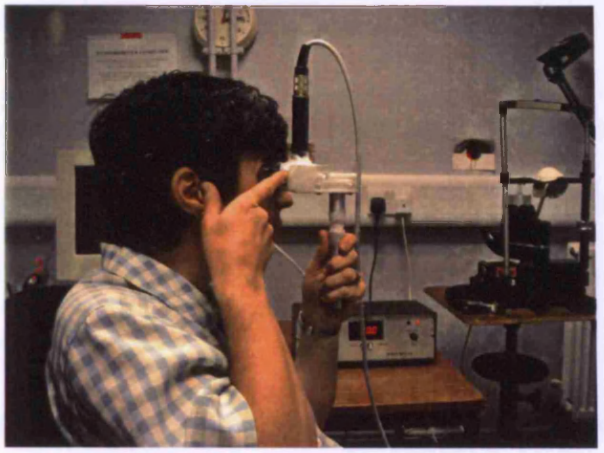


Fig 3.12: The swimming goggle of the evaporimeter attached to the eye for the measurement of the tear film evaporation from the ocular surface.

The evaporation rate was measured on the right eye only. Each measurement of evaporation rate was composed of two measurements of evaporation rate for each eye. Firstly with the eyes closed, and then with the eyes open. The closed eye evaporation readings were used to factor out the contribution to evaporation from the skin of the face within the goggle. It is necessary for calculations of ocular surface evaporation rates to know the exposed ocular surface area within the goggle. A photograph of the subject's eye, with a reference scale, was taken and the area of the eye calculated with the NIH Image II computer program.

The evaporation rates were recorded twice for each subject, for two minutes. Only the measurements recorded in the second minute (stored every 5 Hz) were used in the calculation, to allow the environment within the goggle to stabilise during the first minute.

3.6 Iris Colour Classification System

The subjects are classified according to their iris colour using the Iris Color Classification System by Seddon et al (1990). This system is based on four standard photographs (Fig 3.13). Categories of iris colour are distinguished based on the predominant colour: blue, grey, green, light brown, and brown, and the amount of brown or yellow pigment in the iris, into five groups. The system uses photographs of four standard iris colours to grade any individual's iris.

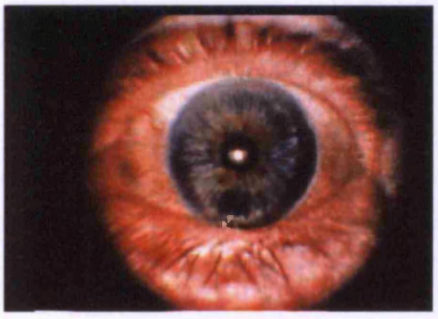


Fig 13 (a): Standard A

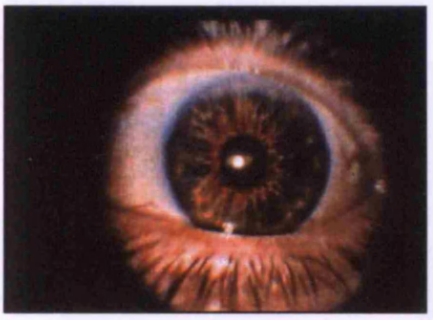


Fig 13 (b): Standard B

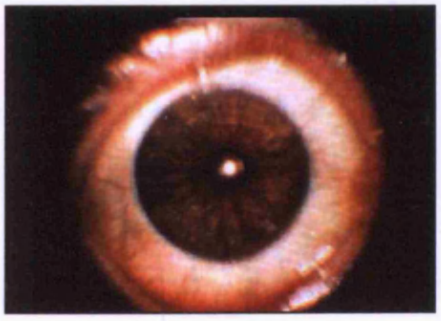


Fig 13 (c): Standard C

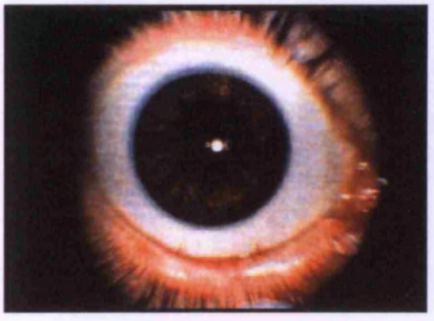


Fig 13 (d): Standard D

Fig 3.13: Iris Color Classification System: Photographs of the four standard iris colours, used to grade an individuals iris (From Seddon et al: Invest Ophthalmol Vis Sci 31: 1592-1598, 1990).

Grade 1: Blue or grey iris with brown or yellow specks equal to (in approximate percentage of total iris area) or less than in standard A (Fig 3.13 a).

Grade 2: Blue, grey or green iris with brown or yellow specks equal to or less than in standard B (Fig 3.13 b) but greater than in standard A.

Grade 3: Green or brown iris with brown or yellow specks equal to or less than in standard C (Fig 3.13c) but greater than in standard B.

Grade 4: Brown iris with colour equal to or less than in standard D (Fig 3.13 d), but greater than in standard C.

Grade 5: Brown iris darker than standard D.

Blue or grey irides: are classified as *grades 1 or 2*. These grades are distinguished by assessing the proportion of total iris area with brown or yellow pigment, compared with standard photographs A and B.

Green iris: are classified as *grades 2 or 3*. These grades depend on the extent of brown or yellow pigment in the iris.

Brown iris: are classified as *grades 3, 4, or 5*. These grades depend on the intensity of the yellow – brown pigment compared with standard photographs C and D.

3.7 Photography of the Eye

3.7.1 Canon Digital Camera

A Canon Power Shot G2 Digital Camera (Canon UK Ltd) was used for photography of each subject's eye and adnexa. These photographs were later used for iris colour classification, and the calculation of the exposed ocular surface area.

Fig 3.14: Digital Canon Camera Power Shot G2, attached to a close-up lens Canon (250D, 58mm), through a conversion lens adapter.

For optimum results a close-up lens (Canon 250D, 58mm) was required, and this was used with a conversion lens adapter (Canon, LA-DC58). A software program (Canon Digital Camera Solutions V8.0) was provided with the camera to download the images taken onto the computer.

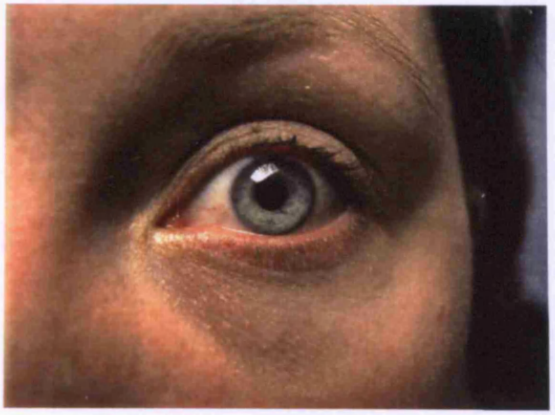


Fig 3.15: Photograph of an eye taken with the Canon Camera, using a Canon close-up lens.

3.8 Recording of Blink Rate

3.8.1 Video Camera Recorder

A Sony DCR-TRV27E Digital Handycam (Sony UK Ltd, Berkshire) was used to record the blinking pattern of the subjects. A digital video format was chosen since it allowed easier transfer and storage of recorded sequences. The camera was hidden, in order the recording of the blink rate of the subjects to be done without their knowledge, as blink rate could be affected (Doane, 1980; Yap, 1991).

Fig 3.16: Sony Digital Video Camera Recorder.

Each subjects sequence was first recorded onto digital video tape using the camera's own recorder. Each tape was then downloaded onto an E Mac computer (E Mac G4, 1.25 MHz) using the iMovie software program (iMovie 4.01, Apple Computer. Inc). Each subject's sequence could then be identified for analysis.

Since blink rate can be affected by the psychological status of the subject, the recording was done without the subject's knowledge, while they were watching an educational film. Once the data collection was completed, all the subjects were made aware of the video recording. The subjects were then asked to sign a second consent form, which explained the purposes of the study and why the subject was only made aware of the camera after the completion of the experiment and not before. If the subject did not agree, the recording was deleted in the presence of the subject and the subject's data removed from the study. There were only two subjects who disagreed with the procedure of the experiment, and the recording data were deleted.

4. An Investigation of the Diurnal Variation in Corneal Sensitivity and Thickness

4.1 Introduction-Purpose

The diurnal variation of corneal sensitivity in humans has been studied in two previous works. Millodot (1972) measured the diurnal variation of central corneal sensitivity to touch using the Cochet-Bonnet Aesthesiometer over a 12-hour period. He found that sensitivity was lowest in the morning, with a slow improvement during the day. du Toit et al (2003) investigated the diurnal variation of both corneal sensitivity and thickness over a 24-hour period and found a 35% variation in sensitivity over this cycle. Central corneal sensitivity was measured using a non-contact pneumatic aesthesiometer (Vega et al., 1999), a device based on the pneumatic aesthesiometer developed by Murphy et al (1996).

This reduction of corneal sensitivity in the morning has been attributed to eyelid closure and to reduced oxygen tension at the anterior ocular surface caused by lid closure (Millodot and O'Leary, 1979). This is accompanied by swelling of the epithelial cells which stimulates the corneal nerve endings and induces neural adaptation (Millodot, 1972).

Diurnal variation of corneal thickness in humans has been monitored over periods ranging from 12 to 48 hours, and using optical or ultrasonic pachymetry (Kiely et al., 1982; Holden et al., 1983; Harper et al., 1996; du Toit et al., 2003). Other studies have measured overnight swelling and diurnal variation of corneal thickness for less

than 12 hours (Mandell and Fatt, 1965; Mertz, 1980; Feng et al., 2001). All of these studies showed that the cornea is thickest immediately after eye opening in the morning, regardless of the instrumentation used or the time points when measurements were taken. However, there was a disparity in the time point at which the thinnest measurement of the cornea was taken, of between 5-10 hours after eye opening.

Corneal thickness measurements are indicative of the metabolic status of the cornea, since they provide information on the corneal hydration status (Mishima et al., 1966). Such measurements give valuable information on the physiological state of the cornea. Evaluation of corneal thickness is important in a wide range of disorders, such as ocular disease (Insler and Baumann, 1986; Mandell et al., 1989; Weston et al., 1995; Larsson et al., 1996; Saini and Mittal, 1996; Auffarth et al., 2000), glaucoma (Copt et al., 1999; Shah et al., 1999; 2000), contact lens related complications (Huff, 1991; Solomon, 1996; Liu and Pflugfelder, 2000), dry eye (Pole and Batzer, 1985; Liu and Pflugfelder, 1999), trauma (Cheng et al., 1988), and hypoxia (Klyce, 1981; Johnson et al., 1985).

Corneal thickness measurement can provide a reference parameter for experimental and clinical research. However, it is important to understand how corneal physiological factors, such as hypoxia, evaporation and intraocular pressure, affect corneal hydration. Hypoxia and lack of tear evaporation combine to produce overnight corneal thickness changes (Mandell and Fatt, 1965; Mertz, 1980; Holden et al., 1983; Tomlinson and Cedarstaff, 1992; Harper et al., 1996; Feng et al., 2001; du Toit et al., 2003). The healthy human cornea experiences hypoxia beneath the closed

eyelid during sleep (Holden et al., 1983; Efron and Carney, 1979). Beneath the closed eyelid, there is a reduction in oxygen levels and an absence of evaporation from the tear film, which are thought to induce corneal anaerobic metabolism and hypotonicity of the tear film, respectively. The anaerobic metabolism causes an accumulation of lactate within the stroma, which produces an osmotic influx of water and a reduction of water in the epithelium (Klyce, 1981). Clinically, an injured epithelium has been shown to result in increased epithelial oedema in the morning, after lid closure during the night and a subsequent reduction of evaporation from the ocular surface (Dohlman, 1987; Feng et al., 2001). Other factors that could influence corneal hydration include intraocular pressure, which increases rapidly after awaking (Frampton et al., 1987; Harper et al., 1996), and the temperature of the body, which decreases during night time sleep and increases again after sleep and throughout the day (Wright, 1992; Harper et al., 1996).

Since corneal sensitivity is known to vary during the day, it is important, for experimental design, that the effect of this diurnal change does not influence the results. Although it is known that corneal sensitivity is lowest immediately after eyelid opening, it has not been clearly established when corneal sensitivity change stabilises. To improve the quality of the results for corneal sensitivity in later studies, it was felt important to establish the diurnal change pattern. The amount of corneal thickness has been included as a control to show that the physiological status of the cornea has changed.

In this study, the pattern of change in corneal sensitivity was assessed using both the Non-Contact Corneal Aesthesiometer (NCCA) and the Cochet-Bonnet

Aesthesiometer. This will allow the assessment of different groups of nerve receptors, both cold and mechanical sensors in the corneal epithelium. In addition, the diurnal variation of corneal thickness measurement will allow an assessment of how corneal sensitivity and thickness inter-correlate during the day.

4.2 Methods

Twenty Caucasian subjects (7 males, 13 females; mean age, 23.7±3.18 years; range, 19-30) were recruited from the student population of Cardiff University. Subjects were excluded if they were contact lens wearers (Ntola and Murphy, 2002), or if they had any ocular or systemic pathology known to affect corneal sensitivity, e.g. ocular surgery, ocular diseases, diabetes, corneal dystrophy (Birndorf and Ginsberg, 1972; Ishikawa et al., 1994; Lyne, 1977; Rosenberg et al., 2000; Schwartz, 1974). Also, pregnant women or women during the premenstruum, menstruation, or ovulating period were excluded, as corneal sensitivity is depressed and corneal thickness increased (Kiely et al., 1983; Martin and Safran, 1988; Millodot, 1984, 1994). Individuals were asked to not consume alcohol for the 24 hours prior to the start of the study, because of the effects of alcohol consumption on corneal thickness (Shiono et al., 1987). Ethical approval was obtained from the School of Optometry and Vision Sciences Research Ethics Committee. After explanation of the purpose of the study, subjects were asked to sign a consent form prior to participating. Subjects were also reminded that they could withdraw from the study at anytime.

Subjects were asked to put a patch on the right eye, to prevent opening of the eye, from the previous night at 11pm, and not to remove it before attending our laboratory the following morning. This was to avoid any changes in corneal sensation and

thickness before measurements and to create a standard starting point for all subjects. The patch was removed five minutes before measurements began. Participants were asked to attend at intervals between 08.00 and 22.00. Measurements of corneal sensitivity and thickness were assessed over a 15 hour period, taking hourly measurements from 08.00 to 12.00, and then every 2 hours until 22.00. All measurements were taken on the *right eye only*. The order of measurements was randomised at each time period, apart from the first visit at 08.00, where corneal thickness was assessed firstly. Each visit lasted fifteen minutes approximately. Subjects were not allowed to sleep during the experiment.

To assess whether measurement of corneal sensitivity with the NCCA has any learning effect for the subjects, the experiment was repeated on a second day (test/re-test group) for the first seven subjects who participated in the study (7 females; mean age, 22.7 ± 3.2 years; range, 20-26).

Corneal sensitivity was assessed at the central area of the cornea using both the Non-Contact Corneal Aesthesiometer (NCCA) and the Cochet-Bonnet Aesthesiometer following the experimental procedure described in section 3.1.1 and 3.1.2.

Corneal thickness was measured using the optical Haag-Streit Pachometer, following the experimental procedure described in section 3.2.

4.3 Results

The distributions of the corneal sensitivity and corneal thickness measurements were assessed for normality (Shapiro-Wilk), using the SPSS11 Statistical Software Program (Lead Tools, Lead Technologies, Inc). The data of corneal sensitivity using both the NCCA and the C-B Aesthesiometer were found to be not normally distributed. The data was log transformed but was found to remain not normally distributed. As a result, non-parametric tests have been used to analyse the raw data. Corneal thickness measurements were found to be normally distributed, allowing the use of parametric statistical tests.

Table 4.1: Median/interquartile range (IR) and mean (± standard deviation) of central corneal sensitivity thresholds and corneal thickness measurements taken over the daily 15 hour period.

Non-Contact Corneal Aesthesiometer (mbars)

	08.00	09.00	10.00	11.00	12.00
Median /IR	0.40/ 0.35-0.6	0.35/ 0.27-0.57	0.32/ 0.25-0.5	0.30/ 0.225-0.45	0.25/ 0.17-0.42
Mean ± SD	0.48±0.17	0.42±0.17	0.37±0.15	0.34±0.15	0.29±0.15

	14. 00	16.00	18.00	20.00	22.00
Median /IR	0.20/ 0.15-0.45	0.20/ 0.15-0.42	0.22/ 0.15-0.4	0.22/ 0.15-0.4	0.20/ 0.15-0.37
Mean ± SD	0.30±0.15	0.29±0.15	0.28±0.15	0.28±0.15	0.26±0.15

Cochet-Bonnet Aesthesiometer (gr/mm^2)

	08.00	09.00	10.00	11.00	12.00
Median /IR	0.40/ 0.4-0.4	0.40/ 0.4-0.4	0.40/ 0.4-0.4	0.40/ 0.4-0.4	0.40/ 0.4-0.4
Mean ± SD	0.41± 0.036635	0.41± 0.030779	0.40± 4.83E-09	0.40± 4.83E-09	0.40± 4.83E-09

	14. 00	16.00	18.00	20.00	22.00
Median /IR	0.40/ 0.4-0.4	0.40/ 0.4-0.4	0.40/ 0.4-0.4	0.40/ 0.4-0.4	0.40/ 0.4-0.4
Mean ± SD	0.40± 4.83E-09	0.40± 4.83E-09	0.40± 4.83E-09	0.40± 4.83E-09	0.40± 4.83E-09

Haag Streit Pachometer (mm)

	08.00	09.00	10.00	11.00	12.00
Median /IR	0.50/ 0.5-0.56	0.52/ 0.48-0.54	0.49/ 0.47-0.54	0.50/ 0.46-0.54	0.50/ 0.44-0.52
Mean ± SD	0.53±0.05	0.51±0.05	0.50±0.05	0.5±0.05	0.49±0.04

	14. 00	16.00	18.00	20.00	22.00
Median /IR	0.48/ 0.46-0.51	0.48/ 0.46-0.54	0.49/ 0.45-0.52	0.50/ 0.46-0.53	0.50/ 0.46-0.53
Mean ± SD	0.49±0.04	0.50±0.05	0.49±0.05	0.50±0.05	0.50±0.04

4.3.1 Corneal Sensitivity - NCCA Aesthesiometer

A significant improvement in C-fibre sensitivity was found during the day, with the sensitivity being lowest in the morning, and gradually increasing towards evening (Kruskal-Wallis test, p = 0.0001) (Fig 4.1).

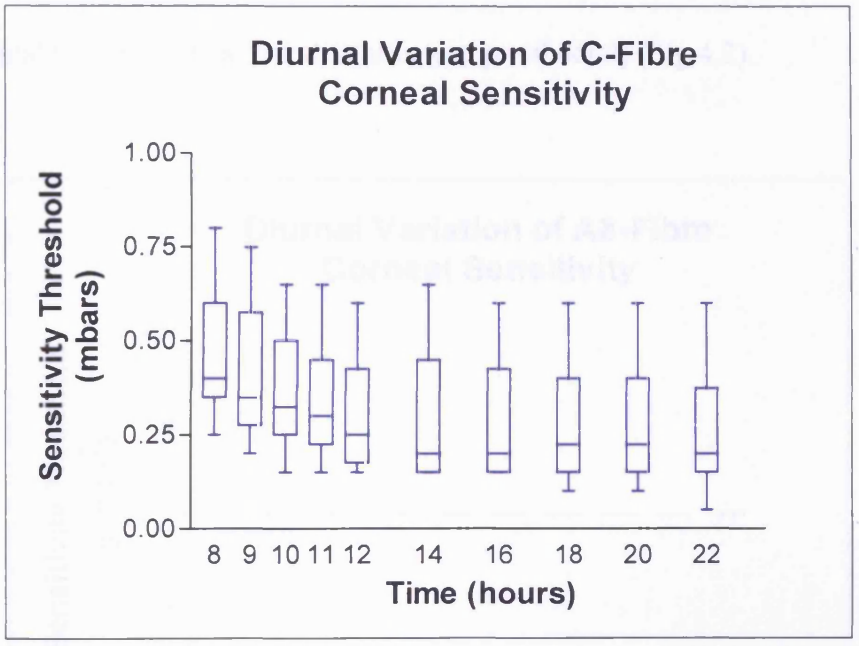


Fig 4.1: Box and whisker plot (median, interquartile range) measurements of C-fibre central corneal thresholds measured every hour from 08.00-12.00, and every 2 hours from 14.00-22.00. A box and whiskers plot shows range and quartiles. The box extends from the 25th percentile to the 75th percentile, with a line at the median (the 50th percentile). The whiskers extend above and below the box to show the highest and lowest values.

Sensitivity continued to increase from 08.00 to 12.00 (Kruskal-Wallis test, p = 0.003), but there was no continuation of this improvement from 14.00-22.00 (Kruskal-Wallis test, p = 0.935). A significant difference was found between the thresholds at 08.00

and 22.00 (Wilcoxon matched pairs test, $p<0.0001$), and the corneal sensitivity increased by 45.8% over the study time period.

4.3.2 Corneal Sensitivity - Cochet-Bonnet Aesthesiometer

No significant change was found in Aδ fibre sensitivity recorded over the experimental time period (Kruskal-Wallis test, $p = 0.0603$) (Fig 4.2).

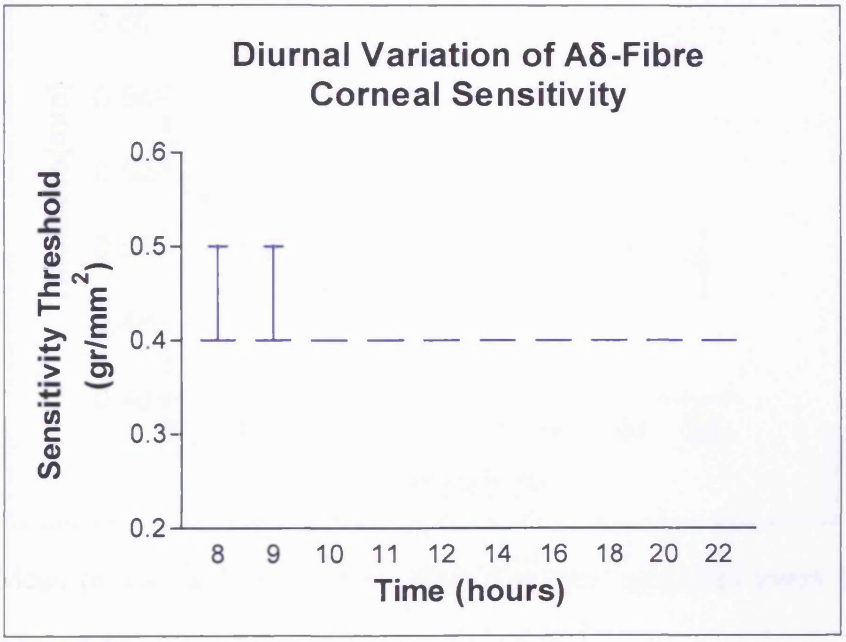


Fig 4.2: Box and whisker plot (median, interquartile range) Aδ fibre central corneal thresholds every hour from 08.00-12.00, and then every 2 hours from 14.00-22.00.

All of the subjects were able to feel the stimulus at the lowest pressure exerted by the longest length of the nylon monofilament thread at the first measurement time. Only two subjects showed a slightly variation in the sensitivity between 08.00-22.00, hence the graph has taken this pattern.

4.3.3 Corneal Thickness

Using the Haag-Streit Pachometer to determine the diurnal variation of corneal thickness, a significant change was found from 08.00 to 12.00 (one-way ANOVA, $p = 0.04$). Fig 4.3 shows the corneal thickness as a function of time.

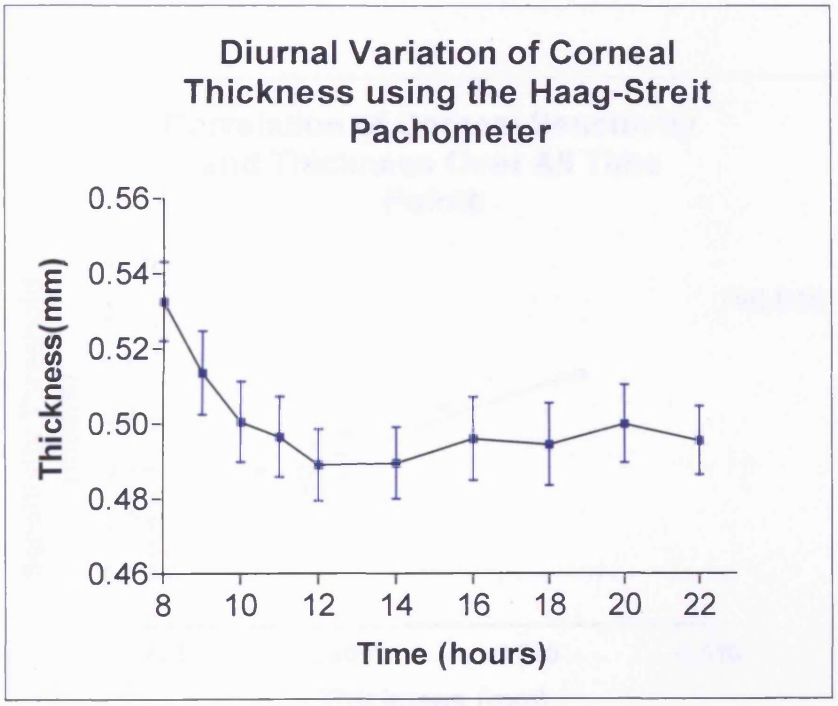


Fig 4.3: Mean (± standard deviation) central thickness measured every hour from 08.00-12.00, and then every 2 hours from 14.00-22.00.

No significant change was found between the 14.00-22.00 measurements (one-way ANOVA, $p = 0.969$). There was no statistical difference over all day time (one-way ANOVA, $p = 0.123$). The corneal thickness decreased during the day by 5.7%.

4.3.4 Correlation between Corneal Sensitivity and Thickness

The relationship between C-fibre corneal sensitivity and thickness was examined over time. An almost significant relationship was found for corneal sensitivity and thickness (Spearman, r = 0.616, p = 0.067), demonstrating a trend that as corneal sensitivity increases, corneal thickness decreases (Fig 4.4).

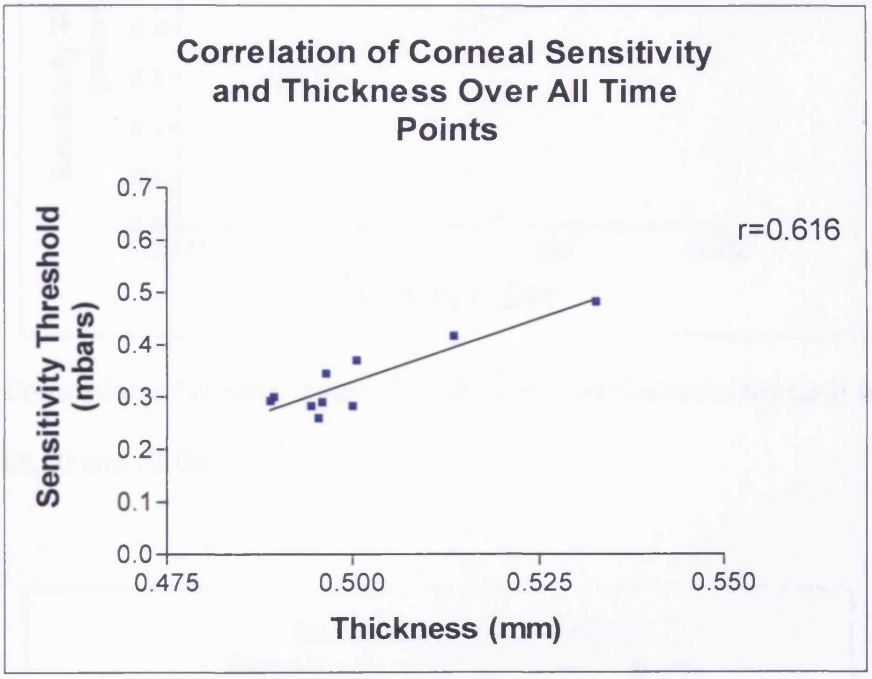


Fig 4.4: Correlation of corneal sensitivity (NCCA) and thickness for all time points between 08.00 and 22.00.

However, the previous analysis of corneal sensitivity and thickness has indicated there are two phases of diurnal variation for both factors: 08.00-12.00, and 14.00-22.00. If the relationship between corneal sensitivity (measured with the NCCA) and thickness is examined between 08.00-12.00, a significant strong correlation is found (Spearman, r = 0.986, p = 0.002) (Fig 4.5). However, when the measurements between 14.00-

22.00 are considered, no significant correlation is found (Spearman, r = 0.35, p = 0.517) (Fig 4.6).

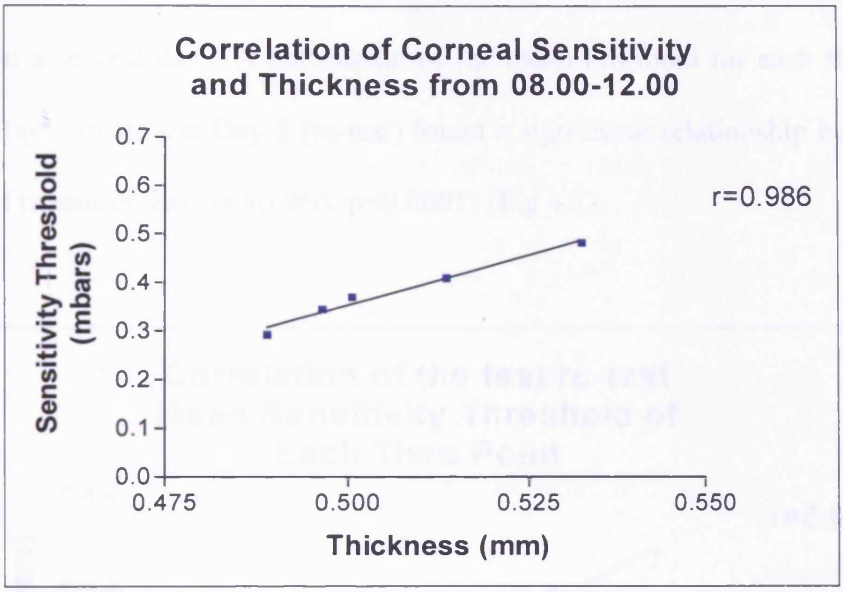


Fig 4.5: Correlation of corneal sensitivity (NCCA) and thickness for each time point between 08.00 and 12.00.

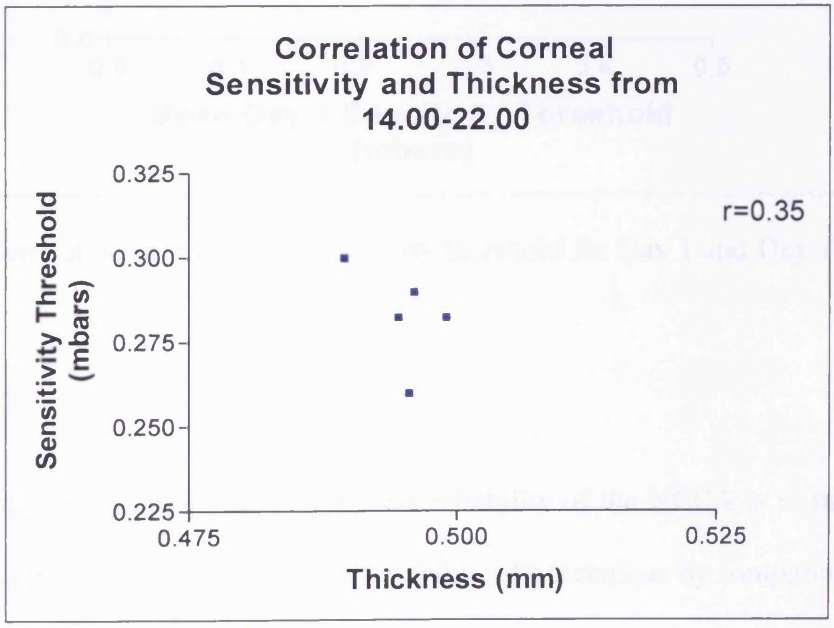


Fig 4.6: Correlation of corneal sensitivity (NCCA) and thickness for each time point between 14.00 and 22.00.

4.3.5 Patient Training with the NCCA

To assess the effect of learning on the measured corneal sensitivity threshold for the NCCA, the corneal sensitivity thresholds, at each time point, were repeated for seven subjects on a second day. A correlation of the mean threshold for each time points between Day 1 (test) and Day 2 (re-test) found a significant relationship between the two sets of measurements ($r = 0.966$, $p < 0.0001$) (Fig 4.7).

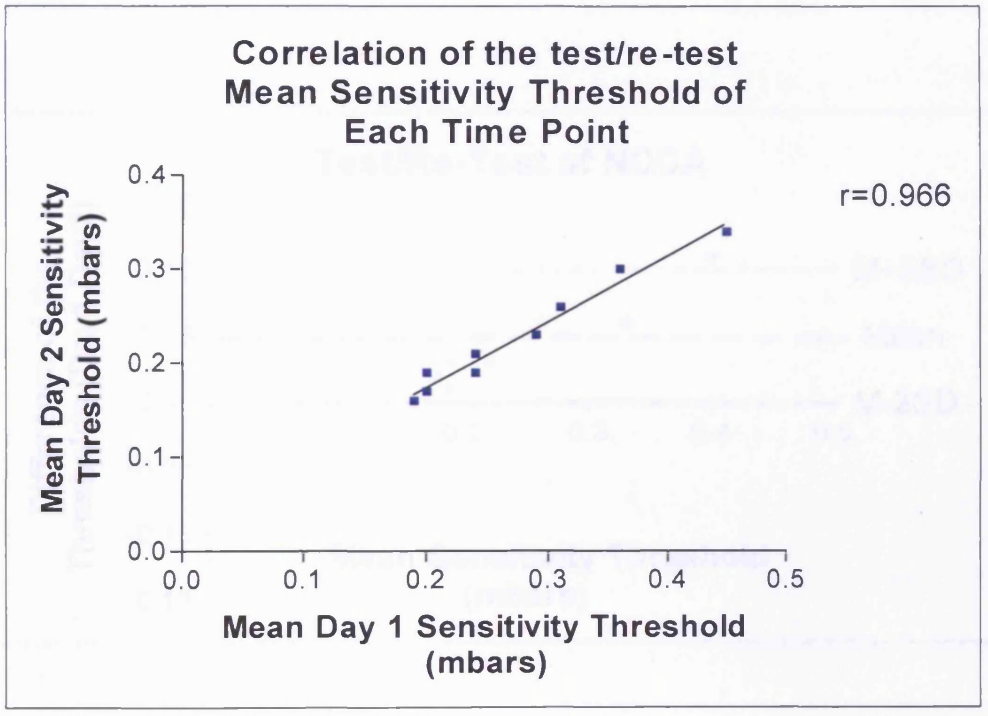


Fig 4.7: Correlation of measured sensitivity threshold for Day 1 and Day 2, using the NCCA.

Another way of comparing the test/re-test reliability of the NCCA is to use a Bland and Altman plot. This assesses the repeatability of a technique by comparing repeated measurements on a series of subjects. The graph produced can be used to check whether the variability or precision of a method is related to the subjects being

measured or to the technique. The Bland and Altman plot for the test/re-test group is shown in Fig 4.8. Two outcomes can be noted from the graph. Firstly, the results show a strong correlation between the measurements taken on Day 1 and on Day 2, as the points are plotted tightly together within a small standard deviation. Secondly, all the measurements of the second day are slightly lower than those of the first day, indicating that the measurements of corneal sensitivity using the NCCA have a small, but consistent learning component.

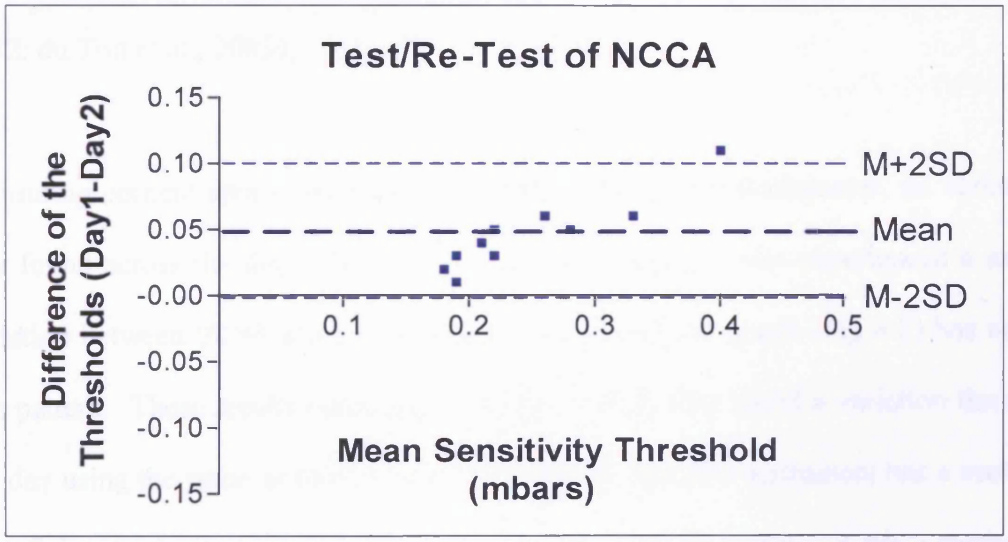


Fig 4.8: Bland and Altman plot of the threshold differences plotted against the average of the two measurements. The horizontal lines represent the mean difference, and the mean difference plus/minus two standard deviations of the mean.

4.4 Discussion

In this study, the diurnal variation of both the corneal sensitivity and thickness were measured, over a period of 15 hours. Assessing corneal sensitivity with the NCCA, a significant change in sensitivity was found over time. This change was characterized by a lower sensitivity in the morning and a progressive increase in sensitivity towards the evening. Central corneal sensitivity was significantly improved one hour after eye opening. This improvement continued, on average, for the next 5 hours up to 12.00, after which no significant change occurred over the rest of the day. This pattern of diurnal change in sensitivity is in accordance with previous investigations (Millodot, 1972; du Toit et al., 2003).

Measuring corneal sensitivity using the Cochet Bonnet Aesthesiometer, no variation was found across the day. There were only two subjects, who experienced a small variation between 08.00 and 10.00, and for this reason the graph (Fig 4.2) has taken this pattern. These results contradict Millodot (1972) who found a variation through the day using the same aesthesiometer. It is known that this instrument has a number of deficiencies in its design that limit the usefulness of its results: it has a truncated stimulus intensity range that prevents measuring of the true sensation baseline and change at high sensitivities; it alters the threshold of the subject by increasing their apprehension; it damages the corneal epithelium by the use of an invasive stimulus; and finally, the nylon monofilament thread is affected by sterilization methods and environmental conditions (Larson, 1970; Murphy et al., 1998).

The diurnal variation of sensitivity may be influenced by factors such as hypoxia, as a result of a prolonged eye closure (Millodot, 1972; Mindel and Mittag, 1978; Millodot

and O'Leary, 1979, 1980; Pesin and Candia, 1982; Tanelian et al., 1982; du Toit et al., 2003). During sleep, the corneal epithelium is exposed to a partial pressure of oxygen of 55 mmHg, whereas when the eyes are open, the epithelium is exposed to a partial pressure of oxygen of 155 mmHg (Heald and Langham, 1956; Hill and Fatt, 1963, 1964; Takahashi and Fatt, 1965; Farris et al., 1965; Polse and Mandell, 1970). Millodot and O'Leary (1979) showed in their study that eyelid closure gives rise to a considerable and progressive loss of corneal sensitivity, whilst corneal thickness increases by a small amount. They attributed this overnight corneal sensitivity decline to a lower oxygen pressure at the corneal surface, and not to the mechanical pressure exerted by the eyelid. They supported this hypothesis with a second experiment where the cornea was exposed to a reduced partial pressure of atmospheric oxygen (Millodot and O'Leary, 1980). They exposed the cornea to two different air mixtures containing 2.1% and 3.15 % oxygen (normal atmospheric oxygen contains 10% oxygen). They found a strong relationship between the time of exposure to a reduced pressure of atmospheric oxygen and a reduced corneal sensitivity. They also found a time delay between the start of the experiment and the reduction in sensitivity. With the 2.1% and 3.15 % oxygen pressures, it took 3 and 4 hours respectively to produce a measurable change in sensitivity.

From these two experiments, it can be concluded that a prime reason for corneal sensitivity reduction must be the change in the oxygen supply to the cornea. However, the mechanism by which the corneal nerves are affected by a reduced oxygen pressure is not clear. There is some evidence that acetylcholine is involved in corneal sensitivity. The corneal epithelium has the highest concentration of acetylcholine in the body. Tanelian et al (1982) showed that when acetylcholine is

instilled into the eye it increases the action potential in the long ciliary nerves of the rabbit cornea. Pesin and Candia (1982) proposed that acetylcholine in the corneal epithelium plays a role in the regulation of Na^+ and Cl^- transport, both of which are necessary in the production of nerve impulses. If this theory is correct, we can explain the changes in sensitivity associated with lid closure, as being due to interference in the synthesis of acetylcholine (perhaps through acetyltransferase, an enzyme used to synthesize acetylcholine) (Mindel and Mittag, 1978, 1979). Since such a situation occurs with lid closure, this may be one of the mediators for a reduced corneal nerve function.

Apart from corneal sensitivity, it was of interest to investigate the diurnal pattern of corneal thickness and how corneal thickness correlates with corneal sensitivity over time. Corneal thickness was found to vary during the day, with central corneal thickness decreasing significantly during the first 5 hours after eye opening. Beyond this time, and for the rest of the day, the thickness did not show any statistically significant change, although slight fluctuations were recorded.

The diurnal variation of corneal thickness may be influenced by factors such as hypoxia, and tonicity changes in the tears (tear hypo-osmolarity) due to the loss of tear evaporation during closed-eye conditions (Harris and Mandell, 1969; Mandell and Polse, 1970; Terry and Hill, 1978; Mertz, 1980). Under the closed eyelid the decreased oxygen pressure mainly affects the endothelial pump function. The endothelial pump function is a major mechanism for removing water from the corneal stroma. Thus under the closed lid the osmotic pressure and the whole hydration are altered (Chan and Mandell, 1975), resulting in stromal lactate accumulation that

causes a movement of water into the stroma (Klyce, 1981). There is also an alteration in the normal tonicity of the pre-corneal tear film, due to the loss of tear evaporation, which causes water movement into the cornea, and is accompanied by swelling of the corneal thickness (Mishima, 1965; Mandell and Fatt, 1965; Hedbys and Mishima, 1966; Mandell and Harris, 1968; Polse and Mandell, 1970). Conversely, when the eyes are open, there is a normal evaporation from the surface of the tears and it is believed that the cornea becomes thinner by about 4%. Evaporation results in a hypertonic tear film, producing an osmotic flow of water from the cornea into the tears and decreasing epithelial oedema (Mishima and Maurice, 1961a, 1961b; Terry and Hill, 1978). Clinically, hypertonic agents are used to treat epithelial oedema (Madigan et al., 1987). Therefore, it is possible that the loss of tear evaporation causes epithelial oedema, whereas the hypoxia mainly affects the stroma, causing stromal oedema.

The measurement of corneal thickness was used to indicate the metabolical and physiological status of the cornea, as it provides an indication of corneal hydration. Transparency of the cornea is maintained with a consistent water hydration of 78%, based on the lattice theory proposed by Maurice (1984). Swelling pressure, a function of stromal hydration that tends to bring water into the cornea, is counter-balanced by two mechanisms, one passive and one active. The maintenance of normal hydration requires the integrity of the barrier function of both the epithelium and endothelium and the constant interaction of these two homeostatic mechanisms against the swelling pressure of the stroma. The epithelium and the endothelium act as barriers to the flow of water into the stroma from either the tears or aqueous humour (Cogan and Kinsey, 1942). The endothelium act as a pump that removes fluid that leaks into the

stroma (Harris and Nordquist, 1955). The loss of the corneal endothelial barrier will result in a much greater increase in thickness than the loss of the epithelial barrier (Cristol et al., 1992). The passive mechanism consists of normal evaporation from the surface of the tears, when the eye is open.

A significant finding from this study is that the diurnal variations of both corneal sensitivity and thickness are influenced by the same effect, which is the prolonged eyelid closure during sleep, although the mechanisms that cause this effect for corneal sensitivity and corneal thickness are different. Both corneal sensitivity and thickness changed significantly for the same time period between 08.00 and 12.00. Corneal sensitivity increased and corneal thickness decreased significantly, having an inverse relationship, indicating that as corneal sensitivity increases, corneal thickness decreases. This high correlation between the two variables implies that diurnal variation in corneal sensitivity and thickness may be physiologically regulated by the hypoxic conditions, causing a reduction in the sensitivity and a corneal swelling. Beyond this time and until 22.00, which was the last measurement, no significant change and correlation was found for these two parameters.

This result is in contrast to Douthwaite and Kaye (1980), who suggested an inverse relationship between corneal sensitivity and corneal thickness. This study found only a trend for thicker corneas to exhibit higher corneal sensitivities ($r = 0.35$, $p= 0.517$) (Fig 4.6). Douthwaite and Kaye took measurements at only one time of the day for each subject, in the afternoon. They suggested that people with thick corneas may possess a relatively low corneal sensitivity, and vice versa, proposing that it would be possible to predict corneal sensitivity by measuring corneal thickness, or alternatively,

corneal thickness from sensitivity measurements. However, there are three things that should be mentioned. Firstly, they took measurements at only one time of the day, without considering diurnal changes; secondly, their correlation coefficient, which was calculated to be -0.665, was placed at the 0.1 per cent level of significant and not at 0.05 as it is usually used; thirdly, they used the Cochet-Bonnet Aesthesiometer, which has been demonstrated to have limitations in its ability to measure the sensitivity threshold.

When comparing our results with the results of du Toit et al (2003), a similar pattern in the change of both corneal sensitivity and thickness during the day was found. Their work differs by the fact that they also measured sensitivity and thickness on the previous night of the experiment, as a baseline measurement. The recovery time for sensitivity and thickness was taken as the time point at which there was no significant difference from the level of the previous night's measurements. They found significant changes of sensitivity and thickness from 07.00 up to 14.00, and then no significant variation for the rest of the day. In contrast, the results from our study suggest that baseline measurements for these parameters can be taken 5 hours after the eye opening. One possible explanation may be the different points of the two experiments. We took measurements from 8am after nine hours of lid closure and they commenced one hour earlier. They also found a high correlation between sensitivity and thickness over the 24 hour period. However, this analysis included the morning measurements when the effects of overnight eyelid closure are still affecting the results.

When comparing our results of corneal thickness with previous investigations, the thinnest corneal measurements were found between 4 and 12 hours after eye opening. In these studies the diurnal variation of thickness was monitored over periods ranging from 12 to 48 hours (Kiely et al., 1982; Holden et al., 1983; Harper et al., 1996) or for periods less than 12 hours (Mandell and Fatt, 1965; Mertz, 1980; Feng et al., 2001). We should also take into account that there are differences in the instrumentation used to measure corneal thickness, and the measurements were not conducted at similar intervals or with the same frequency.

The aim of this study was to gain a better understanding of the diurnal effect on corneal sensitivity and corneal thickness and whether there is an inverse relationship between these two parameters, as previously suggested by Douthwaite and Kaye (1980). For clinical and experimental purposes, our results are very useful as they provide the diurnal time points for the highest sensitivity and thinnest cornea. If the diurnal pattern of a parameter is known, the relevance of taking measurements at certain time points can be assessed. For all subsequent studies in this thesis, where corneal sensitivity will be measured using the NCCA, it was important to establish the point at which baseline measurements can be taken. This study suggests that baseline corneal sensitivity and thickness may be measured five hours after eye opening or thereafter.

5. An Investigation of the Anaesthetic Effect of 0.5% Proxymetacaine Hydrochloride (Proparacaine) on Corneal Sensation

5.1 Introduction-Purpose

Topical ophthalmic anaesthetic agents are among the commonest eye drops used for diagnostic purposes, as well as for selected surgical procedures, in therapeutic ophthalmology. Topical anaesthesia is used during contact or applanation tonometry, gonioscopy, contact lens fitting, foreign body removal and some methods of refractive and cataract surgery (Jose et al., 1983; Vale and Cox, 1985; Brady et al., 1994; Craig, 1994; Shahinian et al., 1997; Hamilton and Claoue, 1998; Bennett et al., 1998).

In ocular topical anaesthesia, the anaesthetic drug (cocaine, tetracaine, benoxinate, proparacaine, bupivacaine) is applied to a mucous membrane, such as the cornea or the conjunctiva, to prevent the generation and conduction of nerve impulses. Their main site of action seems to be the cell membrane, where they block the transient increase in membrane permeability to sodium ions that normally occurs with depolarisation of the membrane (Bryant, 1969; Stoelting, 1991; Catteral and Mackie, 1995). The blockade of sodium transport occurs through binding of the local anaesthetic to a specific binding site located within a voltage-gated sodium channel present in the cell membrane (Catteral and Mackie, 1995). The sodium channel is formed by a large heterotrimeric protein, which contains numerous trans-membrane segments. The greater the hydrophobicity of a local anaesthetic, the greater the affinity for binding (Ragsdale et al., 1994). After instillation, anaesthetics diffuse across the cell membrane in the uncharged (lipid soluble) amine form, but at the site

of action, the charged, substituted ammonium cation interacts with the receptor that is only accessible from the inner membrane surface (Catteral and Mackie, 1995).

The duration of action of local anaesthetics is proportional to the time they are in contact with the nerve tissue (Catteral and Mackie, 1995). Consequently, any agent or procedure that keeps the anaesthetic at its site of action will prolong the period of anaesthesia.

The clinical advantage of local anaesthetics is that their function is reversible. Nerve function recovers completely with no structural damage to nerve fibres or cells and the loss of sensation occurs without loss of consciousness (Covino and Vassalo, 1976; Catteral and Mackie, 1995).

Although most of the commonly used topical anaesthetics are similar in onset, duration, and depth of anaesthesia, several important differences exist. Thus selecting the appropriate topical anaesthetic for individual clinical procedures helps in maximising its effectiveness while minimizing undesirable side effects. The desirable properties of an ideal local anaesthetic are: rapid onset of action, profound depth of anaesthesia, adequate duration for the purpose required, no pain at site of administration, no pain effect after the anaesthetic has worn off, no hypersensitivity or allergic reactions, and no local toxicity (Bryant, 1969; Vale and Cox, 1985).

Proxymetacaine Hydrochloride (proparacaine) is an ester of meta-aminobenzoic acid, and is available in a 0.5% solution, both with and without sodium fluorescein 0.25%. It produces little discomfort or irritation in instillation and is readily accepted by most

patients. Boozan and Cohen (1953) reported that one drop of 0.5% proxymetacaine was less painful in instillation than one drop of 0.5% tetracaine. Bartfield et al (1994) compared 0.5% proparacaine directly with 0.5% tetracaine, and 86% of the patients reported that proparacaine caused less pain on administration. Hamilton and Claoue (1998) compared the use of 0.5% proxymetacaine and 1% tetracaine during small incision phacoemulsification cataract surgery, and found that patients receiving proxymetacaine felt significantly less discomfort during its administration than those receiving tetracaine. Shafi and Koay (1998) in a randomised, masked, double-blind study assessed the duration of the stinging sensation and degree of discomfort (using descriptive and linear analogue methods) caused by the instillation of each anaesthetic in each eye. The study indicated that proxymetacaine is more comfortable on instillation than tetracaine. The pH of anaesthetic agents affects the degree of comfort on instillation. Tetracaine has a pH of 4.54 and proxymetacaine a pH of 4.64 (Bartfield et al, 1994). This probably explains why tetracaine (being more acidic) stings more than proxymetacaine. Although tears can dilute and buffer topical anaesthetic agents, this effect is not sufficient to cope with the immediate, relatively large, volume of the anaesthetic instilled (Shafi and Koay, 1998).

Proparacaine has few side effects. These include:

1) Localised allergic hypersensitivity may develop, but less frequently than with tetracaine (Johnston et al., 1998).

2) Allergic reactions may be characterised by conjuctival hyperaemia and oedema, oedematous eyelids, and lacrimation (Householder and Harris, 1969).

3) Development of a hypersensitivity reaction may result in exacerbation of an existing case of Stevens-Johnson Syndrome (Ward et al., 1978; Dannaker et al., 2001).

4) Allergic contact dermatitis on the fingertips (Liesegang and Perniciaro, 1999).

5) Seizure after ocular instillation of 0.5% proxymetacaine hydrochloride for an existing case of corneal abrasion (Cydulka and Betzelos, 1990).

6) Decreased rate of corneal epithelial desquamation (cell sloughing) (Fullard and Wilson, 1986; Wilson and Fullard, 1988).

Previous researchers have considered the efficacy, duration, and extent of recovery of 0.5% proxymetacaine hydrochloride and found that it provides sufficient anaesthesia to allow the measurement of contact tonometry or foreign body removal. Boozan and Cohen (1953) reported that the onset of anaesthesia, with one drop of 0.5% proxymetacaine hydrochloride, occurred after 6 to 20 seconds with an average of 12.9 seconds, with a duration of action between 6-24 minutes, with an average of 15.2 minutes. Linn and Vey (1955) compared proxymetacaine with benoxinate, tetracaine and sympocaine and found each to have similar anaesthetic qualities. However they did not supply any analysis of their findings. They assessed corneal sensitivity using a series of calibrated aesthesiometers similar to von Frey's Hairs. Polse et al (1978) compared different concentrations of proxymetacaine (0.125, 0.25, and 0.5%) with different concentrations of benoxinate (0.1, 0.2, and 0.4%) using the Cochet-Bonnet Aesthesiometer. They found that the stronger the concentration of both anaesthetics, the longer the durations of action. The onset of anaesthesia for all doses was within the first 2 minutes of instillation and lasted 20 to 60 minutes. 0.4% benoxinate had an average recovery time of 52 minutes, whereas 0.5% proxymetacaine had an average

recovery time of 45 minutes. Draeger et al (1984a) also found that the duration of anaesthesia produced by proxymetacaine hydrochloride was dependent on the concentration used. Recovery of sensitivity to the baseline level occurred after 7 minutes for 0.1%, after 17 minutes for 0.5%, and after 24 minutes for 1% proxymetacaine. Weiss and Goren (1991) assessed the duration of action of 0.5% proxymetacaine in both eyes in 7 subjects with documented unilateral corneal hypoesthesia associated with inactive herpetic disease, using the Cochet-Bonnet Aesthesiometer. The duration of maximal effect of proparacaine in control eyes averaged 11.71 minutes compared to 18 minutes found for hypoesthetic corneas (corneas that normally have a decreased sensitivity to touch and pain, due to a disease usually, e.g. herpetic keratitis). The complete recovery time for the control eyes averaged 34.86 minutes compared to 45.43 minutes for the hypoesthetic eyes. Lawrenson et al (1993) examined the efficacy and duration of action of different concentrations of topically applied proxymetacaine delivered using a novel ophthalmic delivery system (NODS), by measuring corneal sensitivity using the Cochet-Bonnet Aesthesiometer. This method applies the drug by incorporating it into a polyvinyl flag attached to a carrier. When applied to the eye, the flag detaches and gradually dissolves, releasing the drug. They found that by using this modality, even the lowest concentration NODS (44μg) produced longer lasting anaesthesia than the 35μl drop (175μg) of 0.5% proxymetacaine. Higher doses of NODS produced a correspondingly greater increase in anaesthetic duration. The onset of anaesthesia produced by the 35μl of 0.5% proxymetacaine was achieved within 1 minute after instillation, with a duration of 20 minutes.

The Cochet-Bonnet Aesthesiometer has been the standard clinical method for assessing and monitoring the duration of local ocular anaesthesia. However it suffers from several deficiencies in its design, as it has a restricted stimulus intensity range that limits its usefulness (Bonnet and Millodot, 1966; Millodot and Larson, 1967; Millodot and O'Leary, 1981; Millodot, 1984). These drawbacks are reflected in its ability to establish true baseline threshold levels. This is important when considering the recovery time of corneal sensitivity after an ocular topical anaesthetic has been instilled into the eye. Later measurements of sensitivity taken over a period of time after instillation, and compared to a preliminary baseline, can result in the deduction that sensitivity has returned to normal levels earlier than it actually has.

In view of the conflicting results found by previous studies on the duration, depth, and recovery time of the anaesthesia produced using 0.5% proxymetacaine hydrochloride, and in response to the availability of a more subtle method of measuring corneal sensitivity, this study aimed to: 1) assess the onset, duration, depth, and recovery time of corneal anaesthesia produced by the instillation of 20μl of 0.5% proxymetacaine hydrochloride, using the NCCA; 2) establish whether there is any effect in the contralateral control eye, with anaesthetic instillation in the other eye, and; 3) to investigate whether iris colour has any effect on the anaesthetic action of proxymetacaine hydrochloride (Millodot, 1975a).

The results of this study will be useful in the later studies on the blink mechanism. One of the suggested mechanisms for the blink stimulus is a localised change in the tear film stability before a full break-up occurs. This tear thinning is associated with an increased tear evaporation, which produces a localised cooling of the tear film.

This change of the tear film temperature can be detected by the temperature sensitive corneal nerves, and trigger a blink before full break up occurs. Anaesthesia will block that stimulus and may produce an altered blink rate. If any loss in corneal sensation leads to any tear film mediated "trigger to blink" going undetected, then subjects will have a reduced blink rate. Following on from the results of this study, and knowing the maximum depth of the anaesthesia produced by proxymetacaine measured with the NCCA, we will assess whether the loss of corneal sensitivity will alter the blink rate.

5.2 Methods

Seventeen Caucasian subjects (2 males, 15 females; mean age, 26±3.6 years; range, 23-39) were recruited from the student population of Cardiff University. Subjects were excluded if they were contact lens wearers (Ntola and Murphy, 2002), or if they had any ocular or systemic pathology known to affect corneal sensitivity, e.g. ocular surgery, ocular diseases, diabetes, corneal dystrophy (Birndorf and Ginsberg, 1972; Schwartz, 1974; Lyne, 1977; Ishikawa et al., 1994; Ruben, 1994; Murphy et al., 1999a; Rosenberg et al., 2000). Also, pregnant women or women during the premenstruum, menstruation, or ovulating period, were excluded, as corneal sensitivity is depressed (Millodot, 1984, 1994; Martin and Safran, 1988). Subjects were further classified, using the Seddon Iris Color Classification System (Seddon et al, 1990), into two iris colour groups: Group 1 (blue, grey, and green), and Group 2 (Brown or dark brown iris) (Group 1 = 8, Group 2 = 9). Ethical approval was obtained from the School of Optometry and Vision Sciences Research Ethics Committee. After explanation of the purpose of the study, subjects were asked to sign

a consent form prior to participating. Subjects were also reminded that they could withdraw from the study at anytime.

Corneal sensitivity was assessed at the central area of the cornea using the Non-Contact Corneal Aesthesiometer (NCCA) using the procedure described in section 3.1.1.

Measurements of the central corneal sensitivity were always taken for the *right eye only,* and a preliminary assessment was made to establish a baseline. All measurements were made from 12pm and ownwards to avoid any possible diurnal bias.

Subjects were asked to attend the laboratory on four different days and each visit lasted for one hour. Central corneal sensitivity was measured under four different conditions

1) proxymetacaine in both eyes (p-p)

2) proxymetacaine in the right eye and saline for the left eye (p-s)

3) saline in both eyes (s-s)

4) saline in the right eye and proxymetacaine in the left eye (s-p).

20µl of 0.5 % proxymetacaine hydrochloride (Minims, Chauvin Pharmaceuticals, Ltd, UK) or 20µl of unpreserved saline 2% (Minims, Chauvin Pharmaceuticals Ltd, UK) was then instilled in either the right, left, or both eyes at the superior palpebral conjuctiva. A P100 micropipette (Wolf Laboratories Ltd, York, UK) was used to instill a measured dose. The anaesthetic was expelled from a minim into a sterile

Eppendorf tube, from which it was extracted by the micropipette. A new sterile pipette tip was used on each instillation. The 20µl volume was selected because other studies have shown that this is the maximum extra volume that the palpebral aperture can contain before tearing will occur (Ludwig and Van Ooteghem, 1986). By choosing a volume of 20µl, all of the anaesthetic can be expected to remain on the eye surface for maximum absorption.

The subjects were then asked to tilt their head back and look down. The upper eyelid was held open, and the proxymetacaine instilled onto the superior, temporal palpebral conjunctiva. The subjects were asked to close their eyes for a few seconds for better absorption of the anaesthetic or the saline. Following the instillation, the central corneal sensitivity was measured at specific time intervals of: 2, 5, 10, 15, 20, 30, 45, and 60 minutes.

5.3 Results

The distributions of the corneal sensitivity measurements for the four experimental conditions were assessed for normality (Shapiro-Wilk test), using the SPSS11 Statistical Software Program tests (Lead Tools, Lead Technologies, Inc). The results for conditions p-p, s-s, and s-p were normally distributed. However, the results for conditions p-s were found to be not normally distributed. The data was log transformed, but normality testing found the data to still be not normally distributed, consequently non-parametric statistical tests have been used for the analysis of the raw data (Prism: GraphPad Software Inc, San Diego).

Table 5.1: Median/ interquirtile range (IR) and Mean (± Standard Deviation) of central corneal sensitivity thresholds (millibars) for each experimental condition, at each time period.

Prox-Prox

Prox-Prox	Baseline	2 mins	5 mins	10 mins
Median/IR	0.90/ 0.77-1.25	1.75/ 1.07-2.65	2.2/ 1.87-3.42	2.8/ 2.12-4.15
Mean±SD	0.96±0.37	1.84±0.85	2.49±1.08	3.00±1.17

15 mins	20 mins	30 mins	45 mins	60 mins
2.95/ 2.22-4.47	2.7/ 1.8-3.67	1.90/ 1.55-2.92	1.70/ 1.15-2.02	1.55/ 1.0-1.72
3.24±1.27	2.72±1.20	2.21±1.05	1.64±0.69	1.38±0.51

Prox-Sal

Prox-Sal	Baseline	2 mins	5 mins	10 mins
Median/IR	0.90/ 0.7-1.05	1.75/ 1.17-2.17	2.70/ 2.0-3.05	2.90/ 2.4-3.25
Mean±SD	0.90±0.35	1.91±0.97	2.66±1.12	3.11±1.29

15 mins	20 mins	30 mins	45 mins	60 mins
3.2/ 2.72-3.65	2.55/ 2.15-3.2	1.90/ 1.57-2.65	1.55/ 1.25-1.82	1.25/ 1.05-1.57
3.49±1.41	2.90±1.53	2.20±1.118	1.63±0.97	1.37±0.87

Sal-Sal

Sal-Sal	Baseline	2 mins	5 mins	10 mins
Median/IR	0.95/ 0.75-1.07	1.05/ 0.77-1.17	0.95/ 0.75-1.07	0.95/ 0.75-1.1
Mean±SD	0.93±0.32	1.00±0.34	0.94±0.33	0.93±0.32

15 mins	20 mins	30 mins	45 mins	60 mins
0.9/ 0.75-1.07	0.95/ 0.77-1.07	0.95/ 0.75-1.1	0.95/ 0.75-1.07	0.90/ 0.72-1.1
0.92±0.33	0.92±0.34	0.94±0.35	0.93±0.33	0.91±0.32

Sal-Prox

Sal-Prox	Baseline	2 mins	5 mins	10 mins
Median/IR	0.90/ 0.77-1.15	1.30/ 0.82-1.47	1.45/ 0.87-1.72	1.4/ 0.92-1.72
Mean±SD	0.93±0.34	1.17±0.43	1.32±0.52	1.29±0.51

15 mins	20 mins	30 mins	45 mins	60 mins
1.3/ 0.85-1.62	1.10/ 0.85-1.72	1.10/ 0.8-1.57	1.05/ 0.67-1.47	0.95/ 0.7-1.42
1.23±0.49	1.20±0.52	1.11±0.45	1.10±0.48	1.07±0.46

5.3.1 Proxymetacaine-Proxymetacaine (p-p), Proxymetacaine-Saline (p-s)

For both conditions, proxymetacaine-proxymetacaine (p-p) and proxymetacaine-saline (p-s), a significant variation for the reduction of corneal sensitivity was found over time (p-p: Kruskal-Wallis test, $p<0.0001$; p-s: Kruskal-Wallis test, $p<0.0001$) (Figs 5.1, 5.2).

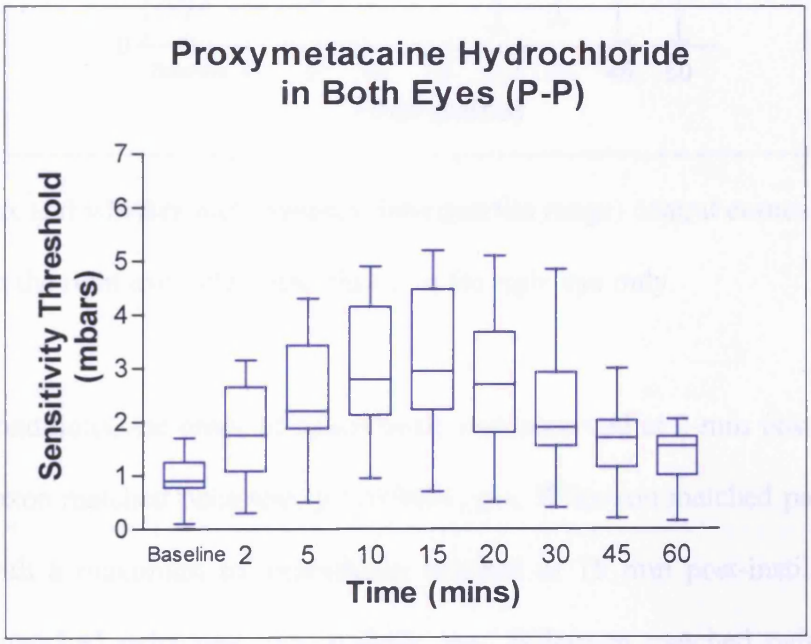


Fig 5.1: Box and whisker plot (median, interquartile range) central corneal sensitivity threshold in the right eye after anaesthesia in both eyes.

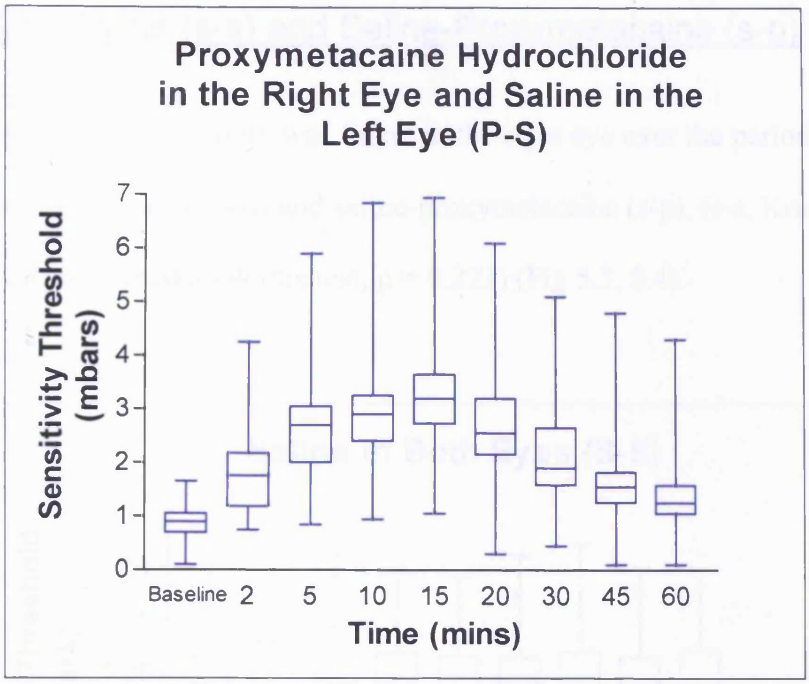


Fig 5.2: Box and whisker plot (median, interquartile range) central corneal sensitivity threshold in the right eye, after anaesthesia in the right eye only.

For both conditions, the onset of anaesthesia was observed at 2-min post-instillation (p-p, Wilcoxon matched pairs test, p = 0.0001; p-s, Wilcoxon matched pairs test, p = 0.0003), with a maximum of anaesthesia reached at 15 min post-instillation (p-p: Wilcoxon matched pairs test, p = 0.0003; p-s: Wilcoxon matched pairs test, p = 0.0003), after which sensitivity began to recover. Corneal sensitivity did not return to pre-instillation levels at 60 minutes post-instillation for both the conditions p-p (Wilcoxon matched pairs test, p = 0.0005), and p-s (Wilcoxon matched pairs test, p = 0.013). Instillation of proxymetacaine hydrochloride in both eyes (p-p) did not show any significant difference in the depth of anaesthesia compared to the anaesthesia produced by the instillation of the anaesthetic in the right eye only (p-s) (Mann-Whitney test, p= 0.221).

5.3.2 Saline-Saline (s-s) and Saline-Proxymetacaine (s-p)

No change in corneal sensitivity was found in the right eye over the period of the trial for conditions saline-saline (s-s) and saline-proxymetacaine (s-p), (s-s, Kruskal-Wallis test, p = 0.928: s-p, Kruskal-Wallis test, p = 0.223) (Fig 5.3, 5.4).

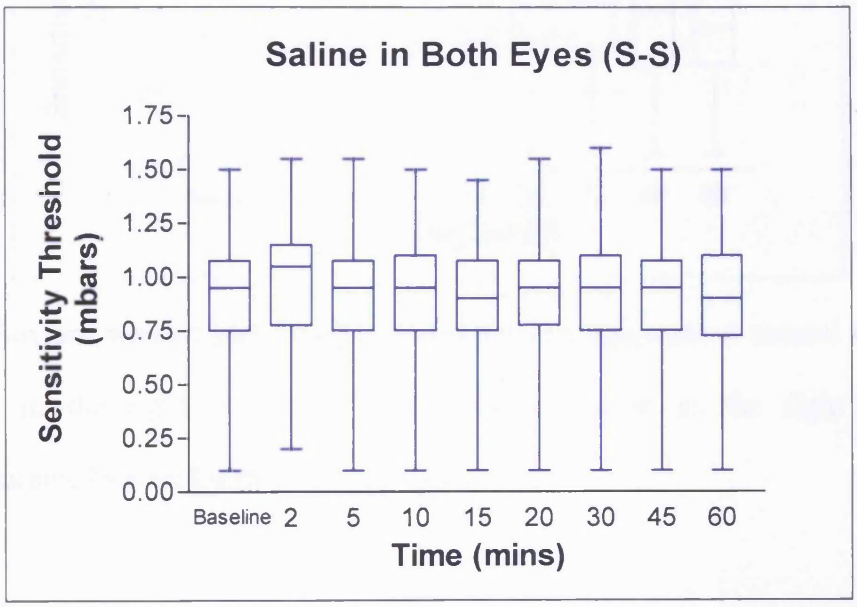


Fig 5.3: Box and whisker plot (median, interquartile range) central corneal sensitivity threshold in the right eye after instillation of saline in both eyes.

For the condition saline-saline, a decrease in sensitivity occurred at 2 minutes (Wilcoxon matched pairs test, p = 0.004) returning to baseline levels for all subsequent measurements (Kruskal-Wallis test, p = 0.999).

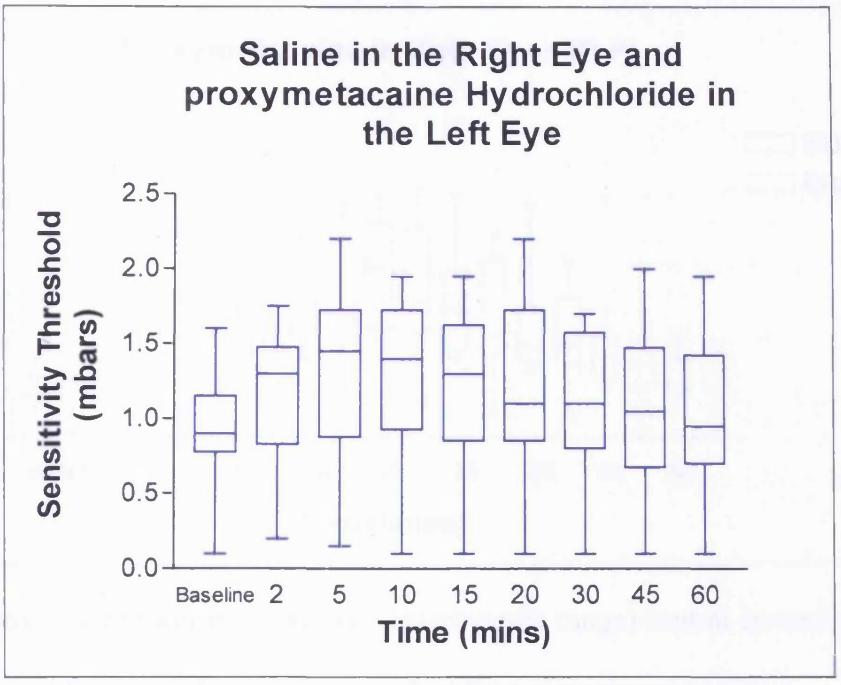


Fig 5.4: Box and whisker plot (median, interquartile range) central corneal sensitivity threshold in the right eye, after instillation of saline in the right eye and proxymetacaine hydrochloride in the left eye.

5.3.3 Iris Colour and Anaesthetic Action

The data were re-analysed for the effect of iris colour on the anaesthetic action of proxymetacaine hydrochloride, classifying the subjects into two groups: blue-grey-green iris and brown iris. No significant difference was found between blue-grey-green and brown eyes for the experimental conditions p-p, and p-s, suggesting that the iris colour of the subject has no effect on the action of this anaesthetic (Mann-Whitney test, $p>0.05$) (Figs 5.5, 5.6).

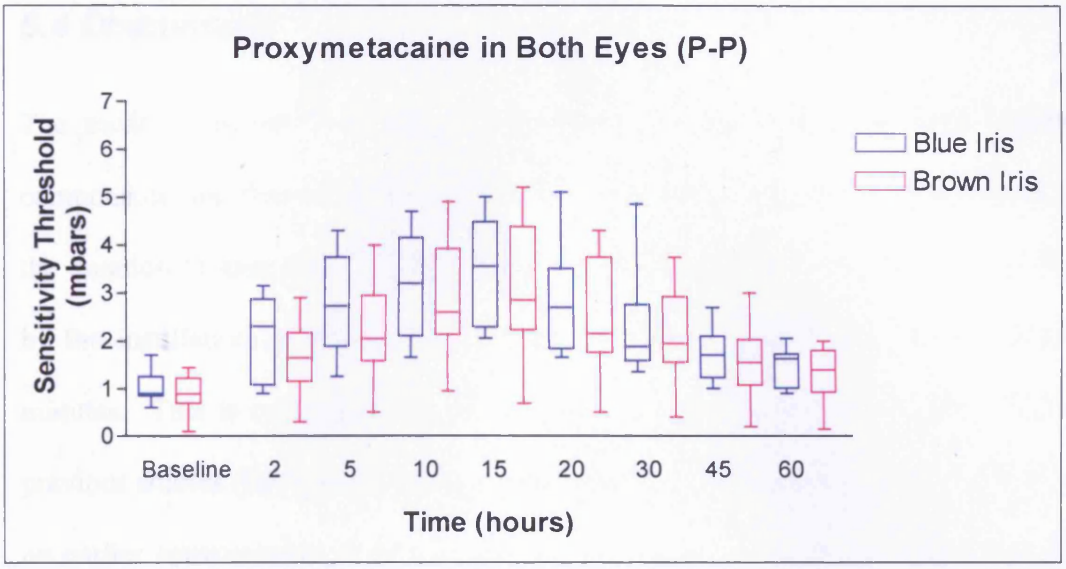


Fig 5.5: Box and whisker plot (median, interquartile range) central corneal sensitivity thresholds as a function of iris colour for the bilateral treatment (p-p).

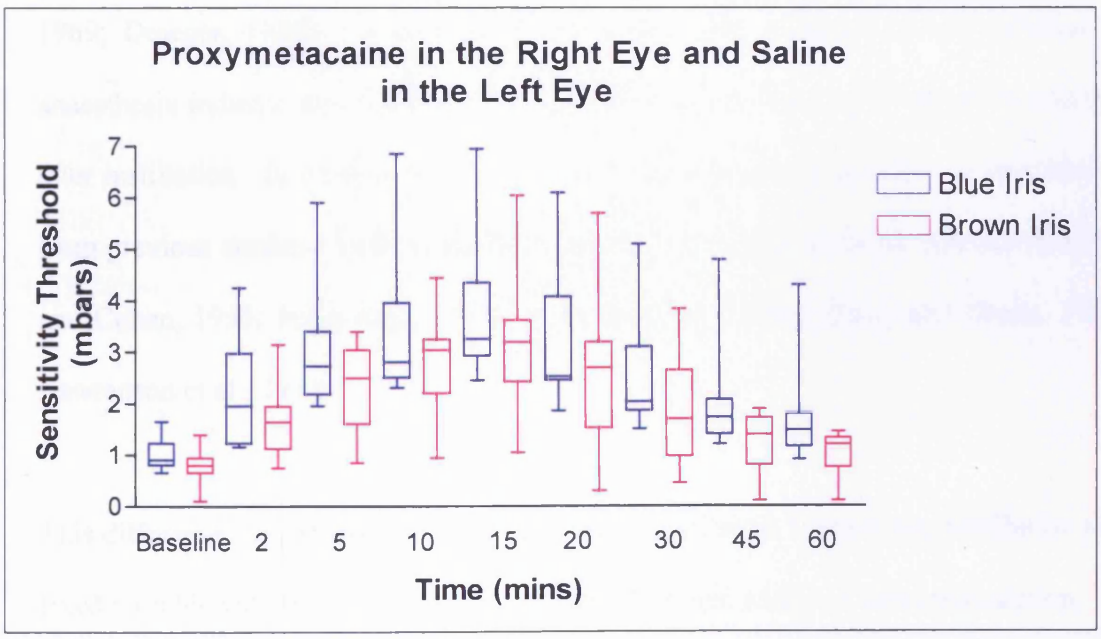


Fig 5.6: Box and whisker plot (median, interquartile range) central corneal threshold as a function of iris colour for the ipsilateral treatment (p-s).

5.4 Discussion

The mode of action of a topical anaesthetic is characterised by three important components: the time of onset, the time of the maximum anaesthesia produced, and the duration of anaesthesia. This study found that the onset of anaesthesia produced by the instillation of 20µl of 0.5% proxymetacaine hydrochloride occurs within 2 minutes. This is comparable with the values of 15 seconds and 1 minute found in previous studies (Boozan and Cohen, 1953; Bryant, 1969; Lawrenson et al., 1993). If an earlier measurement in our experiment had been taken at 1 minute, we would expect to find that the anaesthetic had already begun its action. The maximum depth of anaesthesia occurs at 15 minutes post-instillation. This result compares with the more variable values of 1 minute and 15 minutes found by other researchers (Bryant, 1969; Draeger, 1984a; Lawrenson et al, 1993). The findings on the duration of anaesthesia indicate that sensitivity is still below pre-instillation levels at 60 minutes after instillation. In contrast to our study, the findings on the duration of anaesthesia from previous studies found sensitivity to recover at 15, 20, 35 or 45 minutes (Boozan and Cohen, 1953; Polse et al., 1978; Draeger et al., 1984a; Weiss and Goren, 1991; Lawrenson et al., 1993).

This difference can be explained by considering two main factors: the instillation of a fixed/variable volume of anaesthetic, and the different modes of nerve stimulation.

All of the previously studies, apart from Lawrenson et al (1993), described the volume of the anaesthetic instilled as "a drop". Although this may be a standard volume used frequently in clinical practice, for a controlled study of anaesthetic action it does not provide a repeatable instilled volume. A typical drop from a single dose

preparation, such as a Minim, has a volume of 25-50 μl (Ludwig and Van Ooteghem, 1986). Ludwig and Van Ooteghem showed that the maximum volume of extra fluid that the palpebral aperture can contain is 20μl. In this regard, even the 35μl of 0.5% proxymetacaine instilled by Lawrenson et al (1993) was too much for the palpebral aperture to contain, producing an overflow of tears. As a result, less of the anaesthetic will be available for absorption by the eye. The consequence of this reduced volume absorbed by the eye may be a restricted duration of action, as found in the previous studies.

This phenomenon may also explain the delayed maximum depth of anaesthesia (15 minutes) found in this study. If less anaesthetic is available, the anaesthesia that is produced will be more rapidly broken down and an apparent peak in anaesthesia occurs earlier. This is particularly seen in those studies which found a short duration of action. Lawrenson et al (1993) found a peak in anaesthesia at 1 minute, but duration of action of only 20 minutes. Instilling the correct volume for absorption is vital for controlling the repeatability of anaesthetic action.

The second possible factor that could be responsible for the disparities between this study and the previous published results is the mode of stimulation of the sensory corneal nerves. The NCCA uses a temperature change stimulus produced by a short duration, controlled intensity, air-pulse aimed at the anterior corneal surface, stimulating the C-fibres of the corneal epithelium that are predominantly located in the superficial cell layer (MacIver and Tanelian, 1993a; Belmonte and Gallar, 1996; Muller et al., 1996, 2003). In contrast to this study, all of the previous works used a mechanical stimulus, such as the nylon monofilament thread of the Cochet-Bonnet

Aesthesiometer (Cochet and Bonnet, 1960) or the thin metal wire of the Draeger electronic optic aesthesiometer (Draeger, 1984), to stimulate the Aδ fibres found in the epithelial basal cell layer (MacIver and Tanelian, 1993; Belmonte and Gallar, 1996; Muller et al., 1996, 2003). Thus two different populations of fine nerve endings were stimulated. Also the stimulus intensity range of the Cochet-Bonnet Aesthesiometer is restricted, particularly at lower stimulus intensities. This can lead to an incorrect baseline sensation which is supra-threshold, creating an apparently reduced sensitivity. For the recovery time from the anaesthesia, the sensitivity needs only to return to this raised baseline level, producing a shorter measured duration of action.

The extended duration of anaesthesia found in this study was noted in a previous study that considered the anaesthetic action of 0.4% benoxinate hydrochloride, using the NCCA (Murphy et al., 1997). The sensitivity after the instillation of a single drop of non-preserved 0.4% benoxinate hydrochloride recovered to baseline levels by 60 minutes post-instillation. On that occasion, a similar explanation based on the different modes of stimulation and the design flaws of the Cochet-Bonnet was proposed. Although the volume of instilled anaesthetic was not controlled, and while this may produce excess tearing and loss of some of the instilled anaesthetic, there appeared to be no reduction in the depth or duration of anaesthesia produced. Since both the benoxinate study and the present study found long anaesthetic durations, the reduced volume of anaesthetic may only have a limited effect. It may also be possible that in the benoxinate study, although the tearing produced by the instillation caused a washout of the anaesthetic, the full intensity range of the NCCA stimulus was able to detect even slight changes in corneal sensitivity.

Furthermore, instillation of anaesthetic bilaterally was unable to produce any deeper anaesthesia in the right eye than produced by anaesthesia of the right eye alone.

This study also investigated the effect of iris colour on the anaesthetic action. Although no predictable pattern of iris colour effect on the duration or depth of anaesthesia was produced, a trend can be seen in the results, indicating that the brown eyes were less anaesthetised than the blue. It is likely that, if a greater sample size was used, a significant effect would be observed. The same trend was indicated in the study of Murphy et al (1997) where a greater anaesthetic effect on blue irises, only for the bilateral treatment (benoxinate hydrochloride in both eyes).

The unexpected finding of this study was the contralateral effect of 0.5% proxymetacaine hydrochloride instillation in the non-tested eye. Although no statistically significant reduction in the corneal sensitivity of the measured (right) eye was found when 20µl of the anaesthetic was instilled in the contralateral eye, the graph (Fig 5.4) suggests that a change in corneal sensitivity has occurred. These contralateral alterations may reflect a direct and sympathetic physiological response to the anaesthetic action of 0.5% proxymetacaine hydrochloride.

Similar contralateral effects have been found in previous investigations and have also been attributed to sympathetic responses. Harris and Mandell (1969) found a contralateral corneal swelling of approximately 3% when a rigid contact lens was worn on the other eye. The response was presumed to be due to an osmolarity effect as a result of lacrimation. Fonn et al (1999a) found a swelling of the contralateral control eyes while wearing high Dk silicone hydrogel and low Dk hydrogel lenses on

the other eye, attributing this phenomenon to sympathetic responses. Guzey et al (2002) found a corneal oedema on the contralateral control eyes while wearing high Dk silicone hydrogel and low Dk hydrogel lenses. Ladage et al (2003) examined the rabbit corneal epithelial cell proliferation rate after extended wear of disposable or silicone hydrogel contact lenses or eyelid closure. Although they found a suppression of the cell proliferation rate in corneal epithelium of the tested eyes, the contralateral eyes that served as controls also showed an increased proliferation when the other eye was wearing a high Dk soft or RGP lens, whereas the low and medium Dk lenses showed a reverse effect for the contralateral eyes. You et al (1993) observed electron dense deposits within the Descemet's membrane of both the ablated and unablated eyes of the exposed rabbits, which was not present in animals not exposed to excimer radiation. He suggested that these contralateral alterations may reflect a direct and sympathetic response to the laser radiations or may be due to a systemic factor or hormonal change induced by surgery. Further support for a sympathetic effect has been shown by Dubraix et al (1997) in a study in which excimer laser photoablation induced an increase in the hyaluronan content of treated and untreated contralateral rabbit corneas. Estil et al (2001) reported a similar cross-talk reaction during wound healing. Inducing a wound in one cornea caused an increase in cell proliferation in the contralateral control cornea. Dunhum et al (1994) showed a decrease in intraocular pressure of the contralateral eye after the instillation of timolol in the other eye. They suggested that this was due to systemic absorption.

In view of the different results of this study to previous published studies, does it mean that the current clinical practice and advice given to patients should be modified? As was discussed in the introduction of this chapter, topical

proxymetacaine is used for contact tonometry, foreign body removal and other minor ocular emergencies, as well as in some refractive surgery techniques. Since these procedures are very short, the instillation of 0.5% proxymetacaine hydrochloride is long enough to produce the necessary anaesthesia. Clinicians should be aware that the actual action of the anaesthetic extends up to 60 minutes, longer than the 20 minutes previously thought, and patients should be warned that the risk from an undetected foreign body after topical anaesthesia is present for longer than 20 minutes. Patients should be asked to sit in the waiting room for at least 30 minutes before leaving, a sufficient time to avoid any accidental corneal damage due to an undetected foreign body.

In conclusion, the anaesthetic effect of 20μl of 0.5% proxymetacaine hydrochloride peaks at 15 minutes, has not disappeared fully even 60 minutes after instillation, and is not affected by the subject's iris colour. Our results indicate the need for a review of the anaesthetic action of other ocular anaesthetics with more sensitive techniques, such as the NCCA.

6. An Investigation of the Effect of Iris Colour and Ethnic Origin on Corneal and Skin Sensitivity and on Tear Film Stability and Blink Rate

6.1 Introduction-Purpose

The effect of iris colour on corneal sensitivity has been studied previously by Millodot (1975a; 1976a), and Tota and La Marca (1982). In 1975, Millodot compared corneal sensitivity to touch, using the Cochet-Bonnet aesthesiometer, between Caucasians, Africans, Indians, and Chinese. The results indicated that Caucasians with blue eyes had more sensitive corneas than those with brown eyes, and Caucasians with dark brown irises had more sensitive corneas than non-Caucasians with dark irises. It was also found that the sensitivity diminishes further in non-Caucasians with increasing skin pigmentation. In 1976, Millodot investigated whether this phenomenon could be attributed either to some characteristic of the cornea (differences in thickness or nerve density), or to some central nervous system factor. Corneal sensitivity to touch was determined in people having the same (control) or different iris colour (heterochromia) in their two eyes. No difference in sensitivity was found between the two eyes of each subject, both for the control group and the heterochromic group. This suggested that the variation in sensitivity found for different iris colours was not due to some inherent difference in the cornea, but rather to some higher mechanism of the sensory system.

Further evidence that iris pigmentation alone was not the important factor came from the study that found albinos to have a reduced touch sensitivity. Corneal sensitivity might be expected to be even higher in albinos than blue-eyed Caucasians since they

have non-pigmented irises. However, Millodot (1978) found a reduced corneal sensitivity in albinos, suggesting an inherent deficiency in albinism.

A similar effect from ethnic origin was found with tear film stability, increasing from Chinese, to Africans, to Indians, to Caucasians (Patel et al., 1995). Tear film stability was also lower in Caucasians with brown eyes than those with blue eyes (Patel et al., 1991b). According to Tota and La Marca (1982), subjects with blue eyes produce more tears than subjects with brown eyes, and this may partly explain the higher stability.

Using fluorescein break-up time as an indicator of pre-corneal tear film stability, average values of 13.9 secs (Maudgil et al., 1989), 9.6 secs (Sukul et al., 1983) and 7.8 secs (Chopra et al., 1985) have been reported for normal Indian brown eyes. For normal Chinese eyes, values of approximately 7 secs have been reported (Cho et al., 1992, 1993; Brown et al., 1993; Cho and Yap, 1993), whereas for Malays, assessment of tear film stability using non-invasive methods gave values of 15.8 secs (Mohidin et al., 2002). For Caucasian subjects, reported values range between 15-34 secs (Lemp and Hamill, 1973), 10-60 secs (Rengstorff, 1974), and 19.9 secs (Patel et al., 1995). Generally the average break-up time for non-Caucasian eyes is substantially below the break-up time reported for Caucasian eyes.

Other than genetic differences, several reasons can account for the differences in these studies, such as diet, age, ambient temperature, and humidity. Differences in tear film stability have been reported in age-matched Caucasian and Chinese subjects living in a common environment (Cho and Brown, 1993). Therefore temperature and humidity

can be discounted as the causes for the differences between the two groups. Diet, however, may account for the differences observed. Vitamins A and C can influence tear film quality directly or indirectly (Sommer and Green, 1982; Sommer, 1983a, 1983b; Ubels and MacRae, 1984; Paterson and O'Rourke, 1987; Patel et al., 1993), and a diet devoid of specific trace elements (e.g. zinc, magnesium) may depress tear film stability indirectly (Shreeve, 1982; Paterson and O'Rourke, 1987).

The basis for the variation of corneal sensitivity and iris pigmentation is not clear, but the effect has remained one of the principal physiological factors associated with corneal sensation variation. This study will therefore assess:

1) The effect of iris colour on corneal sensation by assessing a different group of nerve receptors than previously reported, the cold sensitive C-fibres. In addition, by assessing the skin thermal sensitivity to a cooling stimulus, the relationship between corneal sensitivity and skin sensitivity will be considered.

2) The effect of iris colour on tear break-up time by repeating the studies that found tear stability to be influenced by iris colour and ethnic origin (Patel et al, 1991, 1995).

3) The relationship between blink rate, corneal sensitivity and tear film stability among subjects of the same and different racial origins. One model proposed for normal blinking is that changes in the tear film prior to break-up are detected by the corneal nerves. If tear film stability triggers a blink and is influenced by iris colour, then investigating the relationship between blink rate, tear film stability, corneal

sensitivity and iris colour may be useful in understanding the blink mechanism further.

6.2 Methods

Two hundred subjects were recruited from the student population of Cardiff University within the age range of 20-40 years. The subjects were selected to produce four groups of different ethnic origin: I) 100 Caucasians (white) (38 males, 62 females, mean age=23.3±3.6 years, range=19-36) II) 40 Asians (Indian sub-continent) (12 males, 28 females, mean age=21.93±3.45 years, range=19-30) III) 40 Chinese (15 males, 25 females, mean age=25.6±6.45 years, range=19-40), and IV) 20 Black Africans (8 males, 12 females, mean age=25.2±4.43 years, range=19-35).

The unequal division of subjects was naturally created by the variation of iris colour in each ethnic group. Caucasians have the widest range of iris colours from pale blue to brown. Both Indians and Chinese have either brown or dark brown irises, while black Africans typically have only dark brown irises. Consequently, to ensure that we have 20 subjects for each iris grade in each ethnic group, the numbers of subjects required were 100 Caucasian, 40 Asian, 40 Chinese, and 20 black African.

Subjects were excluded if they were contact lens wearers (Ntola and Murphy, 2002), or if they had any ocular or systemic pathology known to affect corneal sensitivity, e.g. ocular surgery, ocular diseases, diabetes, corneal dystrophy (Birndorf and Ginsberg, 1972; Schwartz, 1974; Lyne, 1977; Ishikawa et al., 1994; Ruben, 1994; Murphy et al., 1999a; Rosenberg et al., 2000). Also, pregnant women or women during the pre-menstrual, menstruation, or ovulating period, were excluded, as corneal

sensitivity is depressed (Millodot, 1984, 1994; Martin and Safran, 1988). Ethical approval was obtained from the School of Optometry and Vision Sciences Research Ethics Committee. After explanation of the purpose of the study, subjects were asked to sign a consent form prior to participating. Subjects were also reminded that they could withdraw from the study at anytime.

The blink rate of the subject was recorded over a five minute period using the digital video camera using the procedure described in section 3.8.1.

A photograph of each subject's right eye was taken using the Canon Digital Camera, using the procedure described in section 3.7.1, and each photograph was later used for iris colour classification using the Iris Color Classification System by Seddon et al (1990), as described in section 3.6.

Central corneal sensitivity of the right eye only was measured using the NCCA, as described in section 3.1.1. All measurements were made after 12pm to avoid any possible diurnal bias. The skin sensitivity of the right eye was assessed at the upper closed eyelid using the NCCA. This area of the skin was chosen because it can be easily positioned in front of the slit-lamp mounted NCCA, and should have a high level of sensitivity.

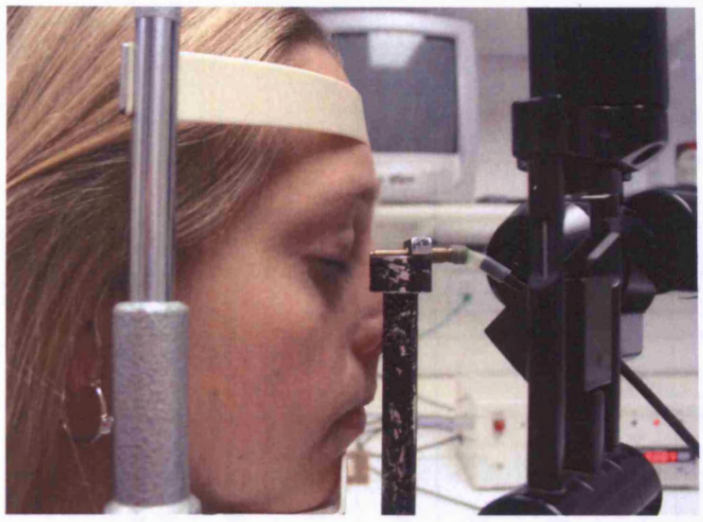


Fig 6.1: Alignment of NCCA with the closed eyelid for the assessment of thermal skin sensitivity.

Tear film stability was assessed using the fluorescein break-up time, as described in section 3.3.1.

6.3 Results

The distributions of the measurements for corneal sensitivity, skin sensitivity, break-up time, and number of blinks were assessed for normality (Shapiro-Wilk test), using the SPSS11 Statistical Software Program (Lead Tools, Lead Technologies, Inc). Normality test found all sets of data not to be normally distributed. The data were log transformed and normality test found all the data to be normally distributed, allowing the use of parametric statistical tests (Prism, GraphPad Software Inc, San Diego).

Table 6.1: Median/ Interquartile Range (IR) and Mean (± Standard Deviation) of measurements taken for all ethnic groups:

CS: Corneal Sensitivity Threshold (mbars) **SS:** Skin Sensitivity Threshold (mbars) **TBUT:** Tear Break-Up Time (secs)

BR: Blink rate: Blinks/min.

		Caucasians Grade 1	Caucasians Grade 2	Caucasians Grade 3	Caucasians Grade 4	Caucasians Grade 5
CS (mbars)	**Mean±SD**	0.66±0.16	0.71±0.22	0.85±0.31	1.01±0.31	1.11±0.34
	Median/ IR	0.7/ 0.52-0.77	0.72/ 0.57-0.87	0.77/ 0.62-0.95	1.05/ 0.87-1.22	1.1/ 0.87-1.37
SS (mbars)	**Mean±SD**	0.63±0.22	0.60±0.16	0.73±0.31	0.75±0.40	0.78±0.39
	Median/ IR	0.65/ 0.5-0.67	0.52/ 0.45-0.62	0.7/ 0.45-0.85	0.7/ 0.55-0.92	0.8/ 0.6-1.0
TBUT (secs)	**Mean±SD**	10.94±7.99	8.56±3.34	11.90±6.0	10.43±5.56	16.17±8.58
	Median/ IR	7.65/ 6.4-11.43	8.64/ 5.7-11.64	9.54/ 7.82-15.31	9.47/ 5.5/15.34	15.51/ 11.52-19.36
BR (Blinks/min)	**Mean±SD**	15.82±12.01	18.25±8.17	12.73±6.62	15.39±9.10	9.07±6.52
	Median/ IR	11.4/ 7.1-24.4	14.5/ 11.5-24.0	12.2/ 7.7-20.2	15.6/ 10.0-22.8	7.5/ 4.9-9.7

		Asians Grade 4	Asians Grade 5	Chinese Grade 4	Chinese Grade 5	Africans Grade 5
CS (mbars)	**Mean±SD**	0.85±0.28	0.94±0.30	0.71±0.30	1.02±0.54	1.23±0.28
	Median/ IR	0.82/ 0.7-0.87	0.85/ 0.77-1.07	0.70/ 0.52-0.82	0.85/ 0.55-1.35	1.17/ 1.0-1.5
SS (mbars)	**Mean±SD**	0.88±0.35	0.76±0.39	0.74±0.44	0.66±0.27	1.21±0.67
	Median/ IR	0.90/ 0.6-1.12	0.62/ 0.52-0.87	0.62/ 0.5-0.8	0.60/ 0.45-0.82	1.17/ 0.6-1.67
TBUT (secs)	**Mean±SD**	11.43±6.78	14.09±7.12	9.25±5.25	10.42±5.48	14.10±8.08
	Median/ IR	8.73/ 5.32-16.59	12.72/ 9.27-16.96	7.61/ 5.59-11.45	10.68/ 5.13-13.95	15.07/ 8.66-20.28
BR (Blinks/min)	**Mean±SD**	15.64±9.20	16.02±10.46	16.11±11.79	15.03±11.2	12.58±9.36
	Median/ IR	14.20/ 10.4-19.5	12.80/ 8.8-22.5	10.65/ 7.5-23.0	10.60/ 5.5-22.0	8.00/ 5.3-17.7

6.3.1 Univariate Analysis of the Results

6.3.1.1 Corneal Sensitivity, Iris Pigmentation and Ethnic Group

Since 13 statistical tests were conducted, a Bonferroni correction was used, thus the statistical significance required was $p<0.0035$ (i.e. 0.05/13).

The graphs of corneal sensitivity have been illustrated using the actual corneal sensitivity threshold measurements and not the log transformed data of corneal sensitivity thresholds. This was done because some of the log-transformed data were negative, hence the graph would not be able to illustrate clearly how corneal sensitivity changes with increasing iris pigmentation. A significant difference in corneal sensitivity was found between the grades of all the ethnic groups (one-way ANOVA, $p<0.0001$), suggesting that corneal sensitivity was affected by iris colour and ethnic origin (Fig 6.2). Table 6.2 shows the mean (standard deviation) of corneal sensitivity for each iris colour grade of each ethnic group.

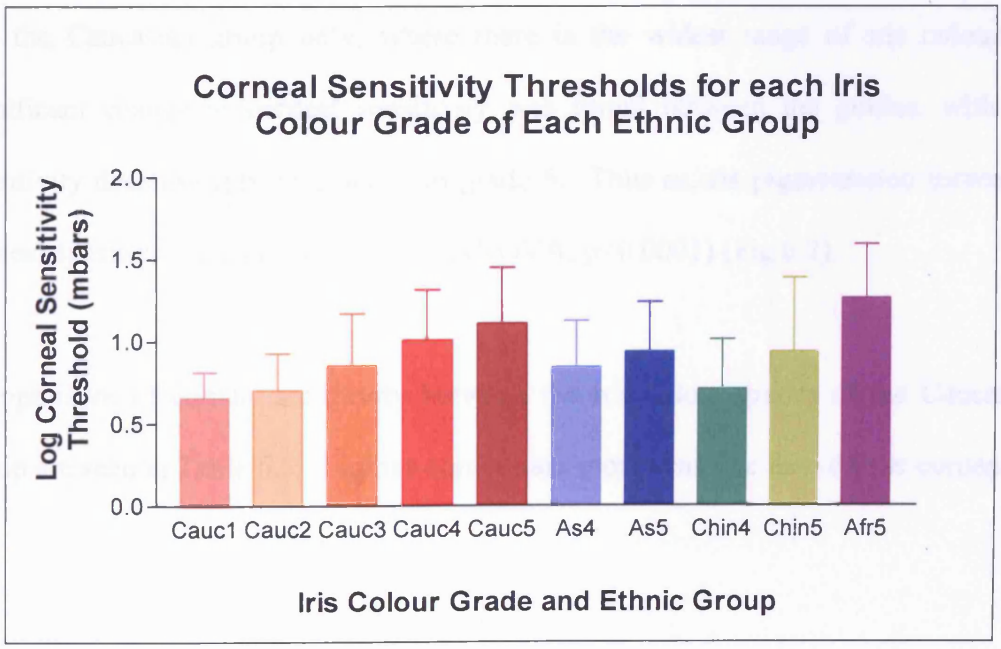


Fig 6.2: Mean (± standard deviation) central corneal sensitivity threshold for each iris colour grade and ethnic group.

	Log Corneal Sensitivity Threshold (mbars) Mean ± SD		**Log Corneal Sensitivity Threshold (mbars) Mean ± SD**
Cauc 1	0.65±0.16	**As 4**	0.84±0.28
Cauc 2	0.7±0.22	**As 5**	0.93±0.3
Cauc 3	0.86±0.31	**Chin 4**	0.7±0.3
Cauc 4	1.01±0.31	**Chin 5**	0.93±0.45
Cauc 5	1.11±0.34	**Afr 5**	1.26±0.33

Table 6.2: Mean (± standard deviation) central corneal sensitivity threshold of each iris colour grade for each ethnic group.

For the Caucasian group only, where there is the widest range of iris colours, a significant change in corneal sensitivity was found between the grades, with the sensitivity decreasing from grade 1 to grade 5. Thus as iris pigmentation increases, corneal sensitivity decreases (one-way ANOVA, $p<0.0001$) (Fig 6.2).

Comparisons of corneal sensitivity between the iris colour grades of the Caucasian group are seen in Table 6.3. Lighter corneas are more sensitive than darker corneas.

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Grade 1					
Grade 2	p>0.0035				
Grade 3	*p<0.0035*	p>0.0035			
Grade 4	*p<0.0035*	*p<0.0035*	p>0.0035		
Grade 5	*p<0.0035*	*p<0.0035*	p>0.0035	p>0.0035	

Table 6.3: Comparison of corneal sensitivity between the five iris colour grades of the Caucasian group (unpaired t-tests).

The same pattern of change was shown for both the Asian and Chinese groups, where there are only two iris colours, brown (grade 4) and dark brown (grade 5). Although the difference in corneal sensitivity between the two grades is not significant, it can be generally seen from the graph that dark brown irises are less sensitive than brown irises (Asian: unpaired t-test, $p = 0.342$; Chinese: unpaired t-test, $p = 0.368$).

The African group, with only dark brown irises, are less sensitive than any other grade 5 group. If corneal sensitivity decreases as the iris colour becomes darker, then the findings for the African group seem to be reasonable, as Africans have even more pigmented irises than the dark brown irises of Caucasians, Asians and Chinese.

Comparing corneal sensitivity changes between the four ethnic groups, and taking into consideration the colour of the iris, only the results for the grade 5 iris colour group can be compared (one-way ANOVA, $p = 0.027$) (Fig 6.3).

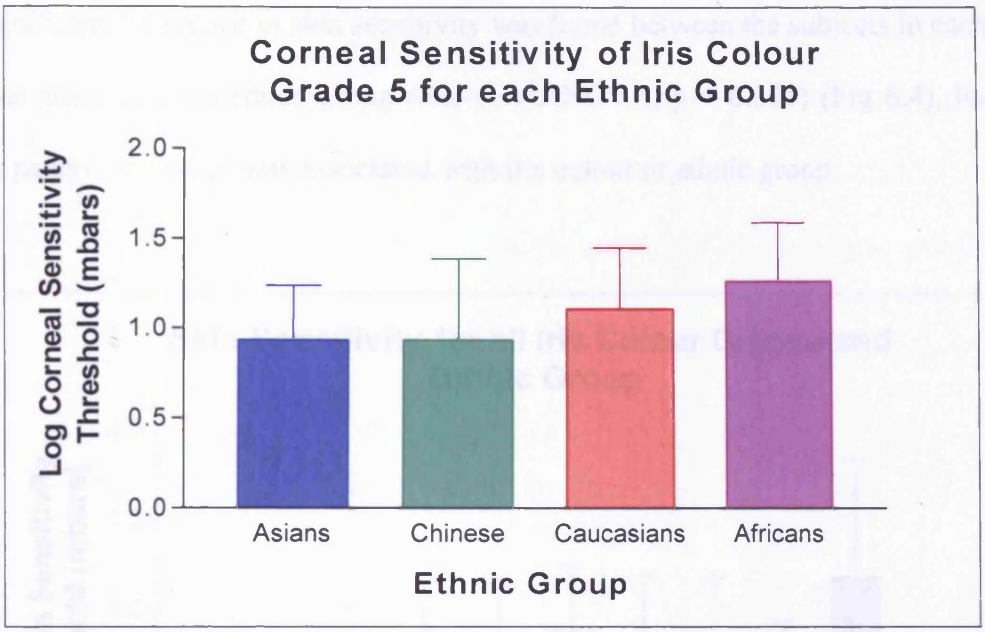


Fig 6.3: Mean (± standard deviation) central corneal sensitivity threshold for iris colour grade 5 between the four ethnic groups.

Summarising the findings, corneal sensitivity for a cooling stimulus was affected by iris colour. As iris pigmentation increased, corneal sensitivity decreased. A difference in corneal sensitivity was also found between ethnic groups, with sensitivity decreasing from Asians/ Chinese, to Caucasians, to Africans.

6.3.1.2 Skin Sensitivity, Iris Pigmentation and Ethnic Group

The graphs of skin sensitivity have been illustrated using the actual skin sensitivity threshold measurements and not the log transformed data of skin sensitivity thresholds. This was done because all the logged data were negative, hence the graph would not be able to illustrate clearly how skin sensitivity changes with increasing iris pigmentation.

A significant difference in skin sensitivity was found between the subjects in each iris colour grade of each ethnic group (one-way ANOVA, p = 0.008) (Fig 6.4), but no clear pattern of change was associated with iris colour or ethnic group.

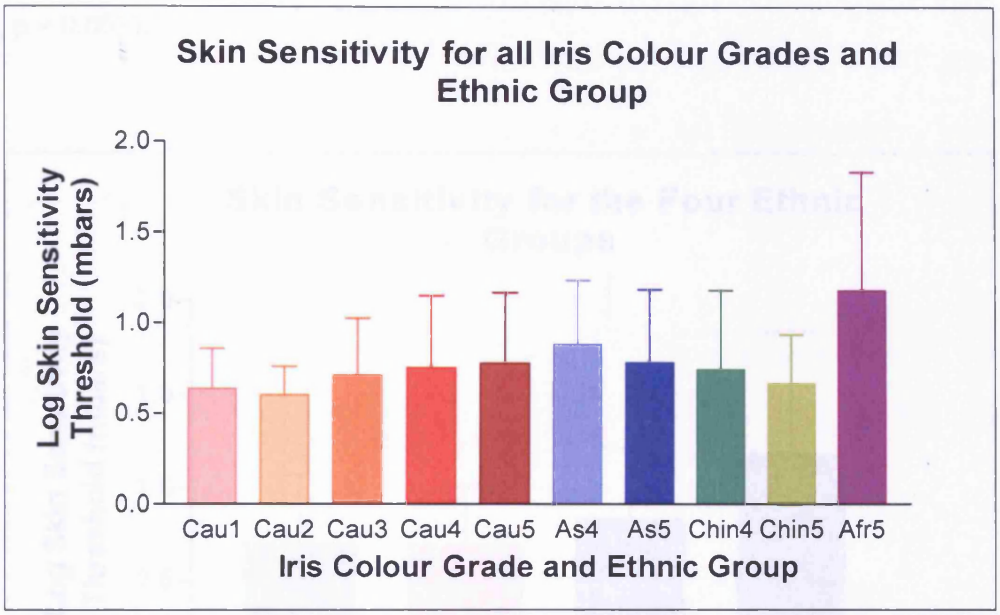


Fig 6.4: Mean (± standard deviation) skin sensitivity threshold for each iris colour grade and ethnic group.

For the Caucasian group, where there is the greatest variety of iris colour, there was a trend for decreasing skin sensitivity with increasing iris pigmentation. However, there was no significant change in skin sensitivity between the five grades (one-way ANOVA, p = 0.478). No difference in skin sensitivity was found between the two iris colour grades of the Asian and Chinese Groups (Asian: unpaired t-test, p = 0.286; Chinese: unpaired t-test, p = 0.559).

Since there was no difference in skin sensitivity with iris colour for each ethnic group, the results were combined and skin sensitivity was compared between the four ethnic

groups. A significant difference between the groups was found (one-way ANOVA, p = 0.0004). The African group had the lowest sensitivity and was significantly different than the skin sensitivity for the Chinese, Caucasians and Asians (Chinese, unpaired t-test, p = 0.003; Caucasians, unpaired t-test, p<0.0001; Asians, unpaired t-test, p = 0.003).

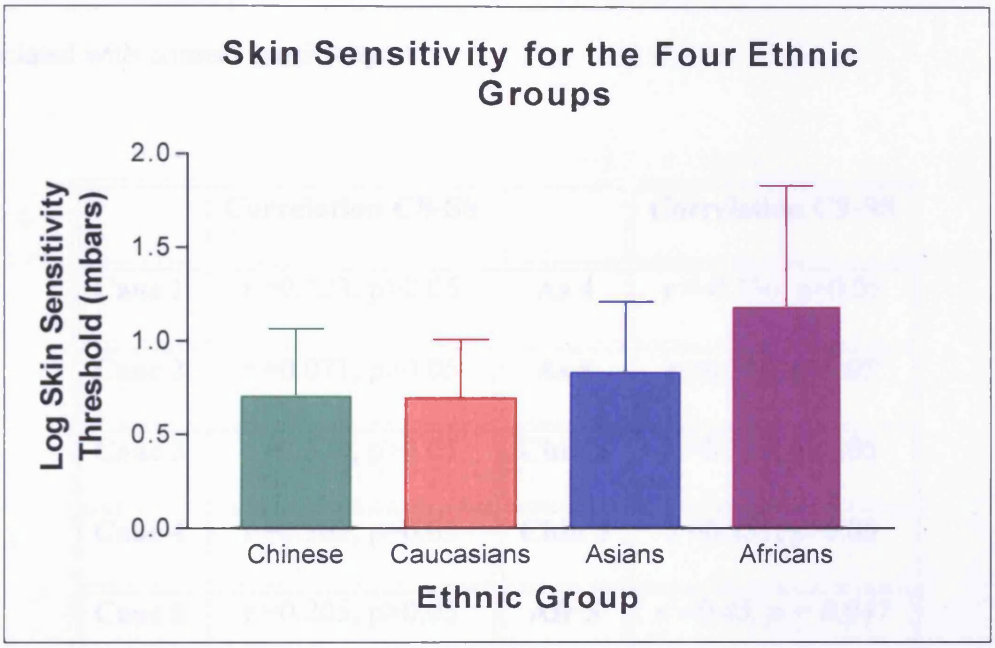


Fig 6.5: Mean (± standard deviation) skin sensitivity threshold for the four ethnic groups.

Summarising the results for skin sensitivity, although there was a significant difference between the iris colour grades for the ethnic groups, this pattern of change was not associated with iris colour. Skin sensitivity differed between the ethnic groups, decreasing from Caucasians, to Chinese, to Asians, to Africans.

6.3.1.3 Correlation between Corneal and Skin Sensitivity

No significant correlation was found between corneal and skin sensitivity for each iris colour grade of every ethnic group, apart from African group. Table 6.4 shows the correlation for all iris colour grades of each ethnic group. Only a weak significant correlation was found when the data for all subjects were compared (Pearson, r = 0.294, $p<0.0001$) (Fig 6.6). These results indicate that skin sensitivity was not associated with corneal sensitivity.

	Correlation CS-SS		**Correlation CS-SS**
Cauc 1	r =0.123, p>0.05	**As 4**	r =-0.336, p>0.05
Cauc 2	r =0.071, p>0.05	**As 5**	r =0.316, p>0.05
Cauc 3	r =0.334, p>0.05	**Chin 4**	r =0.337, p>0.05
Cauc 4	r =0.362, p>0.05	**Chin 5**	r =0.251, p>0.05
Cauc 5	r =0.205, p>0.05	**Afr 5**	*r =0.45, p = 0.047*

Table 6.4: Correlation of corneal sensitivity and skin sensitivity for each iris colour grade and ethnic group (Pearson r correlations).

Considering only the Caucasian group, where there is a variety of iris colour, a significant but weak correlation was found, such that corneal sensitivity decreased, as skin sensitivity decreased (r = 0.278, p = 0.05) (Fig 6.7).

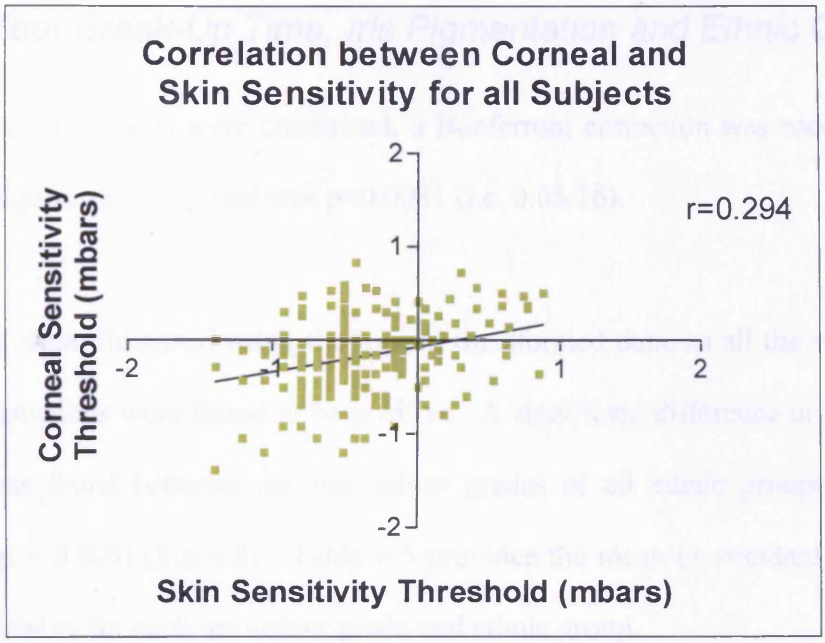


Fig 6.6: Correlation of corneal sensitivity and skin thermal sensitivity for all subjects.

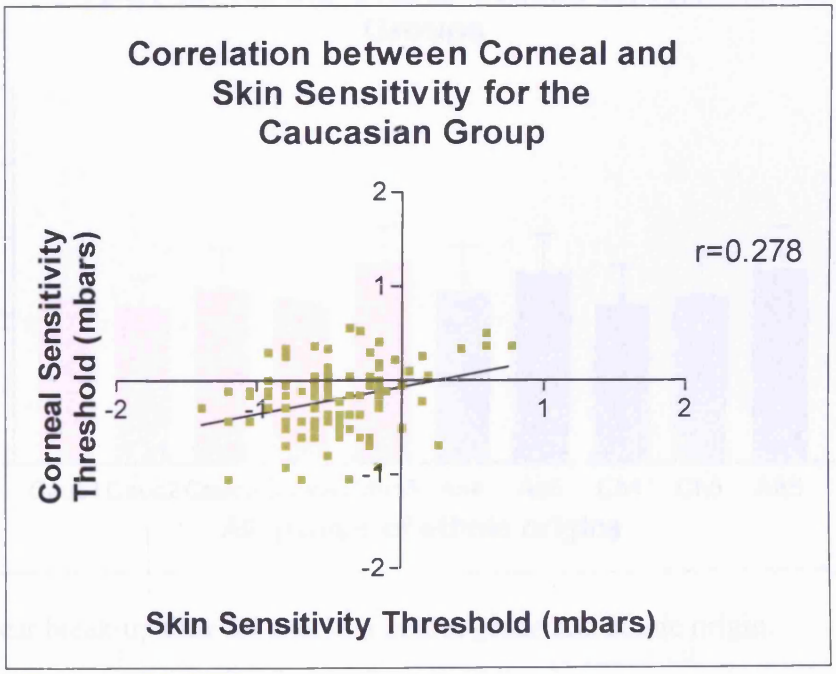


Fig 6.7: Correlation of corneal sensitivity and skin thermal sensitivity for all subjects of Caucasian group.

6.3.1.4 Tear Break-Up Time, Iris Pigmentation and Ethnic Origin

Since 16 statistical tests were conducted, a Bonferroni correction was used, thus the statistical significance required was $p<0.0031$ (i.e. 0.05/16).

The graphs were illustrated using the logged transformed data, as all the values after log transformations were found to be positive. A significant difference in tear break-up time was found between the iris colour grades of all ethnic groups (one-way ANOVA, $p = 0.005$) (Fig 6.8). Table 6.5 provides the mean (± standard deviation) tear film stability for each iris colour grade and ethnic group.

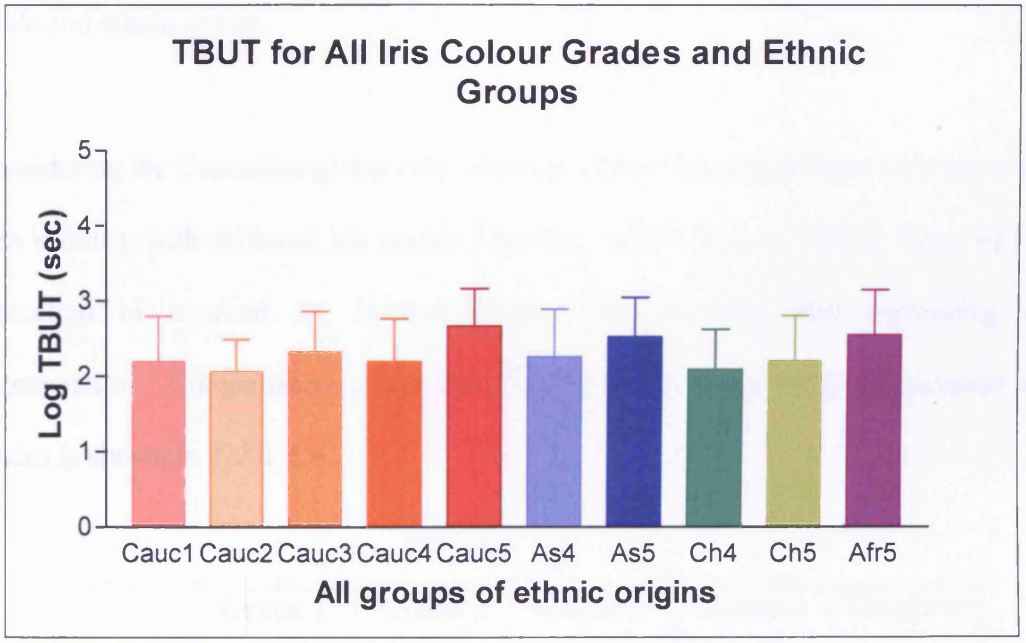


Fig 6.8: Tear break-up time for each iris colour grade and ethnic origin.

	Log Tear Break-Up Time (secs) Mean±SD		Log Tear Break-Up Time (secs) Mean±SD
Cauc 1	2.2±0.6	**As 4**	2.25±0.63
Cauc 2	2.07±0.42	**As 5**	2.52±0.52
Cauc 3	2.33±0.53	**Chin 4**	2.08±0.53
Cauc 4	2.19±0.57	**Chin 5**	2.2±0.6
Cauc 5	2.67±0.49	**Afr 5**	2.55±0.6

Table 6.5: Mean (± standard deviation) tear film break-up time for each iris colour grade and ethnic group.

Considering the Caucasian group only, although there was a significant change in tear film stability with different iris colour (one-way ANOVA, p = 0.007), there was no indication of a trend for increasing tear film stability with increasing iris pigmentation. Comparisons of the tear film break-up times for the Caucasian iris grades is shown in Table 6.6.

	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Grade 1					
Grade 2	p>0.0031				
Grade 3	p>0.0031	p>0.0031			
Grade 4	p>0.0031	p>0.0031	p>0.0031		
Grade 5	p>0.0031	*p<0.0031*	p>0.0031	p>0.0031	

Table 6.6: Comparison of tear film break-up times between the five iris colour grades of the Caucasian group.

For the Asian and Chinese group, there was no significant difference in tear film stability between the grades 4 and 5 (Asian: unpaired t-test, p = 0.152; Chinese: unpaired t-test, p = 0.529).

In order to assess tear film stability between the four ethnic groups, the results for iris colour Grade 5 were compared. No significant change was found between the four ethnic groups (one-way ANOVA, p = 0.061) (Fig 6.9).

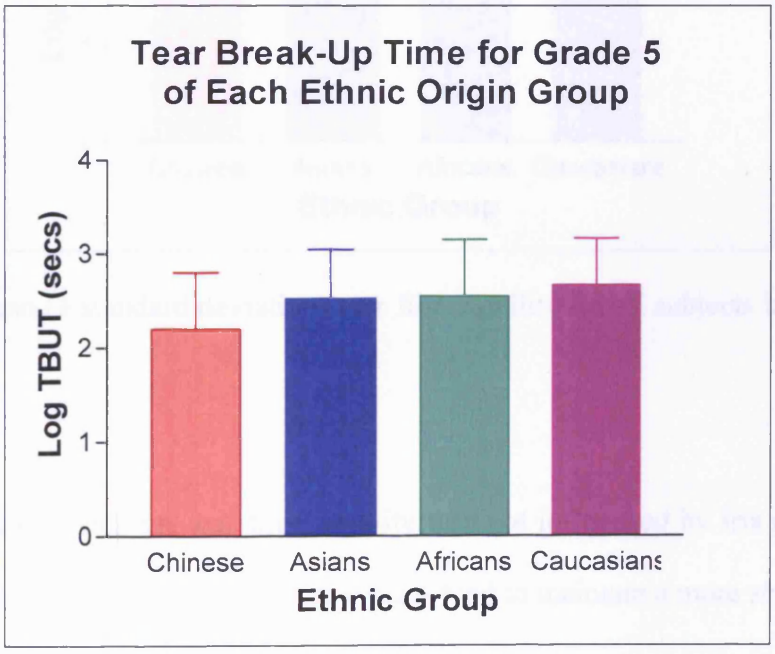


Fig 6.9: Mean (± standard deviation) tear film stability for iris colour grade 5 of the four ethnic groups.

Patel et al (1995) found tear film stability to increase from Chinese, to Africans, to Indians, to Caucasians, without taking into account iris colour. When the results were compared by including all the subjects in each group, as Patel et al did, there was no significant difference between the four ethnic groups (one-way ANOVA, p = 0.056) (Fig 6.10).

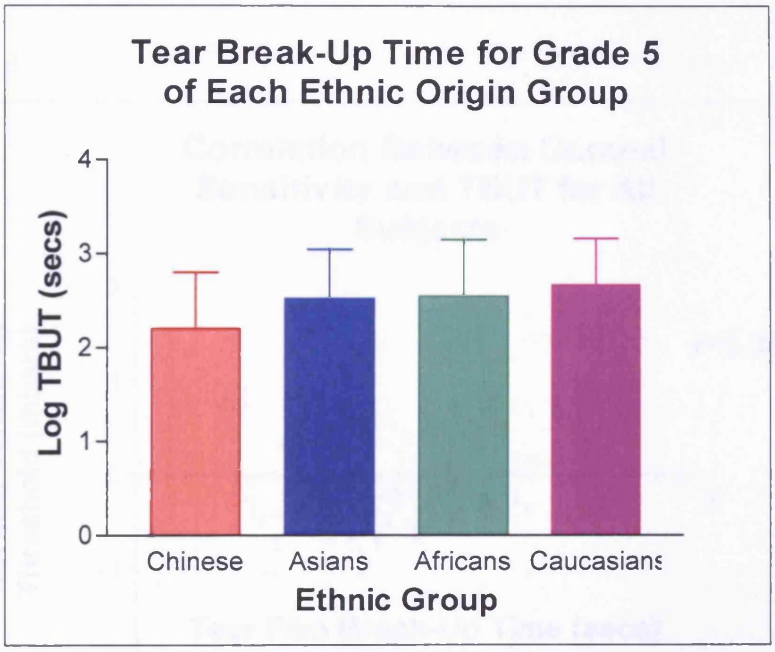


Fig 6.10: Mean (± standard deviation) tear film stability for all subjects in each ethnic group.

Summarising the findings, tear film stability was not influenced by iris pigmentation or ethnic origin, although eyes with dark irises tend to maintain a more stable tear film than eyes with lighter irises.

6.3.1.5 Correlation between Corneal Sensitivity and Tear Film Break-Up Time

Comparing the data of all subjects, a significant but weak correlation was found between corneal sensitivity and tear film break-up time, suggesting that as corneal sensitivity decreases, tear film stability increases (Pearson, r = 0.303, p<0.0001) (Fig 6.11).

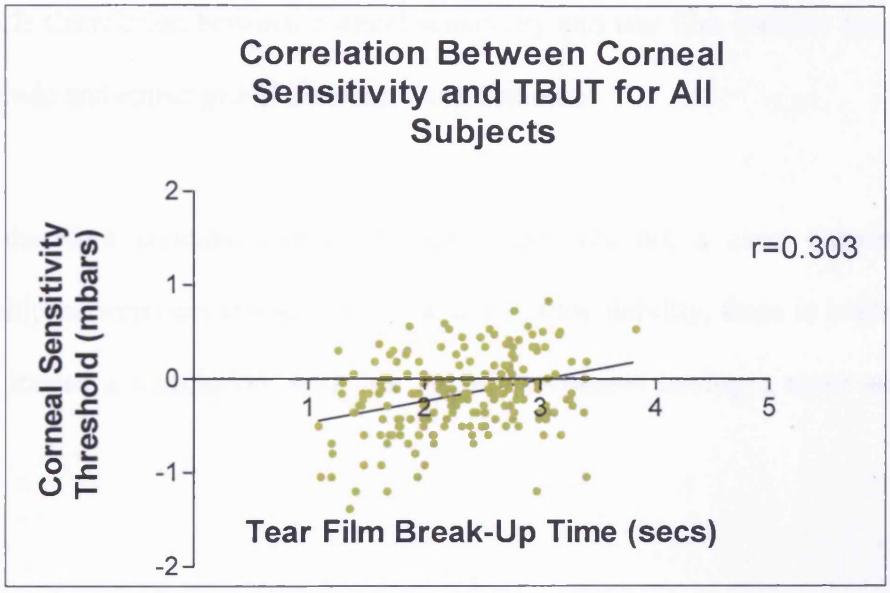


Fig 6.11: Correlation of corneal sensitivity and tear film stability for all subjects.

The comparison was also repeated for each iris grade/ethnic group and the results are shown in Table 6.7

	Correlation CS-TBUT		Correlation CS-TBUT
Cauc 1	r =-0.004, p>0.05	**As 4**	r =0.067, p>0.05
Cauc 2	r =0.189, p>0.05	**As 5**	r =0.225, p>0.05
Cauc 3	r =-0.392, p>0.05	**Chin 4**	*r =0.538, p =0.014*
Cauc 4	*r = 0.459, p = 0.041*	**Chin 5**	r =0.178, p>0.05
Cauc 5	*r= 0.784 , p<0.0001*	**Afric 5**	*r =0.581, p* = 0.009

Table 6.7: Correlation between corneal sensitivity and tear film stability for each iris colour grade and ethnic group (Pearson r correlations).

It was therefore concluded that although there was not a clear pattern of the relationship between corneal sensitivity and tear film stability, there is evidence that there is indeed a correlation, with less sensitive corneas having a more stable tear film.

6.3.1.6 Blink Rate, Iris Pigmentation and Ethnic Origin

Since 14 statistical tests were conducted, a Bonferroni correction was used, thus the statistical significance required was p<0.0035 (i.e. 0.05/14).

The graphs were illustrated using the logged transformed data, as all the values after log transformations found to be positive. A difference in blink rate was found between the iris colour grades of all ethnic groups (one-way ANOVA, p = 0.028) (Fig 6.12).

Looking only at the Caucasian group, a significant change in blink rate was found with different iris colours (one-way ANOVA, $p = 0.002$), but, there was no pattern of change associated with iris pigmentation. Nevertheless, subjects with darker corneas tended to blink less compared to the other grades, and since these subjects also have a more stable tear film, it suggests a correlation might exist between tear film stability and blink rate (Prause and Norn, 1987; Yap, 1991). A comparison of the blink rates between the iris colour grades of Caucasians is shown in Table 6.8.

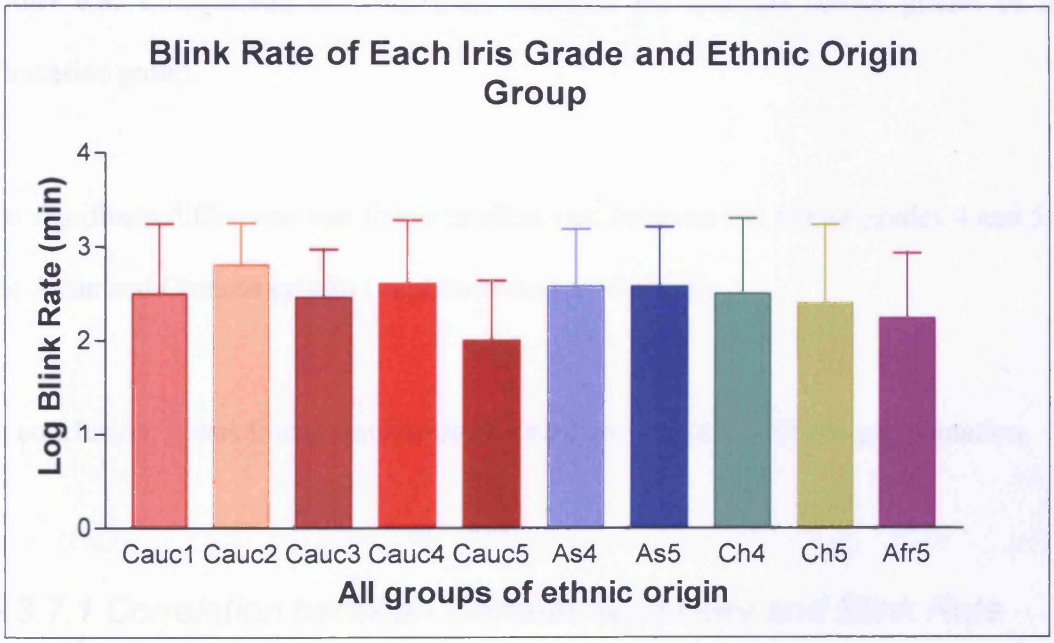


Fig 6.12: Mean (± standard deviation) of blink rate for each iris colour grade and ethnic group.

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Grade 1					
Grade 2	p>0.0035				
Grade 3	p>0.0035	p>0.0035			
Grade 4	p>0.0035	p>0.0035	p>0.0035		
Grade 5	p>0.0035	*p<0.0035*	p>0.0035	p>0.0035	

Table 6.8: Comparison of blink rates between the five iris colour grades of the Caucasian group.

No significant difference was found in blink rate between iris colour grades 4 and 5 in the Asian and Chinese groups (unpaired t-test, p>0.0035).

In conclusion, it was found that the blink rate does not alter with iris pigmentation.

6.3.7.1 Correlation between Corneal Sensitivity and Blink Rate

Comparing corneal sensitivity and blink rate, a significant, but weak correlation was found (Pearson, r = -0.36, p<0.0001) (Fig 6.13), suggesting that as corneal sensitivity decreases, the blink rate also decreases.

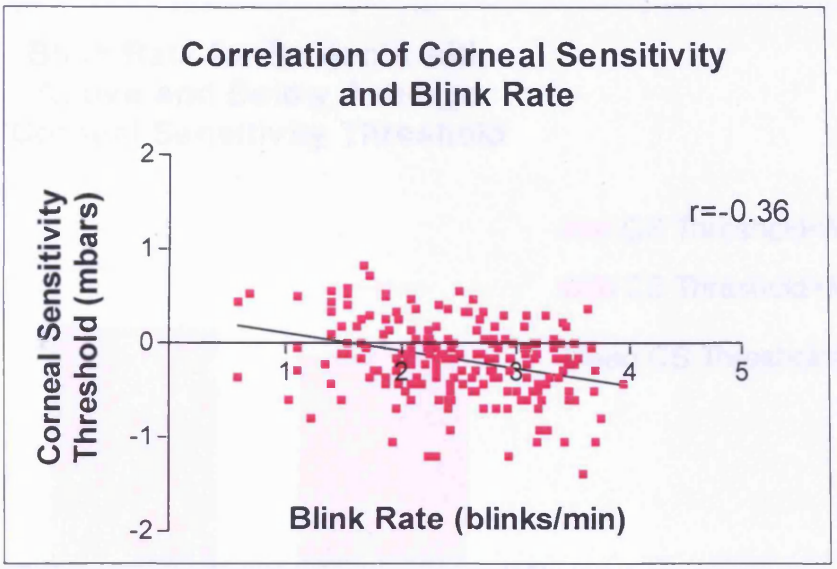


Fig 6.13: Correlation of corneal sensitivity and blink rate for all subjects.

Further analysis of the correlation can be made if the subjects de divided into two groups: Group 1, subjects with corneal sensitivity thresholds less than the mean corneal sensitivity threshold of all subjects; and Group 2, subjects with corneal sensitivity more than the mean corneal sensitivity threshold of all subjects (i.e. Group 1 = more sensitive, Group 2 = less sensitive). The difference between the mean of corneal sensitivity thresholds for the two groups was statistically significant (unpaired t-test, $p = 0.002$) (Fig 6.14), indicating that people having more sensitive corneas blink more than those having less sensitive corneas.

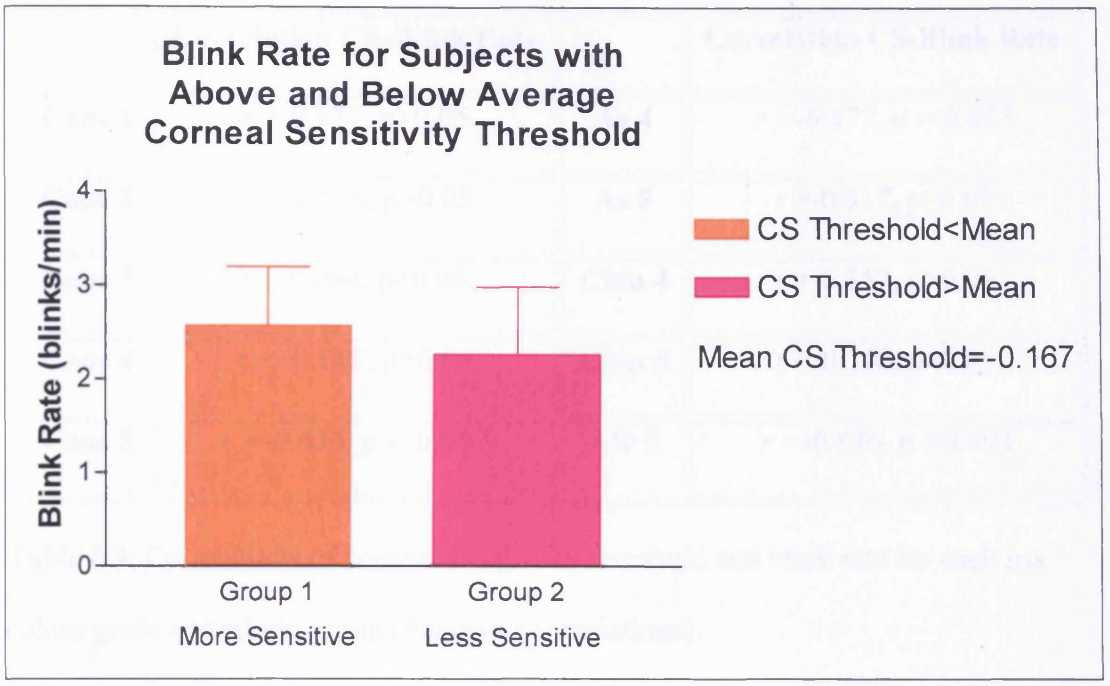


Fig 6.14: Comparison of blink rate for subjects with corneal sensitivity threshold above and below the mean corneal sensitivity threshold.

The correlations between corneal sensitivity thresholds and blink rate for all iris colour grades of each ethnic group are shown in Table 6.9. A significant relationship exists only for grade 5 of the Caucasian and African groups, as well as for grade 4 of the Asian Group.

	Correlation CS-Blink Rate		Correlation CS-Blink Rate
Cauc 1	r =-0.177, p>0.05	**As 4**	*r =-0.477, p = 0.033*
Cauc 2	r = -0.176, p>0.05	**As 5**	r =-0.317, p>0.05
Cauc 3	r = 0.094, p>0.05	**Chin 4**	r =-0.357, p>0.05
Cauc 4	r = -0.118, p>0.05	**Chin 5**	r =-0.246, p>0.05
Cauc 5	*r =-0.656, p = 0.002*	**Afr 5**	*r =-0.636, p =0.003*

Table 6.9: Correlations of corneal sensitivity threshold and blink rate for each iris colour grade and ethnic group (Pearson r correlations).

The findings suggest that corneal sensitivity is involved in the blink mechanism and may control normal involuntary blinking. This is observed when a decreased corneal sensitivity produces a decreased blink rate.

6.3.1.8 Correlation between Tear film Break-Up Time and Blink Rate

The relationship between blink rate and tear film stability was considered for all the subjects to investigate whether tear film stability is a factor in the blink mechanism. A significant correlation was found (Pearson, r =-0.536, p<0.0001) (Fig 6.15), indicating that as tear film stability increases, the blink rate decreases. The correlation was found to be significant, but not strong, due to inter-subject variability. To reduce the variability from iris colour and ethnic origin, only the correlations for Caucasians (grade 5) and Africans (grade 5) were considered (Figs 6.16, 6.17).

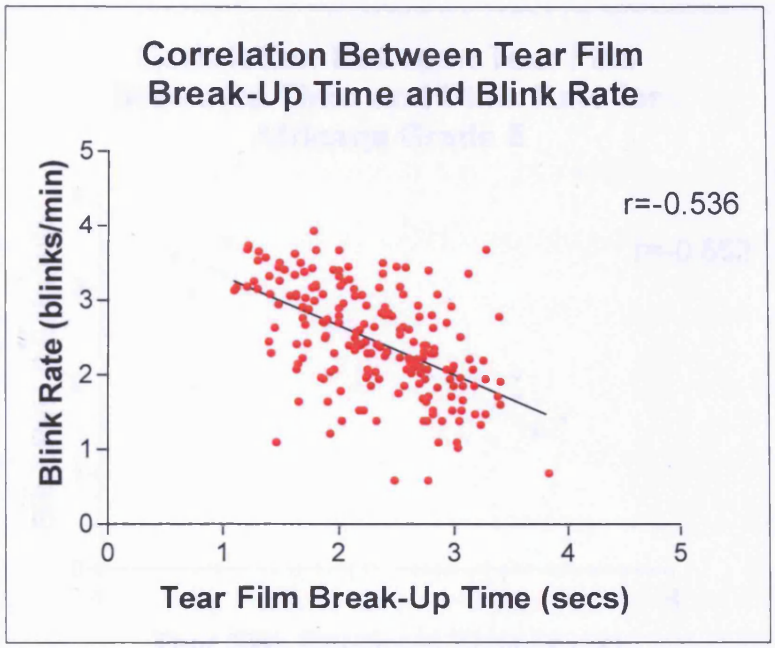


Fig 6.15: Correlation of tear film break-up time and blink rate for all subjects.

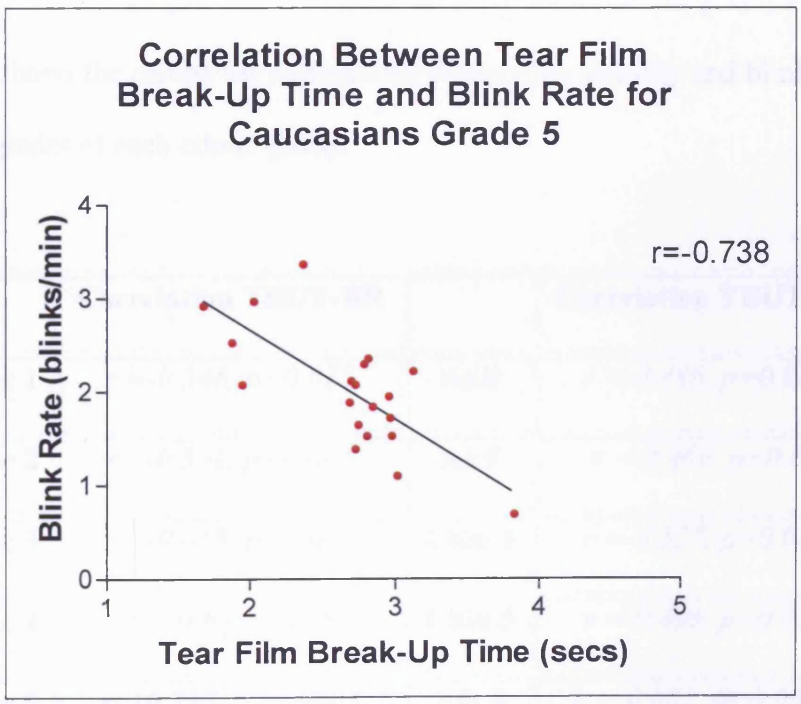


Fig 6.16: Correlation of tear film break-up time and blink rate for Caucasian grade 5 subjects.

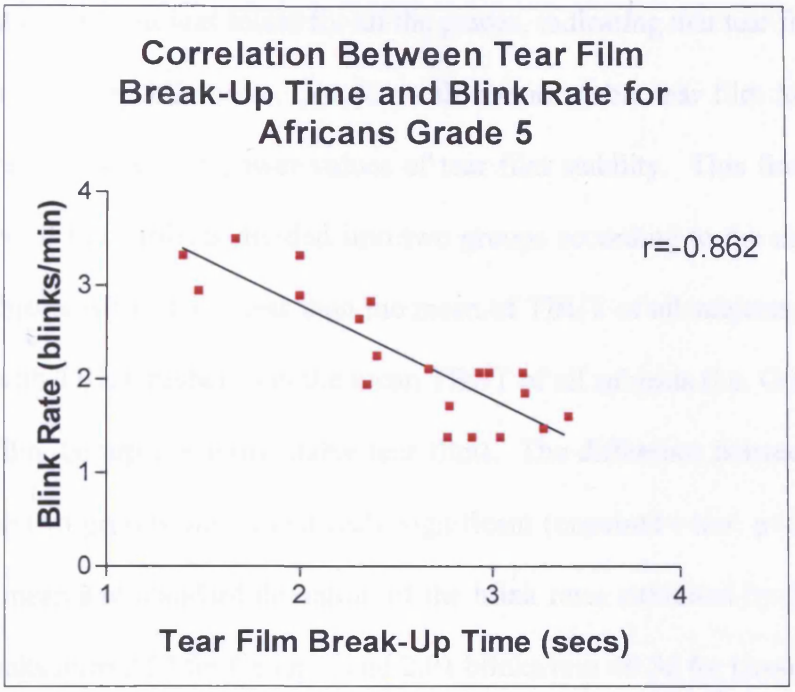


Fig 6.17: Correlation of tear film stability and blink rate for African grade 5 subjects.

Table 6.10 shows the results for correlations of tear film stability and blink rate for all iris colour grades of each ethnic group.

	Correlation TBUT-BR		**Correlation TBUT-BR**
Cauc 1	*r =-0.546, p=0.013*	**As 4**	*r =-0.486, p=0.039*
Cauc 2	*r =-0.581, p=0.007*	**As 5**	*r =-0.468, p=0.04*
Cauc 3	*r =-0.618, p=0.004*	**Chin 4**	*r =-0.527, p=0.017*
Cauc 4	*r =-0,6 p=0.005*	**Chin 5**	*r =-0.495, p=0.026*
Cauc 5	*r=-0.738, p=0.0002*	**Afr 5**	*r =-0.862, p<0.0001*

Table 6.10: Correlation of tear film stability and blink rate for each iris colour grade and ethnic group (Pearson r correlations).

A significant correlation was found for all the grades, indicating that tear film stability influences involuntary blink rate. People with a more stable tear film tend to blink less compared to those with lower values of tear film stability. This finding can be shown clearly if the subjects divided into two groups according to the mean TBUT: Group 1, subjects with TBUT less than the mean of TBUT of all subjects; and Group 2, subjects with TBUT higher than the mean TBUT of all subjects (i.e. Group 1 = less stable tear film, Group 2 = more stable tear film). The difference between the mean TBUT of the two groups was statistically significant (unpaired t-test, $p<0.0001$) (Fig 6.18). The mean and standard deviation of the blink rates exhibited by these groups was 2.81 blinks/min±0.57 for Group 1 and 2.01 blinks/min ±0.56 for Group 2.

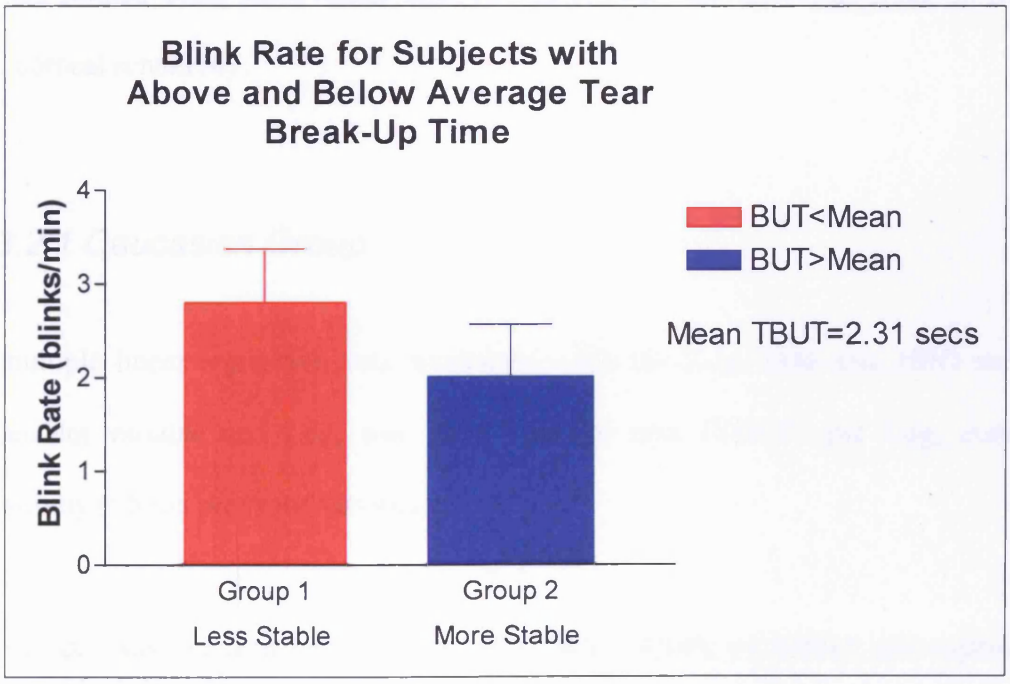


Fig 6.18: Comparison of blink rate for subjects with tear break-up time lower and higher than the mean BUT.

Summarising the results, it was found that tear film stability was closely related to the blink rate, with higher values of tear film stability producing lower blink rates. The findings are reasonable, as a less stable tear film will cause a more frequent eyelid activity to reform the tear film layer, in order to provide a smooth optical sharp image, protect the exposed corneal epithelium from the environment, and moisten and lubricate the ocular surface.

6.3.2 Multivariate Analysis of the Results

The results were also analysed using multivariate analysis for each ethnic group, as well as taking into consideration all the subjects participating in the study. The factors studied using multivariate analysis were blink rate, tear film break-up time, and corneal sensitivity.

6.3.2.1 Caucasian Group

A multiple linear regression was conducted, with the Log_e blink rate (BR) as the dependent variable and Log_e tear film break-up time (TBUT) and Log_e corneal sensitivity (CS) as predictor variables.

The model was found to be significant ($F(2.96) = 45.49$, $p < 0.001$), and explained 48.7% of the variance ($R^2 = 0.487$, adjusted $R^2 = 0.476$). The model was the following:

$$\text{Dependent Variable} = A\,(IV_1) + B\,(IV_2) + C$$

$$Log_e\,BR = -0.212\,(Log_e\,CS) - 0.763\,(Log_e\,TBUT) + 4.173$$

(where Log_e BR, Log_e CS and Log_e TBUT are the log transformed values of BR, CS and TBUT).

The beta coefficients for TBUT was significant (t = -0.847, p< 0.001), but the beta coefficients for CS was not found to be significant (t = -1.521, p = 0.131). Thus TBUT was found to be a unique predictor for BR. The semi partial correlation between TBUT and BR removing the linear effects of CS on TBUT was -0.62. Corneal Sensitivity did not explain any unique variance.

A 3-D cluster graph can illustrate the relationship between BR, TBUT and CS. BR is strongly related to TBUT, indicating that as the TBUT increases, the BR decreases. A relationship can also be seen between CS and BR, indicating that as CS decreases, the BR decreases (Fig 6.19).

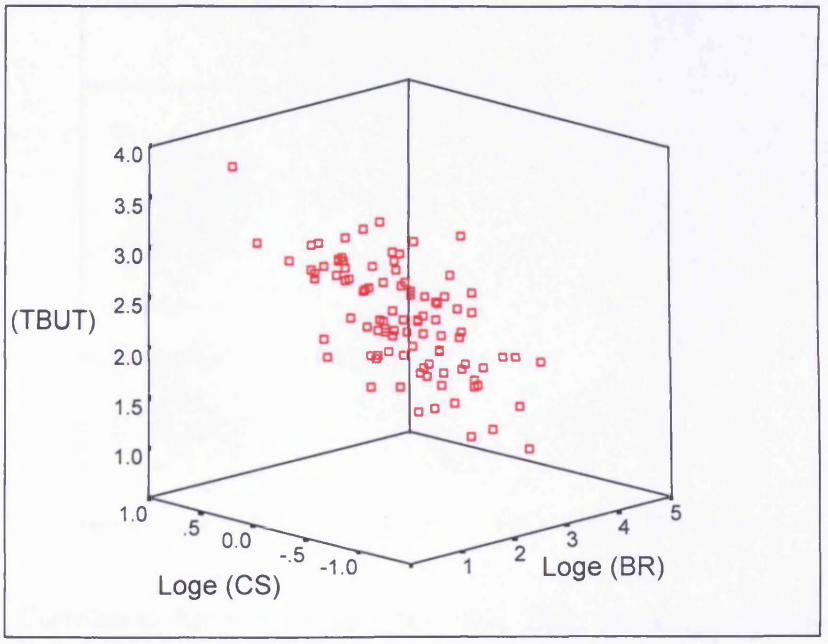


Fig 6.19: 3-D cluster graph illustrating the relationships between blink rate, tear film break-up time and corneal sensitivity for the Caucasian Group.

All the independent correlations between BR, TBUT and CS for the Caucasian group were also investigated using a matrix scatter plot. A significant strong correlation was found between BR and TBUT, suggesting that as the TBUT increases the blink rate decreases (Pearson, r = -0.689, p< 0.001). A significant correlation was also found between BR and CS (Pearson, r = -0.32, p< 0.001), indicating that as corneal sensitivity decreases the blink rate is also decreasing, as well as between CS and TBUT (Pearson, r = -0.31, p< 0.001) indicating that as CS decreases the TBUT increases (Fig 6.20).

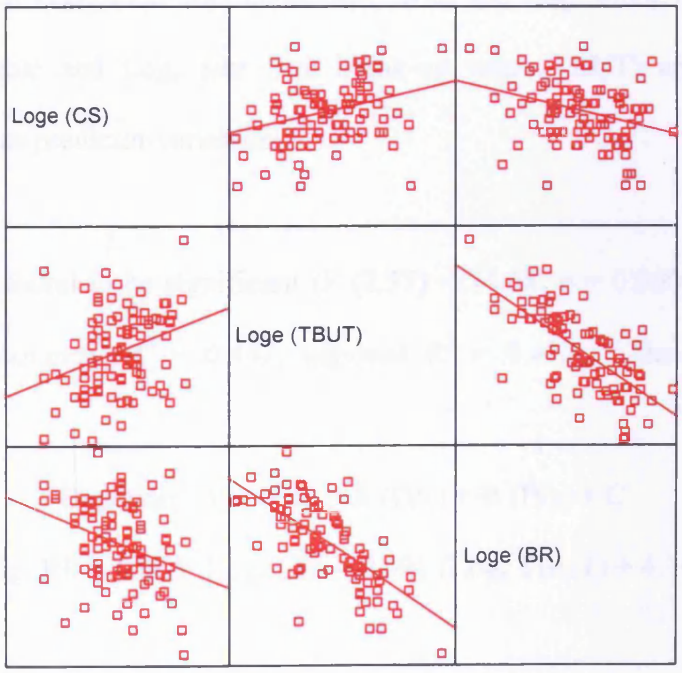


Fig 6.20: Correlation between blink rate, tear film break-up time and corneal sensitivity for the Caucasian Group.

A stepwise multiple regression procedure was also conducted and it only included TBUT in the model as a predictor for blink rate. The adjusted R^2 was 0.469 which is comparable to the linear multiple regression analysis that included TBUT and CS (R^2 = 0.476).

Summarising the results for the Caucasian group, it was found that the blink rate was significantly correlated with corneal sensitivity and tear film break-up time. Linear multiple regression found that the tear film break-up time can be a unique predictor for the blink rate.

6.3.2.2 Asian Group

A multiple linear regression was conducted, with the Log_e blink rate (BR) as the dependent variable and Log_e tear film break-up time (TBUT) and Log_e corneal sensitivity (CS) as predictor variables.

The model was found to be significant ($F(2.37) = 14.68$, $p = 0.000$), and explained 44.2% of the variance ($R^2 = 0.442$, adjusted $R^2 = 0.412$). The model was the following:

$$\text{Dependent Variable} = A(IV_1) + B(IV_2) + C$$

$$Log_e BR = 0.465 (Log_e CS) - 0.691 (Log_e TBUT) + 4.309$$

The beta coefficient for TBUT was significant ($t = -5.312$, $p<0.001$), and the beta coefficient for CS was found to be not significant ($t = 1.85$, $p = 0.072$). TBUT was found to be a unique predictor for BR. The semi-partial correlation between TBUT

and BR removing the linear effects of CS on TBUT was -0.652. Corneal sensitivity did not explain any unique variance.

A 3-D cluster graph illustrated the relationship between BR, TBUT and CS. Only the BR was strongly related to TBUT, indicating that as TBUT increases, the BR decreases. No relationship could be seen between CS and BR (Fig 6.21).

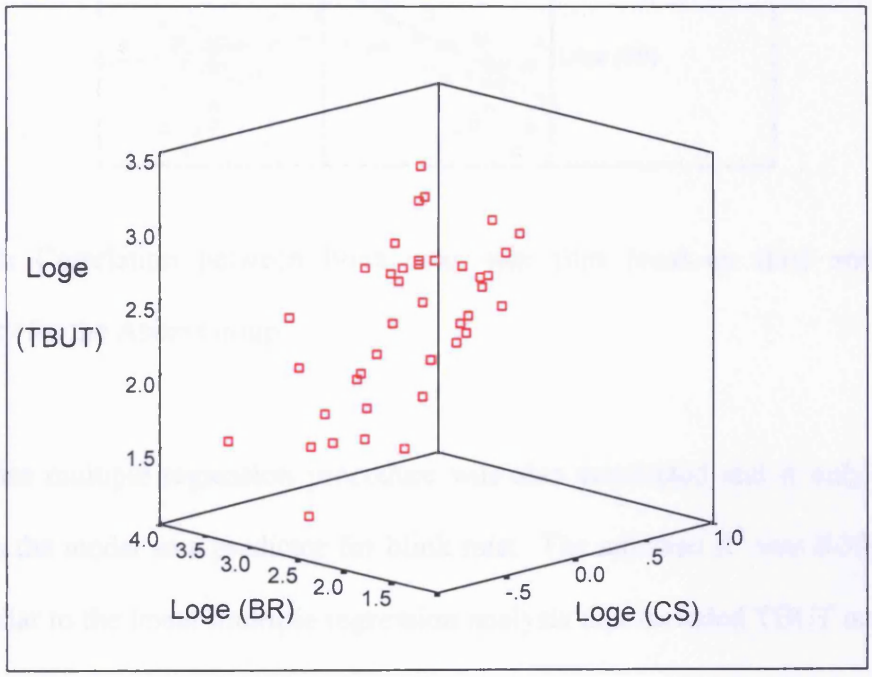


Fig 6.21: 3-D cluster graph illustrating the relationships between blink rate, tear film break-up time and corneal sensitivity for the Asian Group.

All the independent correlations between BR, TBUT and CS for the Caucasian group were also investigated using a matrix scatter plot. A significant strong correlation was found between BR and TBUT (Pearson, $r = -0.625$, $p < 0.001$), but no correlation was found between BR and CS (Pearson, $r = -0.131$, $p = 0.209$), and between CS and TBUT (Pearson, $r = 0.15$, $p = 0.178$) (Fig 6.22).

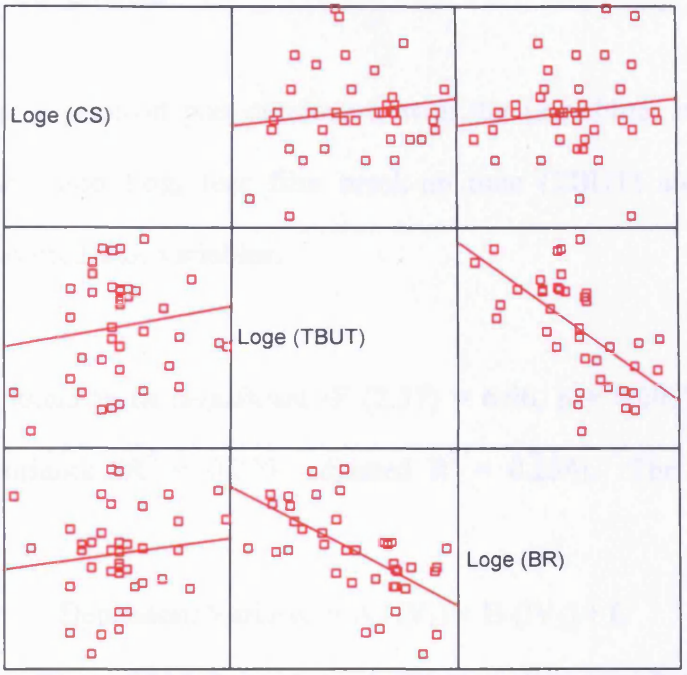


Fig 6.22: Correlation between blink rate, tear film break-up time and corneal sensitivity for the Asian Group.

A stepwise multiple regression procedure was also conducted and it only included TBUT in the model as a predictor for blink rate. The adjusted R^2 was 0.39 which is very similar to the linear multiple regression analysis that included TBUT and CS (R^2 = 0.412).

Summarising the results for the Asian group, it was found that the blink rate was significantly correlated only with tear film break-up time. Linear multiple regression found that the tear film break-up time can be a unique predictor for the blink rate.

6.3.2.3 Chinese Group

A multiple linear regression was conducted, with the Log_e blink rate (BR) as the dependent variable and Log_e tear film break-up time (TBUT) and Log_e corneal sensitivity (CS) as predictor variables.

The model was found to be significant ($F(2.37) = 6.96$, $p = 0.003$), and explained 27.3% of the variance ($R^2 = 0.273$, adjusted $R^2 = 0.234$). The model was the following:

$$\text{Dependent Variable} = A(IV_1) + B(IV_2) + C$$

$$Log_e BR = -0.207 (Log_e CS) - 0.681 (Log_e TBUT) + 3.854$$

The beta coefficients for TBUT was significant ($t = -3.19$, $p = 0.003$), but the beta coefficients for CS was not found to be significant ($t = -0.92$, $p = 0.363$). TBUT was found to be a unique predictor for BR. The semi-partial correlation between TBUT and BR removing the linear effects of CS on TBUT was -0.447. Corneal sensitivity did not explain any unique variance in the model.

A 3-D cluster graph illustrated the relationship between BR, TBUT and CS. BR was related to TBUT, indicating a decrease in the BR as the TBUT increases. Also BR and TBUT was correlated to CS (Fig 6.23), suggesting that as CS decreases the BR decreases and the TBUT increases.

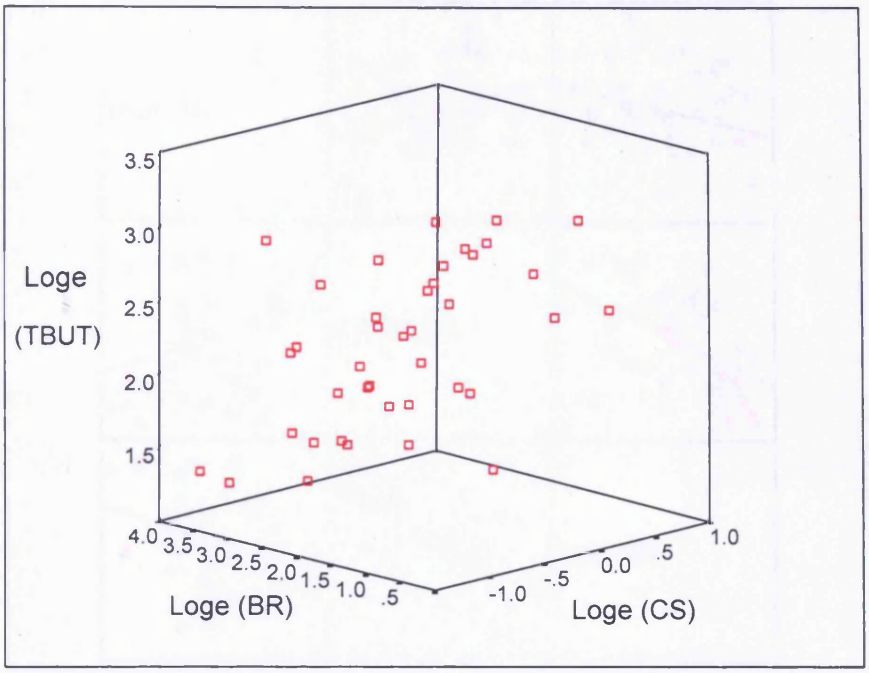


Fig 6.23: 3-D cluster graph illustrating the relationships between blink rate, tear film break-up time and corneal sensitivity for the Chinese Group.

All the independent correlations between BR, TBUT and CS for the Chinese group were also investigated using a matrix scatter plot. A significant strong correlation was found between BR and TBUT, suggesting that as the TBUT increases the blink rate decreases (Pearson, $r = -0.507$, $p < 0.001$). A significant but weak correlation was found between BR and CS (Pearson, $r = -0.271$, $p = 0.045$), indicating that as corneal sensitivity decreases the blink rate is also decreasing, as well as between CS and TBUT (Pearson, $r = -0.291$, $p = 0.000$) indicating that as CS decreases the TBUT increases (Fig 6.24).

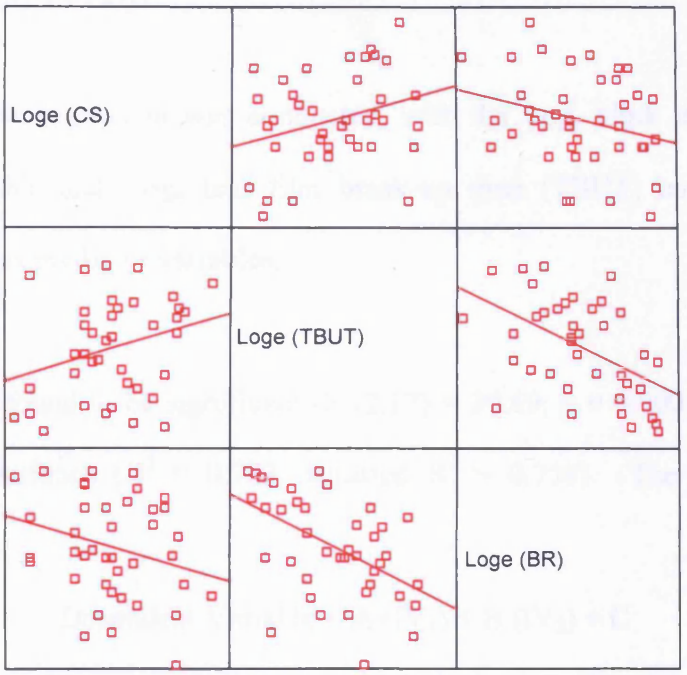


Fig 6.24: Correlation between blink rate, tear film break-up time and corneal sensitivity for the Caucasian Group.

A stepwise multiple regression procedure was also conducted and it only included TBUT in the model as a predictor for blink rate. The adjusted R^2 was 0.237 which is comparable to the linear multiple regression analysis that included TBUT and CS (R^2 = 0.234).

Summarising the results for the Chinese group, it was found that the blink rate was strongly correlated with tear film break-up time and less strongly with corneal sensitivity. Linear multiple regression found that the tear film break-up time can be a unique predictor for the blink rate.

6.3.2.4 African Group

A multiple linear regression was conducted, with the Log_e blink rate (BR) as the dependent variable and Log_e tear film break-up time (TBUT) and Log_e corneal sensitivity (CS) as predictor variables.

The model was found to be significant ($F(2.17) = 30.69$, $p = 0.000$), and explained 78.3% of the variance ($R^2 = 0.783$, adjusted $R^2 = 0.758$). The model was the following:

$$\text{Dependent Variable} = A\,(IV_1) + B\,(IV_2) + C$$

$$Log_e\,BR = -0.707\,(Log_e\,CS) - 0.826\,(Log_e\,TBUT) + 4.49$$

The beta coefficients for TBUT was significant ($t = -5.1$, $p<0.001$), and the beta coefficients for CS was found to be not significant ($t = -1.79$, $p = 0.091$). TBUT was found to be a unique predictor for BR. The semi-partial correlation between TBUT and BR removing the linear effects of CS on TBUT was -0.576. Corneal sensitivity did not explain any unique variance in the model.

A 3-D cluster graph illustrates the relationship between BR, TBUT and CS. Strong relationships can be seen between BR and TBUT, BR and CS, as well as between TBUT and CS (Fig 6.25).

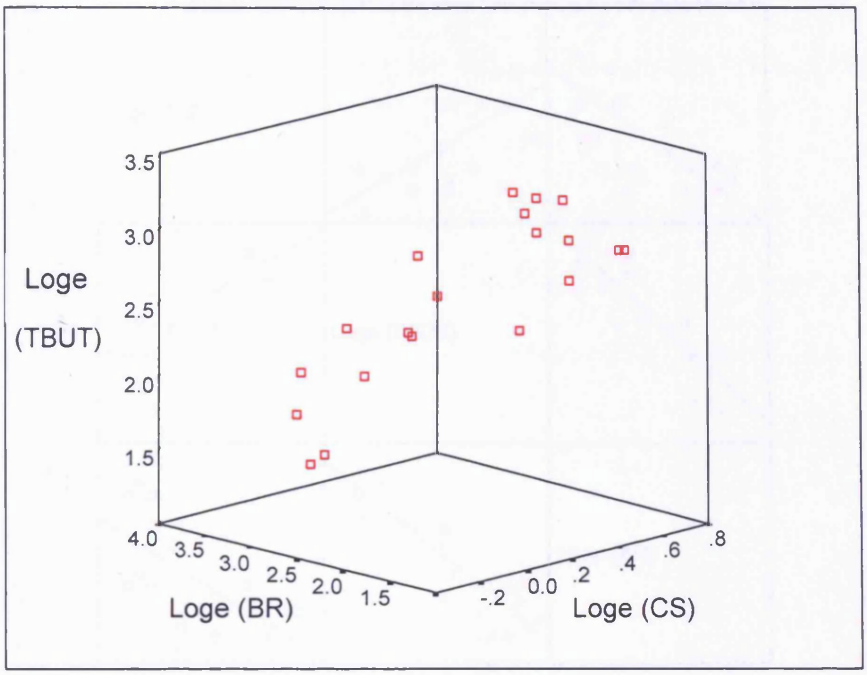


Fig 6.25: 3-D cluster graph illustrating the relationships between blink rate, tear film break-up time and corneal sensitivity for the Chinese Group.

All the independent correlations between BR, TBUT and CS for the Chinese group were also investigated using a matrix scatter plot. A significant strong correlation was found between BR and TBUT (Pearson, $r = -0.861$, $p < 0.001$), as well as between BR and CS (Pearson, $r = -0.672$, $p = 0.001$), indicating that as corneal sensitivity decreases the blink rate is also decreasing. A strong correlation was also found between CS and TBUT (Pearson, $r = -0.59$, $p = 0.003$) (Fig 6.26).

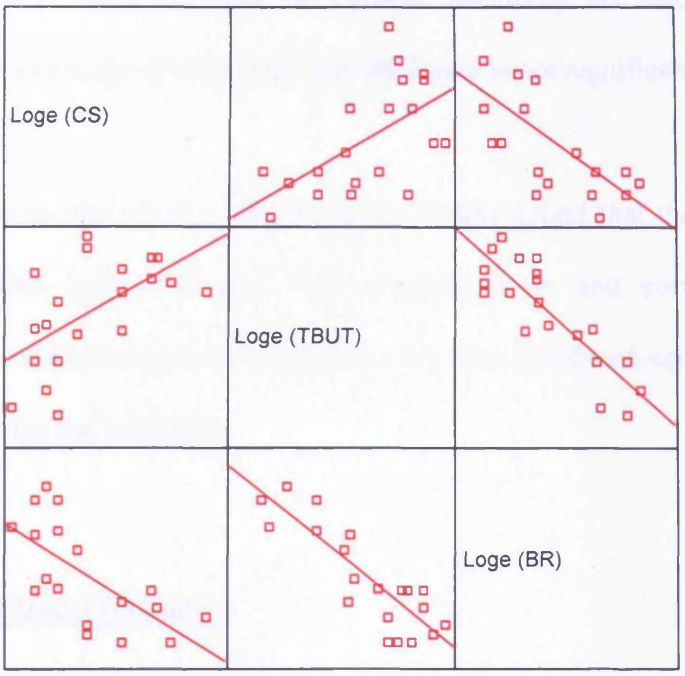


Fig 6.26: Correlation between blink rate, tear film break-up time and corneal sensitivity for the African Group.

A stepwise multiple regression procedure was also conducted and it only included TBUT in the model as a predictor for blink rate. The adjusted R^2 was 0.728 which is comparable to the linear multiple regression analysis that included TBUT and CS (R^2 = 0.758).

Interestingly, although a significant correlation was found between corneal sensitivity and blink rate in the matrix scatter plot, corneal sensitivity was removed in the stepwise multiple regression. This suggests that this relationship is not an important factor and it does not affect the model, although the unstandardised beta coefficient of corneal sensitivity in the model was found to be very strong. This finding indicates that corneal sensitivity is strongly correlated to blink rate, only when the linear effects of tear film break-up time on corneal sensitivity are considered. When the linear

effects of tear film break-up time on corneal sensitivity are removed, then the relationship between corneal sensitivity and blink rate is not significant in the model.

Summarising the results for the African group, it was found that the blink rate was strongly correlated both with tear film break-up time and corneal sensitivity. However, linear multiple regression found that the tear film break-up time is the only unique predictor for the blink rate.

6.3.2.5 *All Ethnic Groups*

A multiple linear regression was conducted, with the Log_e blink rate (BR) as the dependent variable and Log_e tear film break-up time (TBUT) and Log_e corneal sensitivity (CS) as predictor variables.

The model was found to be significant ($F(2.196) = 68.67$, $p = 0.000$), and explained 41.2% of the variance ($R^2 = 0.412$, adjusted $R^2 = 0.406$). The model was the following:

$$\text{Dependent Variable} = A\,(IV_1) + B\,(IV_2) + C$$

$$Log_e\,BR = -0.122\,(Log_e\,CS) - 0.733\,(Log_e\,TBUT) + 4.134$$

The beta coefficients for TBUT was significant ($t = -10.62$, $p < 0.001$), but the beta coefficients for CS was not significant ($t = -1.23$, $p = 0.218$). TBUT was found to be a unique predictor for BR. The semi-partial correlation between TBUT and BR removing the linear effects of CS on TBUT was -0.576. Corneal sensitivity did not explain any unique variance in the model.

A 3-D cluster graph illustrates the relationship between BR, TBUT and CS. Strong relationships can be seen between BR and TBUT, BR and CS, as well as between TBUT and CS (Fig 6.27).

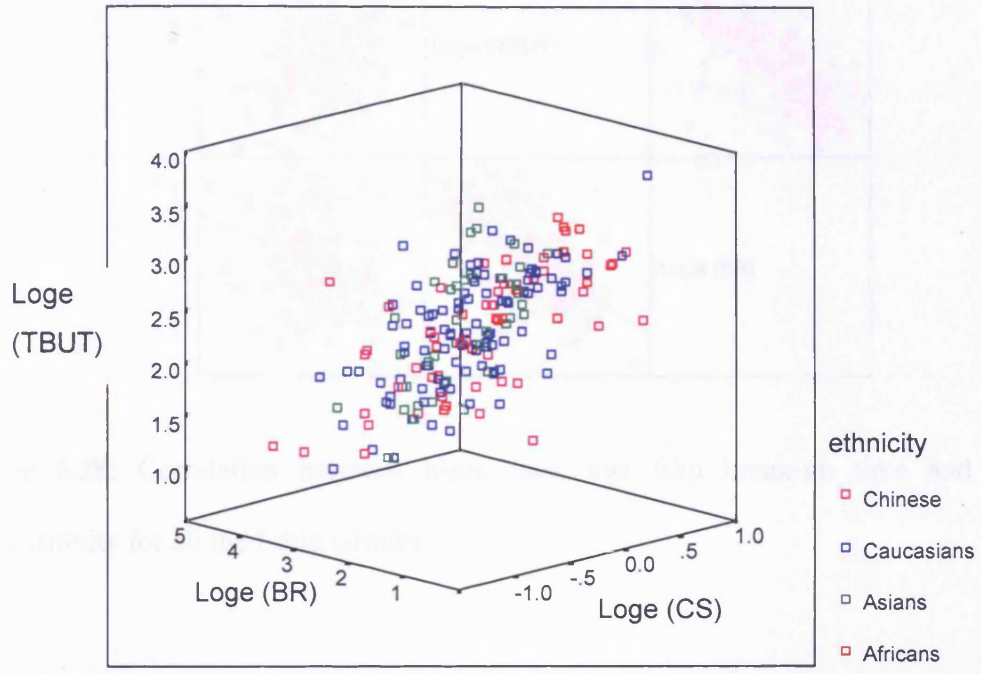


Fig 6.27: 3-D cluster graph illustrating the relationships between blink rate, tear film break-up time and corneal sensitivity for all the Ethnic Groups.

All the independent correlations between BR, TBUT and CS for all the ethnic groups were also investigated using a matrix scatter plot. A significant strong correlation was found between BR and TBUT (Pearson, $r = -0.638$, $p < 0.001$), as well as a less strong correlation between BR and CS (Pearson, $r = -0.271$, $p = 0.000$), and CS and TBUT (Pearson, $r = -0.324$, $p = 0.000$) (Fig 6.28).

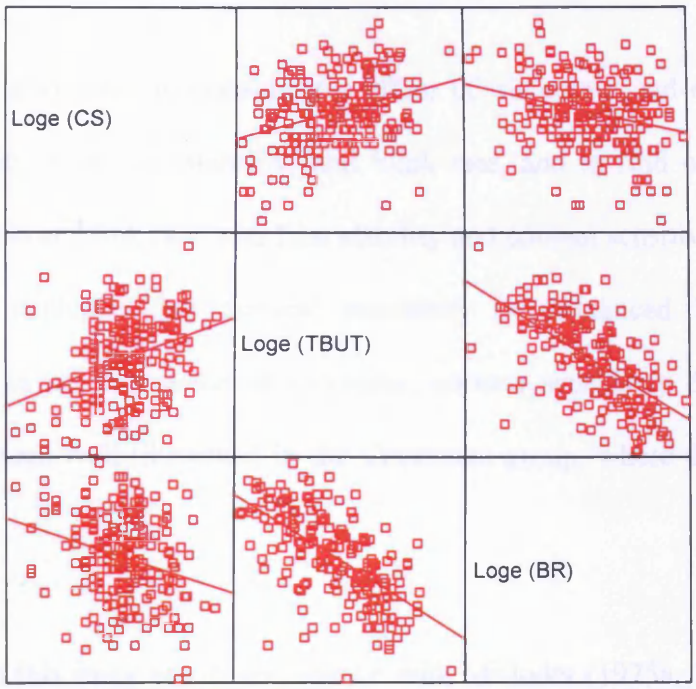


Fig 6.28: Correlation between blink rate, tear film break-up time and corneal sensitivity for all the Ethic Groups.

A stepwise multiple regression procedure was also conducted and it only included TBUT in the model as a predictor for blink rate. The adjusted R^2 was 0.404 which is comparable to the linear multiple regression analysis that included TBUT and CS (R^2 = 0.406).

Summarising the results for all the ethnic groups, it was found that the blink rate was correlated both with tear film break-up time and corneal sensitivity. Corneal sensitivity was significantly correlated to tear film break-up time. However, linear multiple regression found that the tear film break-up time is the only unique predictor for the blink rate.

6.4 Discussion

The aim of this study was to consider the effect of iris colour and ethnic origin on corneal sensitivity, tear film stability, and blink rate, and to find out any possible relationships between blink rate, tear film stability and corneal sensitivity. The results provide strong evidence that corneal sensitivity is influenced by iris colour, suggesting that as iris pigmentation increases, corneal sensitivity decreases. This phenomenon is seen well illustrated in the Caucasian group, where there is a variety of iris colour.

The results from this study are in accordance with Millodot (1975a, 1976a) and Tota and La Marca (1982), who found that people with blue eyes have more sensitive corneas than those with brown eyes. For these earlier studies, corneal sensitivity was measured using the Cochet-Bonnet Aesthesiometer, which stimulates the mechanical nerve receptors of the corneal epithelium. The fact that the same variation in corneal sensitivity with different iris pigmentation was found for both the mechanical and cold sensors of the corneal epithelium is a good indicator that the different nerve receptor types in the corneal epithelium are affected by the same factors.

A variation in corneal sensitivity was also found between different ethnic groups, with corneal sensitivity decreasing from Asians and Chinese, to Caucasians and then Africans (Fig 6.3). These results are reasonable, since the dark brown eyes of Africans are more pigmented in comparison to the dark brown eyes of Chinese, Asians, and Caucasians. The results also partially agree with the findings of Millodot (1975a) who reported that sensitivity diminishes further in non-whites with darker pigmented eyes.

These differences between the previous studies and the current study might be due to the different methods used to assess corneal sensitivity, as well as to the different ways of classifying iris colour. In this study the Non-Contact Corneal Aesthesiometer was used to assess corneal sensitivity and the Iris Color Classification System (Seddon et al., 1990) was used to classify each subject's iris colour. In contrast, the previous studies (Millodot 1975a, 1976a; Tota and La Marca, 1982) used the Cochet-Bonnet Aesthesiometer and separated the subjects into different iris colour group according to their own subjective criteria.

The reduction in corneal sensitivity with darker pigmentation is not easily explained. It has been suggested that melanin in the iris might be correlated to the amount of neuro-melanin in areas of the central nervous system (Hale et al., 1980; Martin and Safran, 1988). It may therefore be conceivable that differences in corneal sensitivity between light and dark-eyed subjects arise from differences in central nervous system function.

To assess whether skin sensitivity was also affected by a similar neuro-melanin influence on the central nervous system, and whether this is responsible for the variation in corneal sensitivity associated with iris pigmentation, measurements of skin thermal sensitivity were assessed. No variation in skin sensitivity was found with increasing iris pigmentation, indicating that skin sensitivity is not involved in the corneal sensitivity variation, but skin sensitivity was found to be influenced by ethnic origin, with skin sensitivity decreasing from Caucasians, to Chinese, to Asians, to

Africans. This pattern appears to follow an apparent progression of increasing skin pigmentation.

The study also found that tear film stability was not influenced by different iris pigmentation, except for eyes with dark irises which tended to maintain a more stable tear film. The results contradict Patel et al (1991) who also investigated the effect of iris colour on tear film stability. They found that people with blue eyes have a more stable tear film than those with brown eyes. This difference in result may be due to the small number of subjects (20 subjects) who participated in the Patel study, or to the different instrumentation (Baush and Lomb keratometer) used to assess tear film stability.

The results from this study, that there is no relationship between iris colour and tear film stability, are more likely to be correct than those from Patel et al since there is no obvious explanation for any relationship to actually occur. However, there may be biochemical or biophysical differences in the tears between eyes with light or dark irises. In particular, dark eyes may have a thicker lipid layer, which produces a lower evaporation from the tear film and higher tear film stability (Patel et al., 1995).

Tear film stability was influenced by ethnic origin, but the change did not follow the results of Patel et al (1995), who found the tear thinning time to be increasing from Chinese, to Africans, to Indians, to Caucasians. However, the difference is only in swapping the African and Asian group around in the order. The common finding of both sets of results was that tear stability was lowest in Chinese eyes and highest in

Caucasians eyes. This is supported by the results of other researchers (Cho et al., 1992, 1993; Brown et al., 1993; Cho and Yap, 1993).

The morphological uniqueness of the Chinese palpebrae may influence the way in which the pre-corneal tear film is established during and after a blink, thus producing a physically different pre-corneal tear film in terms of relative organization and structure, thus producing a lower tear film stability. Also, the reported large differences in the biochemistry of Meibomian gland secretions between Caucasians may be account for the higher values of tear film stability found for this group (Tiffany, 1978; Nicolaides et al., 1981). These differences, in conjunction with the quality and secretory rates of the major tear components, may be the key factors for differences between the ethnic groups.

Turning now to the blink rate, no effect from ethnic origin was found on the rate of blinking, although subjects with dark brown irises had a tendency to blink less than those with lighter coloured irises. Since subjects with dark brown irises also have a more stable tear film, this suggests that there is a relationship between the two factors.

Indeed, a strong and significant correlation between TBUT and blink rate was found for every iris colour grade of each ethnic group. This significant negative correlation between the two variables suggests that people with low tear film stability blink more frequently. Multiple linear regression showed that tear film stability is a unique predictor for blink frequency. Taking this hypothesis one step factor, it suggests that periodic blinking is dependent not only on central control, but also on the stability of the pre-corneal tear film. It may be that blink frequency is adjusted by tear stability to

promote reformation of the tear film layer, in order to provide a sharp optical image, protect the exposed corneal epithelium, and moisten the ocular surface.

These findings agree with the results of Yap (1991) and Abdulmunem (2001) who found a strong relationship between TBUT and blink frequency. However, other investigations (Prause and Norn, 1987; Collins et al., 1989; Patel et al., 1991b; Yolton et al., 1994) found a weak correlation between TBUT and blink rate. The different results for these studies may be due to the experimental method used to record the blink rates. The psychological and perceptual factors that affect blinking were minimised in the present study. Blink rate was measured while subjects watched a film. Although performing such a task has the potential to alter a subject's attention state and thus their blink rate (Goldstein et al., 1985), the subject matter chosen limited this effect. In any case, by asking all subjects to undergo the same viewing conditions, the test could be standardised.

Blink rate was also correlated with corneal sensitivity to determine whether corneal sensitivity is a factor in physiological blinking. It has been suggested previously that tear thinning during the inter-blinking interval produces a localised cooling of the tear film which can be detected by the temperature sensitive corneal nerves before a full break-up occurs. Using univariate analysis, a significant, but weak correlation was found between corneal sensitivity and blink rate, suggesting that corneal sensitivity is involved in the blink mechanism. Previous studies anaesthetised the cornea and found a reduced blink rate (Collins et al., 1989; Moore and Kardon, 1997). It was proposed that blink frequency is primarily determined by the total sensory input of the two eyes, which is then modulated by central processing. In this study the strongest

correlations were found only for Caucasian (R^2 = 0.476) and Africans (R^2 = 0.533) subjects with iris colour grade 5, and they illustrate the pattern that subjects with higher corneal sensitivity blink more often. This function supports the hypothesis that the corneal nerves are detecting early changes in the tear film triggering an early blink. More sensitive eyes are better able to detect these changes, and so trigger more blinks.

Indeed, using multivariate analysis, it was found that corneal sensitivity was strongly correlated with blink frequency in the Caucasian, Chinese and African Group, but it was not found to be the unique predictor for blink frequency in the linear regression model. These findings are important and indicate that the role of corneal sensitivity in blinking is dependent on the effects of tear film stability. A very good example can be seen in the African group, who experience low sensitivity and stable tear film. Both of these factors found to be significant in the linear regression model, but stepwise regression removed the relationship between corneal sensitivity and blink frequency. When the linear effects of tear film stability on corneal sensitivity are taken into account in the model, the correlation between corneal sensitivity and blink frequency is significant in the model. However, when the linear effects of tear film stability on corneal sensitivity are removed, the correlation between corneal sensitivity and blink frequency is not significant, hence stepwise regression removes corneal sensitivity from the model. These findings assist in the hypothesis that corneal nerves are detecting early changes in the tear film.

In conclusion this study found corneal sensitivity to be influenced by iris colour and ethnic origin. As iris pigmentation gradually increases, corneal sensitivity gradually

decreases. Skin sensitivity was not found to account for these variations, suggesting that variation in corneal sensitivity arises from differences in the central nervous system and not from skin sensitivity variations. Tear film stability was not found to be influenced by iris colour. Dark eyes appeared to maintain a more stable tear film, producing lower blink rates. Statistical correlations found that stability of the tear film is a key factor in the blink stimulus. Corneal sensitivity is involved in the mechanism controlling normal involuntary blinks, by detecting changes in the tear when it destabilises between blinks.

7. An Investigation of the Ocular Surface Sensory Trigger for Blinking

7.1 Introduction-Purpose

The pre-ocular tear film is a dynamic structure that is inherently unstable. Frequent blinking is necessary to prevent drying of the ocular surface. Although blinking plays an important role in the maintenance of the integrity of the ocular surface, by contributing to the maintenance of eye surface humidity, drainage of tears, expression of lipids from Meibomian glands, and spreading of tear lipids across the pre-corneal tear film (Holly, 1980, 1985; Doane, 1981; Tiffany, 1985; Korb et al., 1994), the factors involved in the control of spontaneous blinking are not well understood, although it is likely to involve central and peripheral triggers.

There have been many investigations of blinking and the different factors that influence it, as discussed in the Introduction. Stern et al (1984) reported that blink activity is under cortical control and thus is affected by psychological and perceptual factors such as attention, level of concentration, stress and anxiety. In addition to the cortical control, physiological factors have also been associated with blinking. Prause and Norn (1987) found a significant but low negative correlation (r =-0.33) between blink rate and tear film break-up time. Yap (1991) found a stronger negative correlation (r =-0.69) between blink rate and TBUT supporting the hypothesis that tear film break-up is a stimulus for normal involuntary blinking. Collins et al (1989) suggested that corneal sensitivity must play an important role in the mechanism mediating normal involuntary blinks by demonstrating a reduction in blink rate when corneal sensation was blocked using a local anaesthetic. Holly (1973) suggested that

dry spot formation triggers an involuntary blink because it irritates the nerve endings, but the mechanism for this association was not clarified. Mori et al (1997) also suggested that evaporation-mediated cooling, which occurs during the process of tear break-up, may be detected by thermo-sensitive corneal afferents and provide the signal for a blink to reform the tear film.

To investigate the hypothesis that changes in the local tear film stability trigger a blink, this study will focus at several ocular surface conditions and their relationships with blink frequency.

1) Tear Film Stability: To assess the relationship between tear break-up time and blink rate. The hypothesis being tested is that, if a breakdown in the tear-film is the "trigger to blink", then subjects with a lower tear-break-up time will have a higher blink-rate.

2) Corneal Sensitivity: To assess the relationship between corneal sensitivity and blink rate. The first hypothesis being tested is that, if any changes in the tear film prior to break-up are detected by the sensory nerve endings in the cornea, then subjects with a higher corneal sensitivity will have a higher blink rate. The second hypothesis being tested is that, if any loss in corneal sensitivity (produced by local anaesthetics) leads to any tear-film mediated "trigger to blink" going undetected, subjects will have a reduced blink rate.

3) Temperature of the Anterior Ocular Surface: Using the thermal imaging camera, analysis of the change in ocular surface temperature in the inter-blink period will be

made. As the tear-film gradually destabilises following a blink, the ocular surface temperature also decreases. The hypothesis being tested is that subjects with a greater reduction in ocular surface temperature will have a higher blink-rate, if a localised temperature change is the "trigger to blink".

4) Evaporation of the Anterior Ocular Surface: To assess the relationship between blink rate and ocular surface evaporation rate. The hypothesis being tested is that, if early evaporative tear loss is the "trigger to blink", then subjects with a higher evaporation rate will have a higher blink rate.

7.2 Methods

Twenty Caucasian subjects (9 males, 11 females; mean age, 22.85±2.21 years; range, 19-28) were recruited from the student population of Cardiff University. The age range was between 20-40 years, as corneal sensitivity (Millodot, 1977a, 1984; Murphy et al., 2004), corneal temperature (Alio and Padron, 1982a; Girardin et al., 1999; Horven, 1975; Morgan et al., 1999), and tear film stability (Patel et al, 1989) have been found to decrease with age. Subjects were excluded if they were contact lens wearers, pregnant women or women during the premenstruum, menstruation, or ovulating period, as corneal sensitivity is depressed in these situations. Subjects with any ocular or systemic pathology known to affect corneal sensitivity were also excluded. Ethical approval was obtained from the School of Optometry and Vision Sciences Research Ethics Committee. After explanation of the purpose of the study, subjects were asked to sign a consent form prior to participating. Subjects were also reminded that they could withdraw from the study at anytime.

All the subjects taking part in this study had a minimum tear film break-up time of 8 seconds, for comfort purposes, as the assessment of dynamic temperature change across the ocular surface area required 8 seconds without the subject blinking.

Subjects were asked to attend the laboratory on two different days and each visit lasted for one hour. All measurements were made after 12pm to avoid any possible diurnal bias in corneal sensitivity (Millodot, 1972; du Toit et al., 2003; Chapter 4), tear film stability (Patel et al., 1988), corneal temperature (du Toit et al, 1998), or tear evaporation rate (Tomlinson and Cedarstaff, 1992). Humidity and room temperature were maintained at stable levels during the experiment (room temperature 24.42°C ± 1.21; room humidity 31.93% ± 4.01%), since humidity will affect tear evaporation from the ocular surface and, in turn, the ocular surface temperature (Schwartz, 1965; Mapstone, 1968b; Kolstad, 1970; Freeman and Fatt, 1973; Horven, 1975; Hata et al., 1994, 1995).

All ocular measurements were made on the *right eye only*. An initial baseline set of measurements was made at the first visit: blink rate, corneal sensitivity, ocular surface temperature, tear evaporation rate, and tear film stability using sodium fluorescein. Tear evaporation and ocular surface temperature were then re-measured to consider the effect of fluorescein on tear film stability.

At the second visit measurements of corneal sensitivity, ocular surface temperature, and tear evaporation rate were taken to create baseline levels for the second visit. 20µl of 0.5% proxymetacaine hydrochloride (Minims, Chauvin Pharmaceuticals Ltd, UK) was then instilled in both eyes. Two minutes-after instillation ocular surface

temperature and evaporation rate were re-measured to consider the effect of proxymetacaine hydrochloride instillation. At 20 minutes post-instillation, the ocular surface temperature and tear evaporation rate were re-measured for third time.

7.3 Results

The distributions of the measurements of blink rate, inter-blink interval, corneal sensitivity, tear film break-up time, ocular surface temperature, and tear evaporation rate were assessed for normality (Shapiro-Wilk test), using the SPSS11 Statistical Software Program (Lead Tools, Lead Technologies, Inc). Normality testing found data both normally and not normally distributed for both visits. The data was log transformed, but normality testing again found data to be not normally distributed, and so non-parametric statistical tests were used for statistical analysis (Prism: GraphPad Software Inc, San Diego).

Table 7.1: Median/ Interquartile Range (IR) and Mean (± Standard Deviation) of all the measurements taken both Visit 1 and Visit 2.

Visit 1

	CS (mbars)	TBUT (secs)	Baseline Evap ($g/m^2/h$)	Evap after Fluor ($g/m^2/h$)	BaselineTemp (°C)	Baseline Temp Fluor (°C)
Median/ IR	0.52/ 0.37-0.75	15.17/ 9.49-19.24	69.47/ 50.72- 87.66	85.42/ 65.25-92.83	35.15/ 34.80-36.00	35.45/ 34.94-36.15
Mean±SD	0.61±0.32	15.96±7.31	76.21±33.37	85.93±27.3	35.46±0.91	35.55±0.7

Temp Cooling after 8 secs (°C)	Temp Cooling after 8 secs Fluor (°C)	Temp Change (1/2 life) Fluor (secs) after 8 secs	Temp Change (1/2 life) (secs) after 8 secs	Blinks/min	IBI (secs)
0.81/ 0.5-1.02	0.69/ 0.49-0.8	0.82/ 0.69-1.44	0.95/ 0.68-1.31	14.7/ 6.3-18.9	4.11/ 3.16-9.51
0.83±0.41	0.75±0.49	0.98±0.5	0.99±0.39	14.05±7.64	6.06±3.9

Visit 2

	CS (mbars)	Baseline Evap ($g/m^2/h$)	Evap 2min Anaes ($g/m^2/h$)	Evap 20 min Anaes ($g/m^2/h$)	Baseline Temp (°C)	Temp 2 min Anaes (°C)	Temp 20 min Anaes (°C)
Median/ IR	0.45/ 0.35-0.67	60.43/ 38.43-78.11	69.63/ 56.97-90.95	73.49/ 65.45-98.08	36.18/ 35.61-36.59	36.04/ 35.63-36.83	35.89/ 35.43-36.44
Mean±SD	0.56±0.29	61.01±28.81	74.01±22.1	85.35±28.24	35.97±0.87	36.02±0.95	35.81±0.9

Temp Cooling after 8 secs (°C)	Temp Cooling 2 min Anaes (°C)	Temp Cooling 20 min Proxy (°C)	Temp Change (1/2 life) (secs) after 8 secs	Temp Change (1/2 life) (secs) 2min Anaes after 8 secs	Temp Change (1/2 life) (secs) 20 min Anaes after 8 secs
0.67/ 0.56-0.72	0.69/ 0.63-0.96	0.80/ 0.61-0.9	0.88/ 0.5-1.17	1.12/ 0.57-1.34	0.84/ 0.76-0.96
0.73±0.32	0.81±0.36	0.85±0.34	0.94±0.49	1.04±0.43	0.88±0.28

Blinks/Min 15 min Anaes	IBI (secs)
6.90/ 4.36-8.4	9.35/ 7.16-13.71
6.89±3.55	11.67±6.93

7.3.1 Visit 1

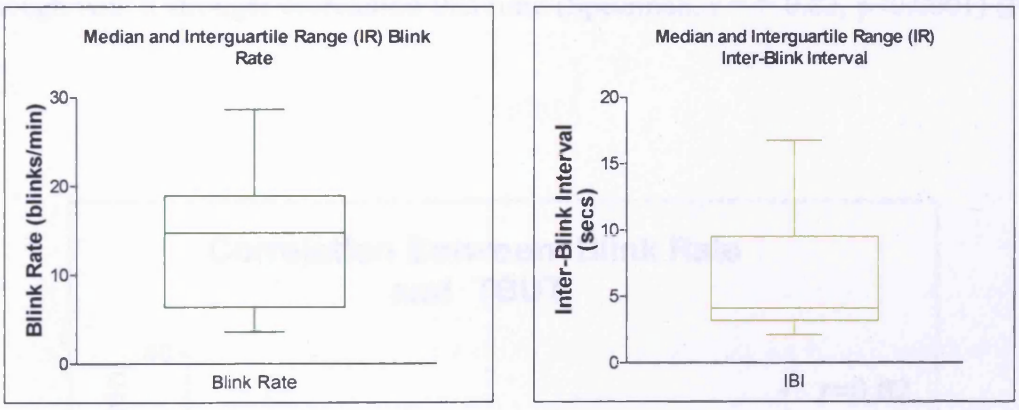
7.3.1.1 Blink Rate or Inter-Blink Interval

Blink frequency can be analysed in two ways:

1) Blink Rate (BR) – the average number of blinks per minute.

2) Inter-Blink Interval (IBI) – the average time between blinks.

It follows that a subject with a high blink rate will have a low inter-blink interval. In contrast to the data in the previous chapter, blink frequency for this study was analysed in both formats.



(a) (b)

Fig 7.1: Median and interquartile range (IR) for blink rate (a) and inter-blink interval (b).

The median/ interquartile range (IR) for the BR was 14.7/ 6.3-18.9 blinks/min, and for the IBI was 4.11/ 3.16-9.51 secs. Both analysis methods provide a measure of the blink frequency. However, while the BR may appear a more intuitive measure, it is affected by the time taken to collect the data. For this study, the BR was measured over five minutes, which effectively reduces the number of measurement, for

calculating the average BR, to five. In contrast, the IBI measurement has an average of 14 measurements per minute, or 70 minutes in total (for the full 5 minute measurement time). This sampling error difference is demonstrated in the size of the interquartile range, where the BR interquartile range is 14.7/ 6.3-18.9 blinks/min and the IBI interquartile range is 4.11/ 3.16-9.51.

The improved quality of the IBI data over the BR data can also be illustrated when comparing blink frequency and tear stability. The strong correlation between blink frequency and tear film stability was shown in the previous study (6.3.8). For this study, the same correlation was found between tear break-up time and blink rate, although with a stronger correlation this time (Spearman, r = = 0.82, p<0.0001) (Fig 7.2).

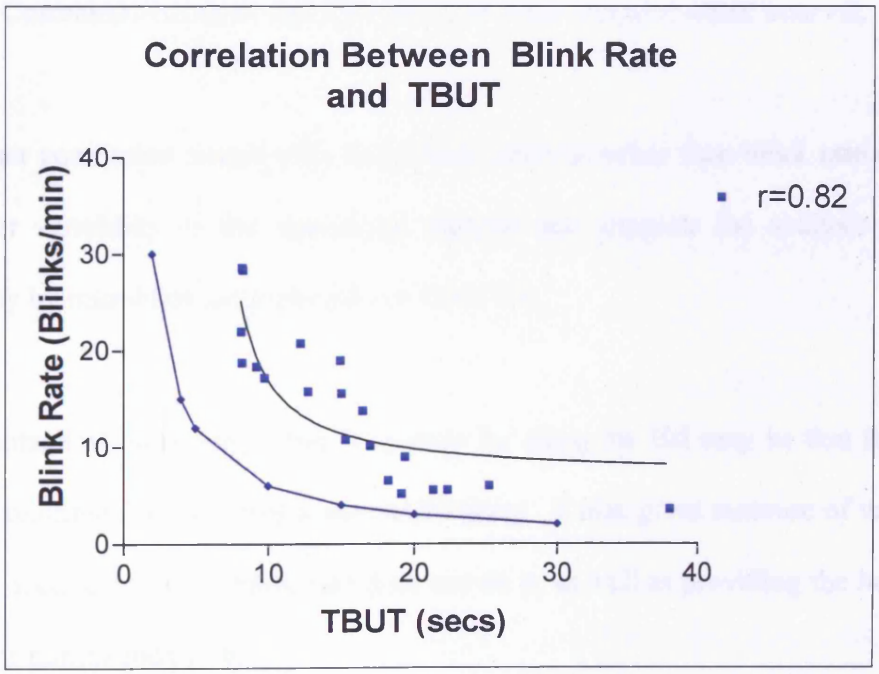


Fig 7.2: Correlation between the tear film break-up time (TBUT) and blink rate (BR). The blue line represents the line that matches blink rate with tear film break-up time, indicating that blinking occurs before a fill break-up time.

However, if tear film break-up time is correlated with the IBI, a stronger relationship is found (Spearman, r = 0.926, p<0.0001) (Fig 7.3).

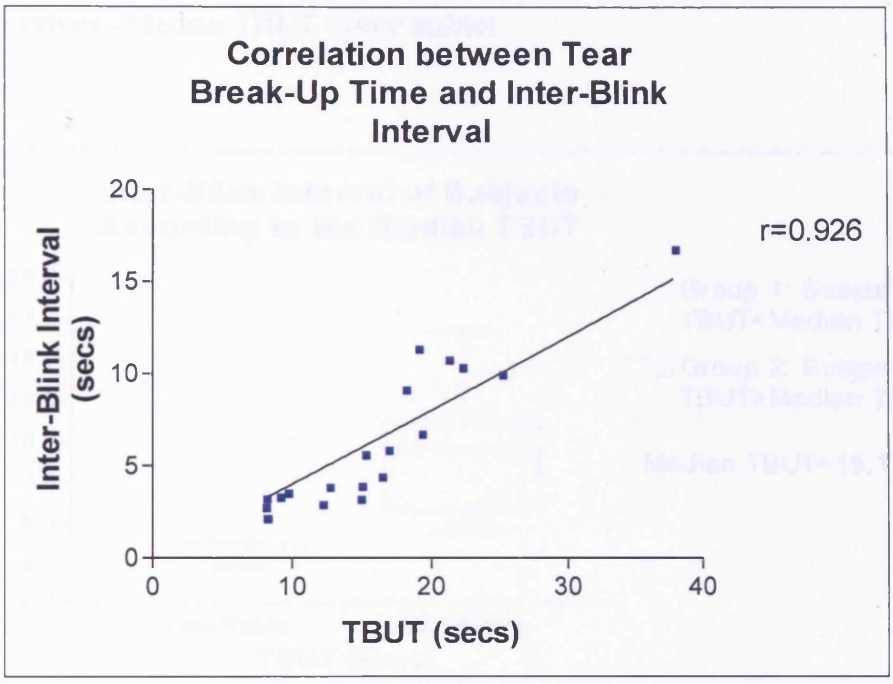


Fig 7.3: Correlation between tear film break-up time and inter-blink interval.

The closer correlation found with inter-blink interval rather than blink rate is due to the lower variability of the inter-blink interval and supports the analysis of blink frequency by inter-blink interval and not blink rate.

An advantage of measuring blink frequency by using the IBI may be that it reduces the time required for recording a subject blinking. It also gives measure of variability and thus accuracy that the blink rate does not do it, as well as providing the number of blinks per minute indirectly.

Before leaving this section it is important to note the finding that subjects with a more stable tear film blink less frequently than those subjects with a less stable tear film,

emphasising that periodic blinking is associated with tear film stability. Another way of expressing this relationship is to divide the subjects into two groups according to the mean tear film break-up time. Group 1: values <Median TBUT (less stable) and Group 2: values >Median TBUT (more stable).

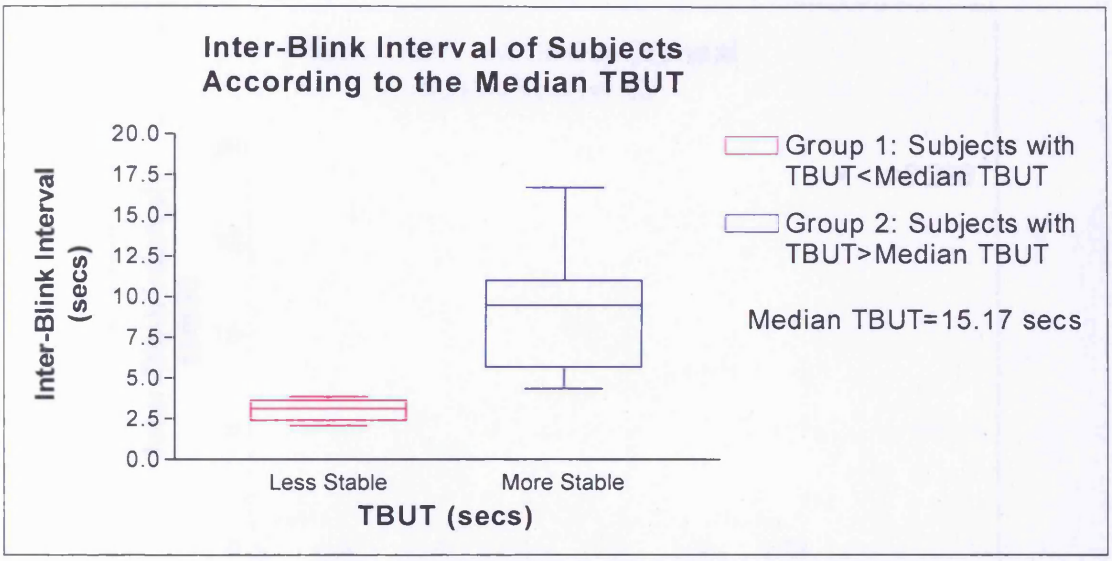


Fig 7.4: Comparison of inter-blink interval for subjects grouped according to the median tear film break-up time. Group 1: values lower than the median TBUT; Group 2: values higher than the median TBUT.

The difference between the mean TBUT of the two groups was statistically significant (Mann-Whitney-test, $p<0.0001$), but the correlation between TBUT and IBI was maintained for both group 1 (Spearman, $r = 0.712$) and Group 2 (Spearman, $r = 0.794$).

7.3.1.2 Correlation between Corneal Sensitivity and Blink Rate

The strong correlation between blink frequency and tear film stability suggests that the changes in the tear film prior to break-up may have a role in triggering the next

involuntary blink. If these changes in the tear film are detected by the sensory nerve endings in the cornea, then subjects with a higher corneal sensitivity will have a higher blink frequency. However no such relationship was found between corneal sensitivity and IBI (Spearman, r = 0.236, p = 0.315) (Fig 7.5).

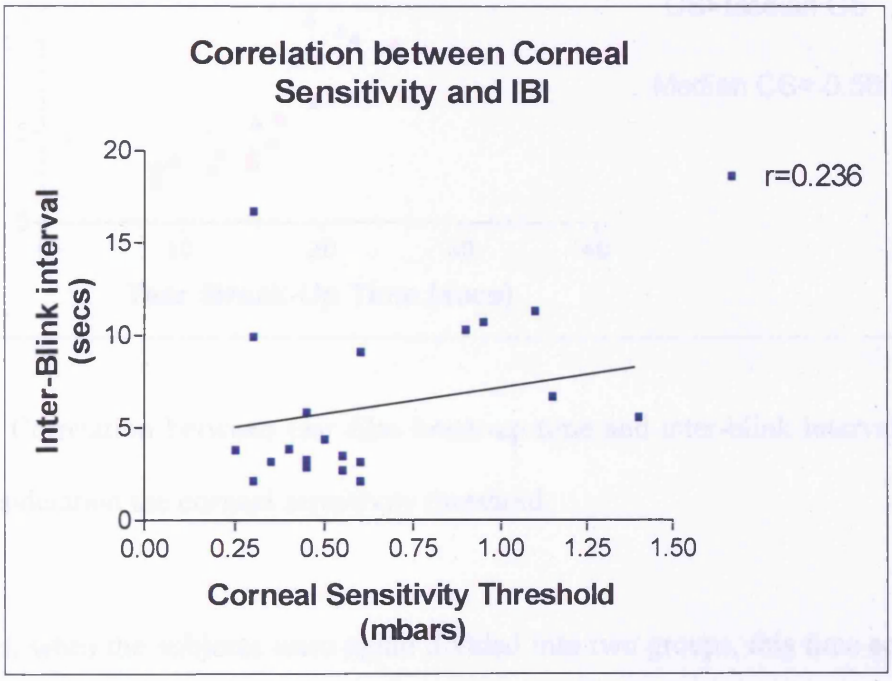


Fig 7.5: Correlation between corneal sensitivity threshold and inter-blink interval.

Further investigation of the potential role for corneal sensory nerves in detecting changes in tear film stability prior to break-up was considered by correlating TBUT with the IBI, taking into consideration the corneal sensitivity of each subject. Using the two corneal sensitivity threshold groups described earlier, a graph was produced (Fig 7.6) but no effect from corneal sensitivity was determined.

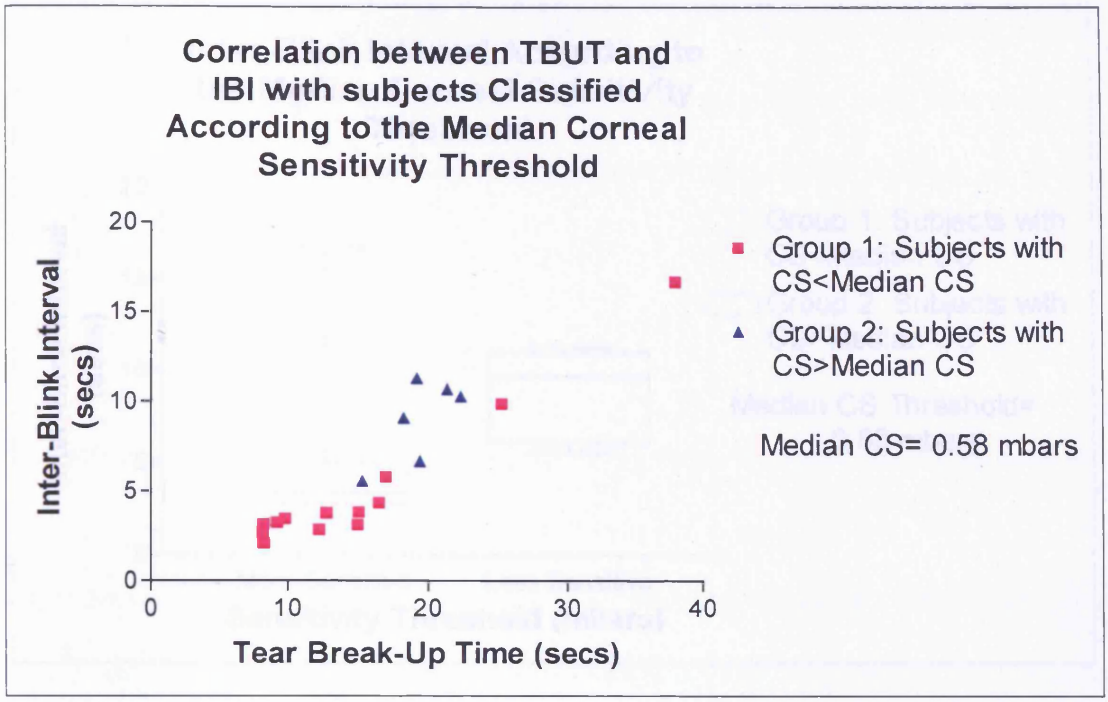


Fig 7.6: Correlation between tear film break-up time and inter-blink interval, taking into consideration the corneal sensitivity threshold.

However, when the subjects were again divided into two groups, this time according to the median value of corneal sensitivity threshold, Group 1: values <Median corneal sensitivity threshold (more sensitive) and Group 2: values >Median corneal sensitivity threshold (less sensitive), a difference in IBI was found (Fig 7.7).

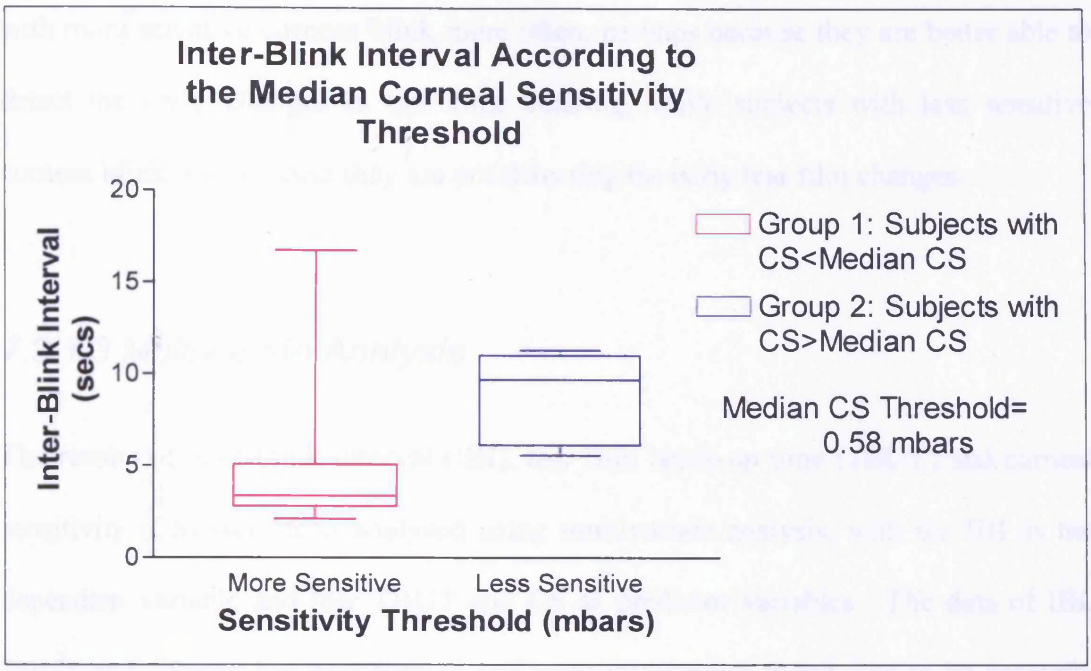


Fig 7.7: Division of subjects into two groups according to the median corneal sensitivity threshold. Group 1: values lower than the median corneal sensitivity; Group 2: values higher than the median corneal sensitivity.

The median/interquartile range IBI for Group 1 was 3.38/ 2.79-5.09 secs and for Group 2 was 9.7/ 6.13-11.03, which was statistically different (Mann-Whitney test, p= 0.009), demonstrating that subjects with more sensitive corneas have a shorter IBI. Correlation between corneal sensitivity and IBI for Group 1 ($r = -0.45$, $p = 0.111$) and Group 2 ($r = 0.429$, $p = 0.419$) was not significant.

The lack of statistical significance may be due to the small number of subjects (6 subjects) in Group 2 or to the low media corneal sensitivity threshold of the 20 subjects who participated (0.58/ 0.37-0.75 mbars).

From these results the role of corneal sensitivity, as a factor in the blink mechanism, appears to be revealed best in those subjects with less sensitive corneas. Subjects

with more sensitive corneas blink more often, perhaps because they are better able to detect the early changes in tear film thinning, while subjects with less sensitive corneas blink less because they are not detecting the early tear film changes.

7.3.1.3 Multivariate Analysis

The results of inter-blink interval (IBI), tear film break-up time (TBUT) and corneal sensitivity (CS) were also analysed using multivariate analysis, with the IBI as the dependent variable and tear TBUT and CS as predictor variables. The data of IBI, TBUT and CS was log transformed and normality testing found data to be normally distributed, allowing the use of linear multiple regression.

The model was found to be significant ($F(2.17) = 37.08$, $p<0.001$), and explained 81.4% of the variance ($R^2 = 0.814$, adjusted $R^2 = 0.792$). The model was the following:

$$\text{Dependent Variable} = A(IV_1) + B(IV_2) + C$$

$$\text{Log}_e(BR) = 0.067(\text{Log}_e CS) - +1.34(\text{Log}_e TBUT) - 1.95$$

The beta coefficients for TBUT was significant ($t = 8.61$, $p<0.001$), but the beta coefficients for CS was not found to be significant ($t = 0.484$, $p = 0.634$). TBUT was found to be a unique predictor for BR. The semi partial correlation between TBUT and BR removing the linear effects of CS on TBUT was 0.902. Corneal Sensitivity did not explain any unique variance.

A 3-D cluster graph can illustrate the relationship between IBI, TBUT and CS. BR is strongly related to TBUT, indicating that as the TBUT increases, the BR decreases. A relationship can also be seen between CS and BR, indicating that as CS decreases, the BR decreases (Fig 6.20).

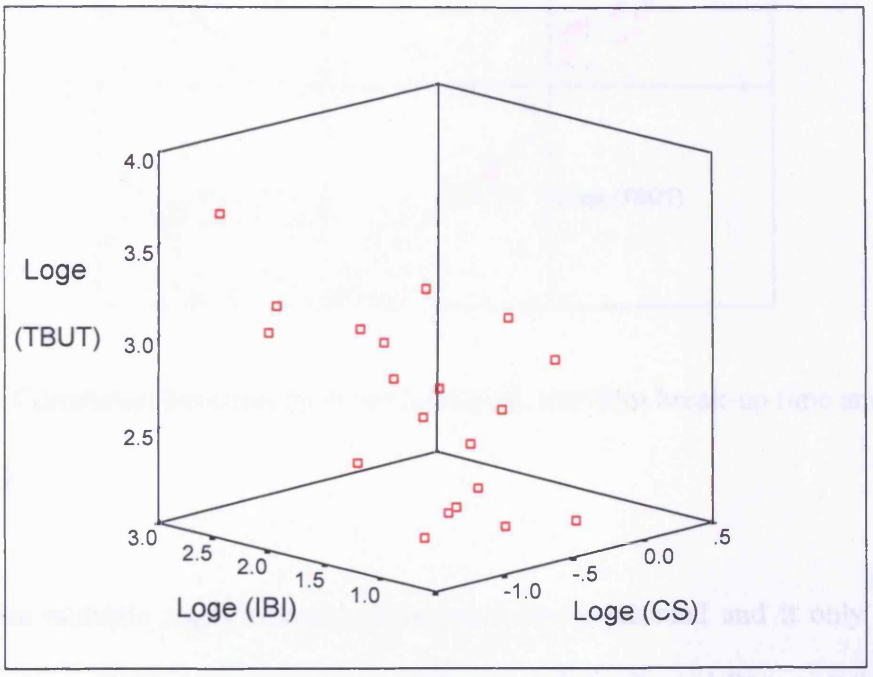


Fig 6.20: A 3-D cluster graph illustrating the relationships between inter-blink interval, tear film break-up time and corneal sensitivity.

All the independent correlations between BR, TBUT and were also studied using a matrix scatter plot. A significant strong correlation was found between IBI and TBUT, suggesting that as the TBUT increases the inter-blink interval increases (Pearson, r = 0.901, p = 0.000). There was no significant correlation found between IBI and CS (Pearson, r = 0.016, p = 0.474), and CS and TBUT (Pearson, r = -0.039, p = 0.435) (Fig 6.21).

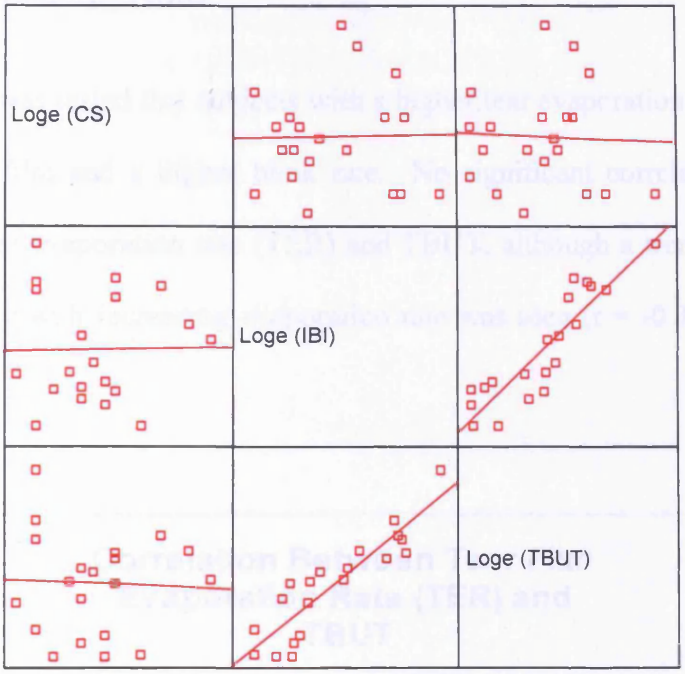


Fig 6.21: Correlation between inter-blink interval, tear film break-up time and corneal sensitivity.

A stepwise multiple regression procedure was also conducted and it only included TBUT in the model as a predictor for blink rate. The adjusted R^2 was 0.8 which is comparable to the linear multiple regression analysis that included TBUT and CS (R^2 = 0.792).

Summarising the results, it was found that the blink rate was significantly correlated only with tear film break-up time. Linear multiple regression found that the tear film break-up time can be a unique predictor for the blink rate.

7.3.1.4 Evaporation Rate

The hypothesis was tested that subjects with a higher tear evaporation rate will have a less stable tear film and a higher blink rate. No significant correlation was found between tear film evaporation rate (TER) and TBUT, although a trend of decreasing tear film stability with increasing evaporation rate was seen (r = -0.38, p = 0.1) (Fig 7.8).

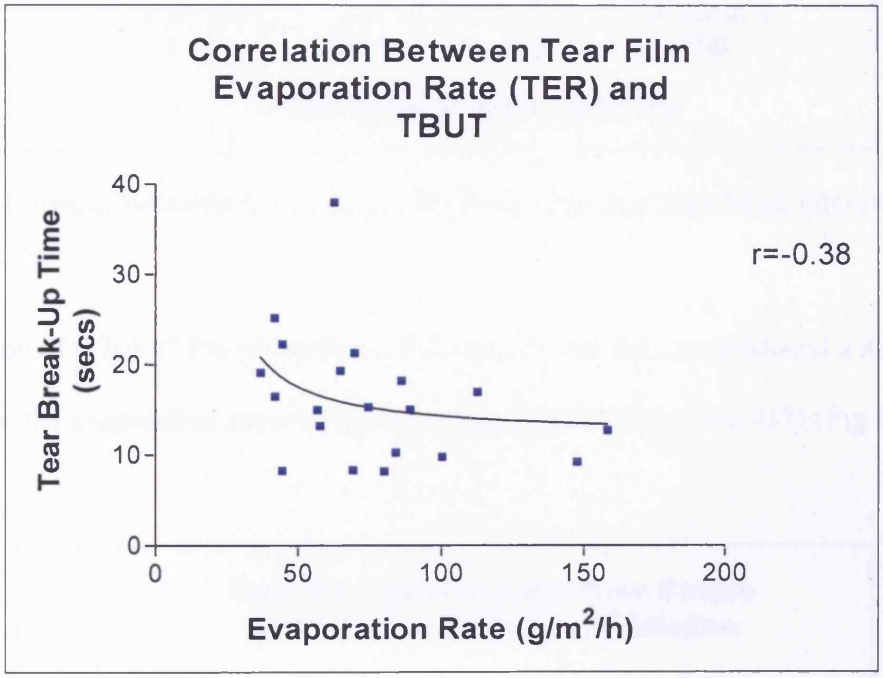


Fig 7.8: Correlation of tear film break-up time and tear film evaporation rate.

No significant correlation was found between TER and IBI (r = -0.272, p = 0.246) (Fig 7.9).

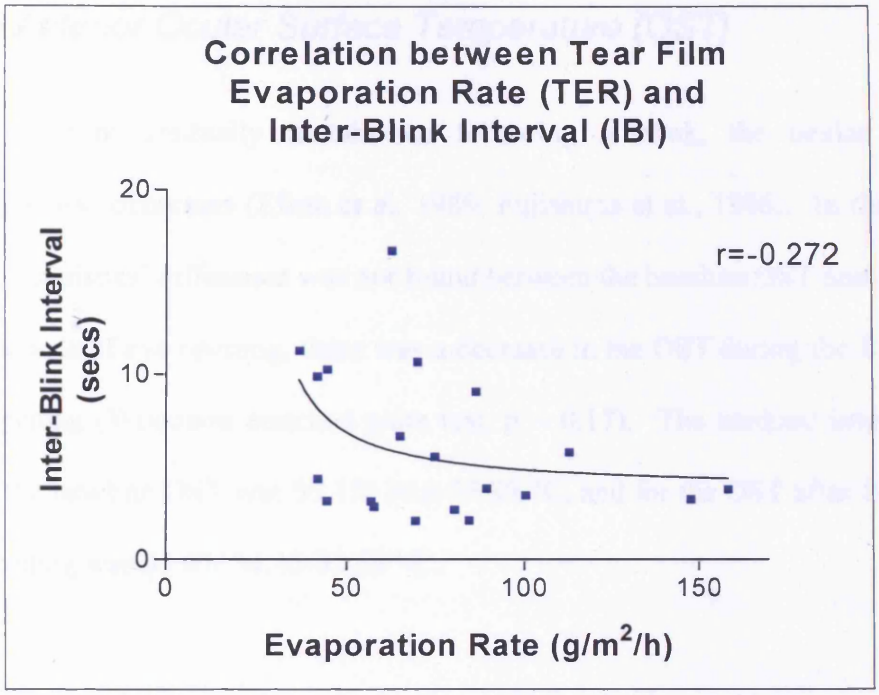


Fig 7.9: Correlation between tear film evaporation rate and inter-blink interval.

Instillation of 0.7µl of 2% unpreserved fluorescein into the eye produced a significant change in the evaporation rate (Wilcoxon matched pairs test, p = 0.037) (Fig 7.10).

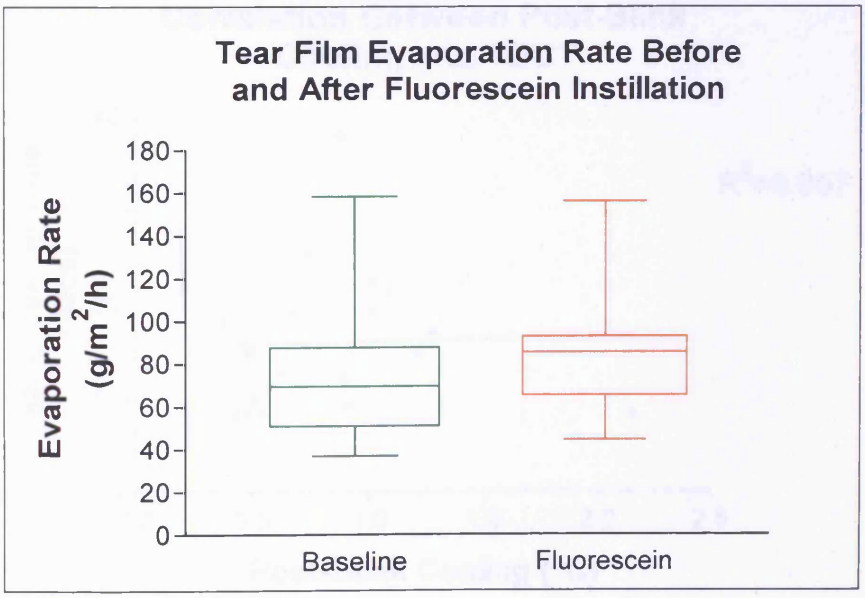


Fig 7.10: Median/ interquartile range evaporation rate from the ocular surface before and after the instillation of 0.7µl of 2% sodium fluorescein.

7.3.1.5 Anterior Ocular Surface Temperature (OST)

As the tear-film gradually destabilises following a blink, the ocular surface temperature also decreases (Efron et al, 1989; Fujishima et al., 1996). In this study, although a statistical difference was not found between the baseline OST and the OST after 8 seconds of eye opening, there was a decrease in the OST during the 8 seconds of eye opening (Wilcoxon matched pairs test, $p = 0.17$). The median/ interquartile range of the baseline OST was 35.15/ 34.8-35.99 °C, and for the OST after 8 seconds of eye opening was 35.07/ 34.45-35.59 °C.

This study considered the hypothesis that subjects having a higher temperature change during the IBI will have a less stable tear film and a higher blink rate. No significant correlation was found between the post-blink cooling and tear break-up time (Spearman, $r = 0.241$, $p = 0.307$) (Fig 7.11).

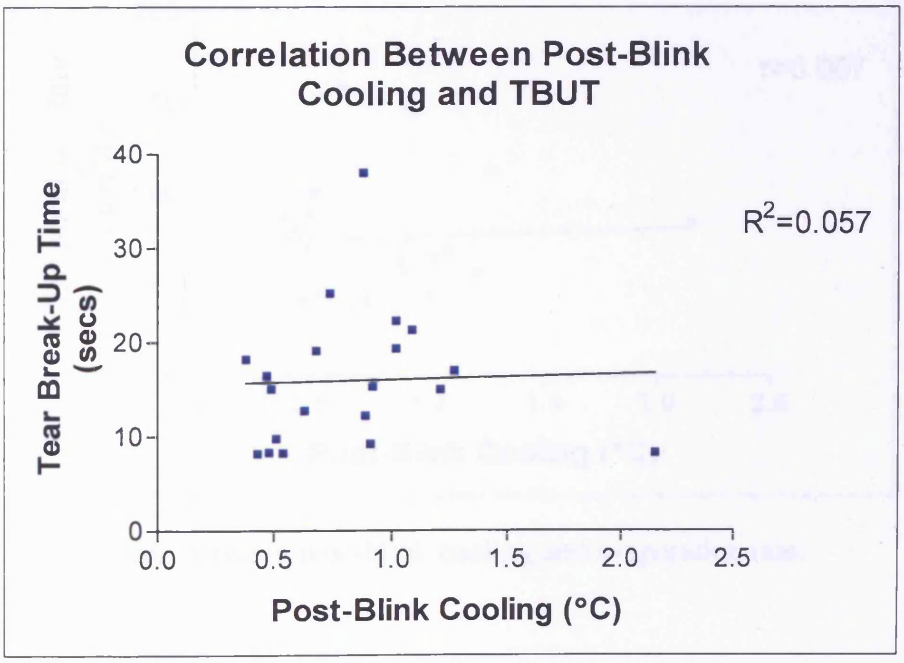


Fig 7.11: Correlation between post-blink cooling and tear film break-up time.

There was no relationship found between either the IBI and post-blink cooling (r = -0.017, p = 0.945) or the IBI and the rate of temperature change (rate of temperature change is described as the time taken to reach half of the overall temperature change, also called the temperature half-life time) (Spearman, r =-0.038, p = 0.874). The results suggest that subjects with a greater, or more rapid reduction, in the ocular temperature do not have a shorter IBI than those experiencing a lower, or less rapid, reduction.

To test whether people with a greater change in post-blink cooling also have a higher evaporation rate, the correlation between post-blink cooling and evaporation rate was considered. No significant correlation was found (Spearman, r =0.007, p = 0.975) (Fig 7.12).

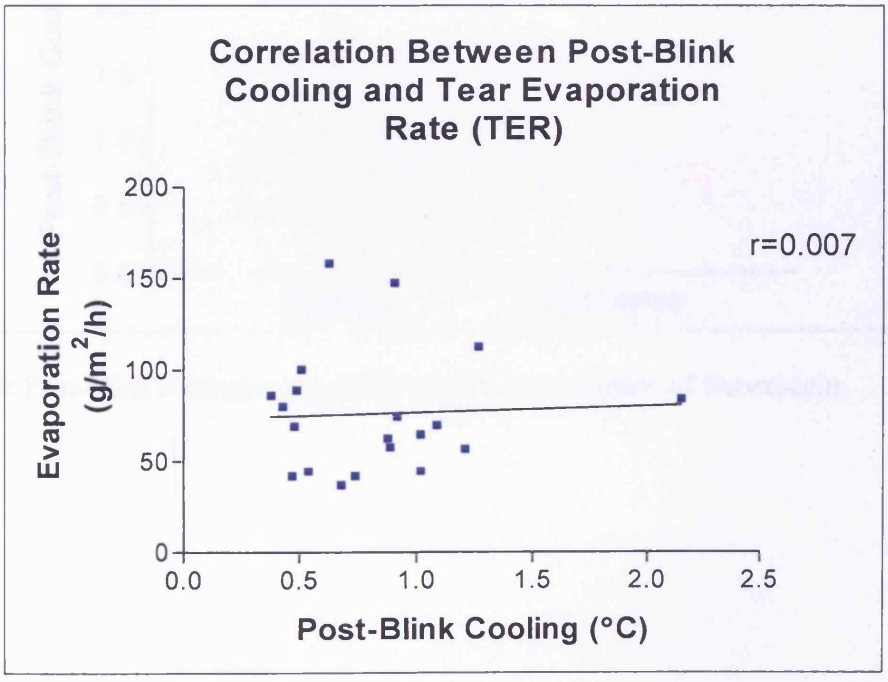


Fig 7.12: Correlation between post-blink cooling and evaporation rate.

Instillation of 0.7µl of 2% fluorescein into the eye did not alter either the baseline temperature of the OST (Wilcoxon matched pairs test, p = 0.133) (Fig 7.13), or the

rate of temperature change (Wilcoxon matched pairs test, p = 0.716). It may be expected that instillation of the fluorescein will cause an initial decrease in temperature, as it is a cooler fluid going into the eye. The lack of change may be due to the slight amount of fluorescein instilled into the eye, which was quickly mixed with the tear film, or because the temperature measurement was not made immediately after the instillation, but rather after the assessment of tear film stability, i.e. blinking had already restored the "normal" OST.

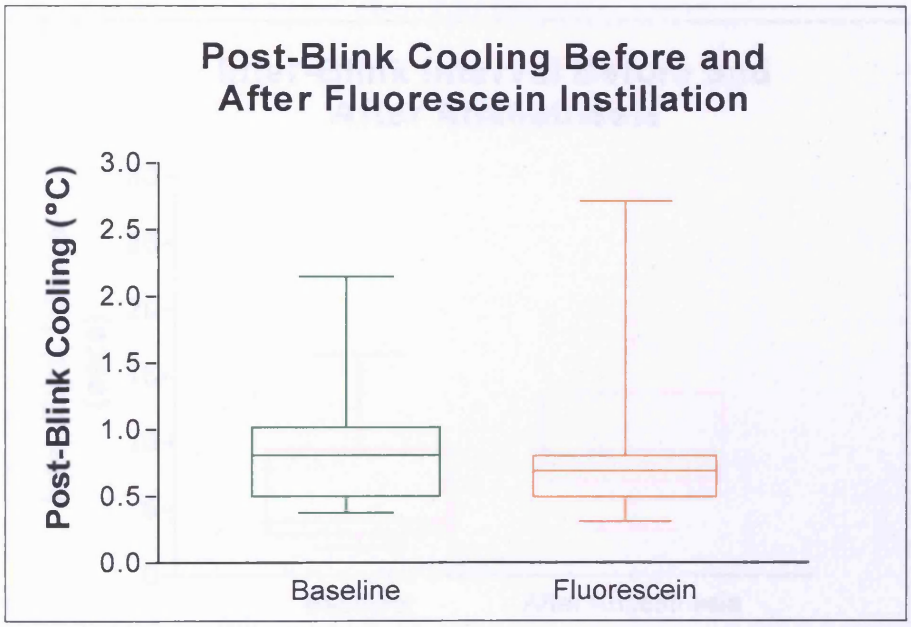


Fig 7.13: Post-blink cooling before and after the instillation of fluorescein.

7.3.2 Visit 2

7.3.2.1 Inter-Blink Interval after Anaesthesia

Comparing the baseline IBI of subjects taken at the first visit with the IBI after anaesthesia, a significant increase in the IBI after anaesthesia was found (Wilcoxon matched pairs test, $p<0.0001$). The median/ interquartile range baseline IBI was 4.11/ 3.16-9.51secs, and for IBI after anaesthesia was 9.35/ 7.16-13.71 secs.

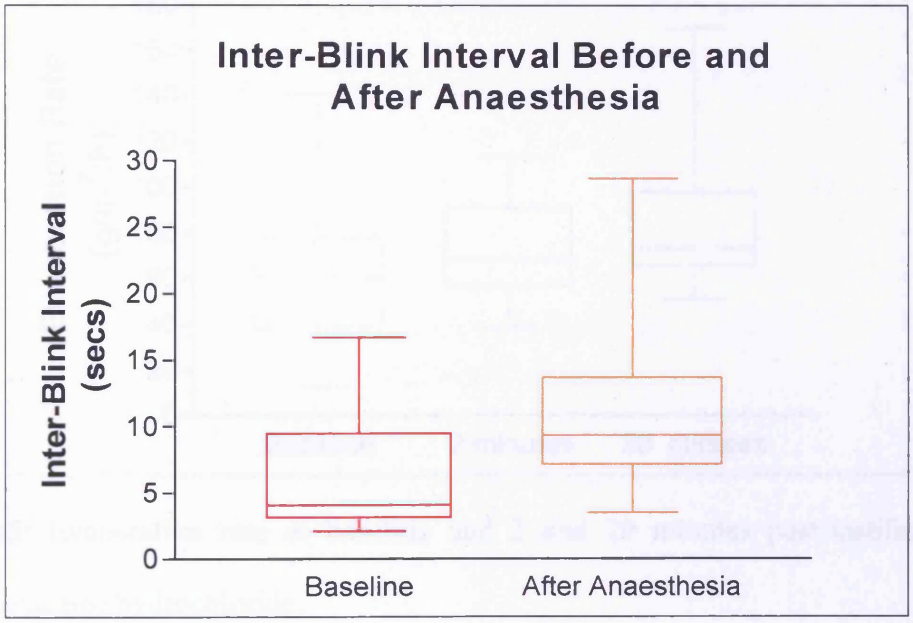


Fig 7.14: Median/ interquartile range inter-blink interval of subjects before and after anaesthesia.

7.3.2.2 Tear Film Evaporation Rate after Anaesthesia

Measurements of tear film evaporation rates were made before anaesthetic instillation and after 2 and 20 minutes of anaesthesia. A significant difference in the evaporation rate was found between baseline, 2 minutes and 20 minutes post-instillation (Kruskal-

Wallis, p = 0.029) (Fig 7.15). There was a significant difference in evaporation rates between baseline and 2 minutes post-instillation (Wilcoxon matched pairs test, p = 0.018), baseline and 20 minutes post-instillation (Wilcoxon matched pairs test, p = 0.003) and among 2 and 20 minutes post-instillation (Wilcoxon matched pairs test, p = 0.002).

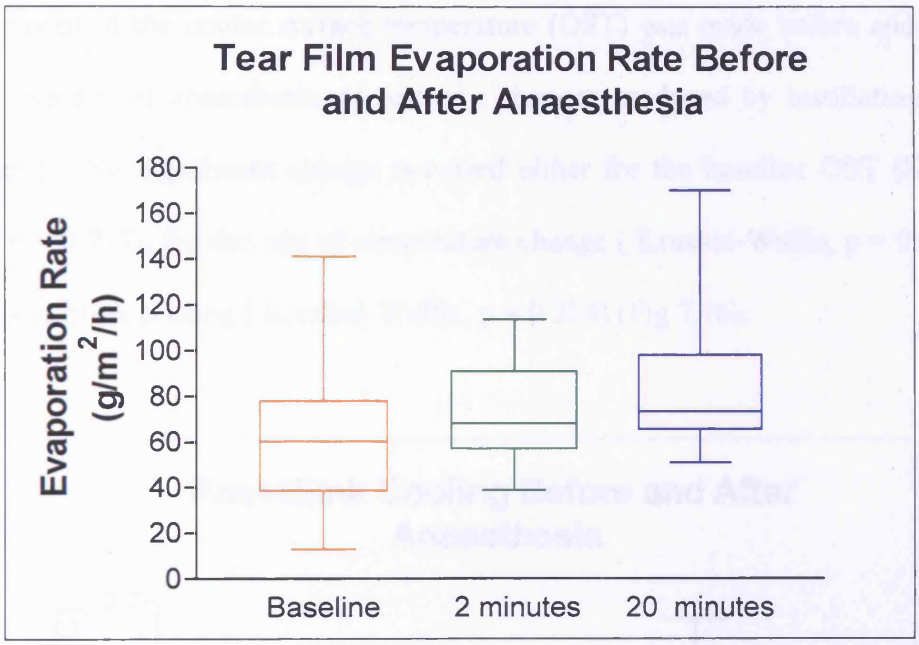


Fig 7.15: Evaporation rate at baseline and 2 and 20 minutes post-instillation of proxymetacaine hydrochloride.

It is proposed that the increase in evaporation rate exhibited 2 minutes after instillation arises from the instability in the tear film, produced by disturbance of the lipid layer (Lemp and Hamill, 1973; Trees and Tomlinson, 1990; Craig and Tomlinson, 1997; Greiner et al., 2002).

The evaporation rate at 20 minutes post-instillation was greater again than the baseline and 2 minutes measurements. Assuming that the tear film layer lipid layer

has stabilised, it can be proposed that the anaesthetic has reduced corneal sensitivity, producing longer inter-blink intervals, thereby delaying reformation of the tear film after break-up and causing increased evaporation.

7.3.2.3 Anterior Ocular Surface Temperature after Anaesthesia

Measurement of the ocular surface temperature (OST) was made before and after 2 and 20 minutes of anaesthesia, to test any changes produced by instillation of the anaesthetic. No significant change occurred either for the baseline OST (Kruskal-Wallis, p = 0.717), for the rate of temperature change (Kruskal-Wallis, p = 0.169) or for the post-blink cooling (Kruskal-Wallis, p = 0.224) (Fig 7.16).

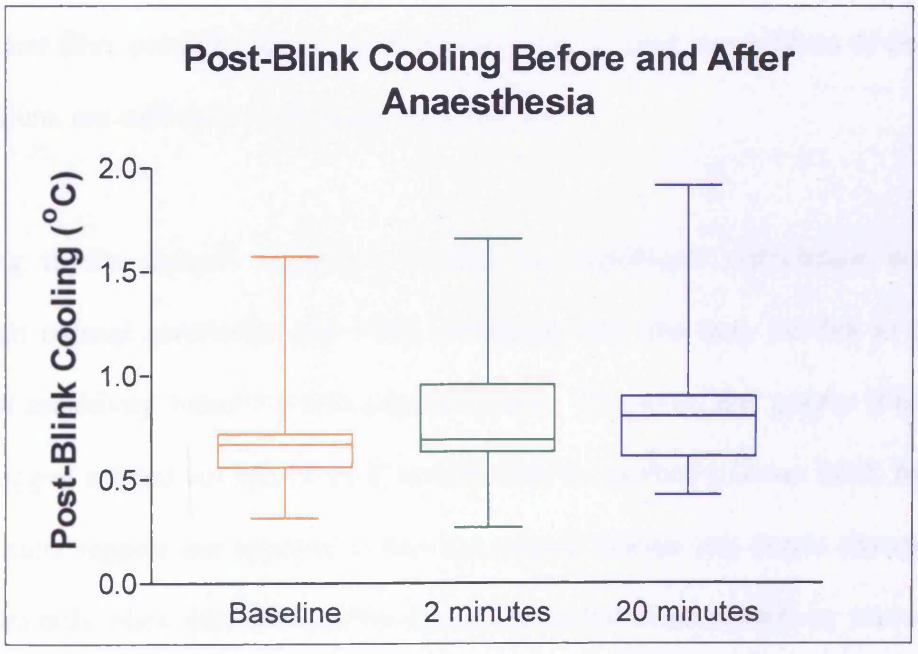


Fig 7.16: Post-blink cooling before and after 2 and 20 minutes of anaesthesia.

7.4 Discussion

This study assessed the hypothesis that a localised cooling in the tear film produced by tear thinning prior to tear break up, allows increased evaporation, which is detected by the corneal nerves, thereby initiating a blink.

The results showed a close and strong relationship between tear film stability and blink frequency, where blink rate increased as tear film stability decreased. This repeats the findings of our previous study (Chapter 6). The crucial question is whether the blink is triggered by break-up of the tear film or by some other change in the tear film before break-up occurs. From Fig 7.2 it can be seen that all of the subjects blinked before a full break-up occurred, indicating that the blink rate is adjusted to maintain tear stability and prevent dry spot formation. Thus early changes in the tear film, possibly perceived by the sensitive C cold nerve fibres of the corneal epithelium, are sufficient to stimulate a new blink.

Turning to the corneal sensitivity results, no significant correlation was found between corneal sensitivity and blink frequency, but this may be due to the good corneal sensitivity found for this subject cohort. However, the graphs (Fig 7.5 and 7.7) suggest a trend for low corneal sensitivities to produce a lower blink frequency. The results support the hypothesis that the corneal nerves can detect changes in the tear film only when they are sufficiently sensitive, but when sensitivity starts to drop, early changes in the tear film are not detected, and the subject blinks less frequently.

To verify the involvement of the corneal nerves in the blink mechanism, corneal sensitivity was blocked by anaesthesia and a significant increase in the inter-blink

interval was recorded, producing lower blink rates, suggesting that the tear film changes between blinks were not detected. Previous investigations which have considered the effect of blocking corneal sensation on the blink rate (Collins et al., 1989; Moore and Kardon, 1997; Nakamori et al., 1997) also found a significant decrease in the blink rate.

Localised tear film cooling, produced by tear film evaporation during the process of tear film thinning and break-up, may be detected by the thermo-sensitive corneal afferents, and this process is considered as a possible signal for a blink. However, no significant correlation was found between tear film evaporation rate and inter-blink interval, or between the tear film evaporation rate and the tear film break-up time. Similar results were found by Craig and Tomlinson (1997), and Craig et al (1997, 2000). They found that the lipid layer thickness controls both the evaporation from the ocular surface area and the stability of the tear film, and they attributed the lack of correlation between the stability of the tear film and the evaporation rate to the fact that the evaporation measurement took into account the whole of the exposed ocular surface area, whereas tear film break-up is only assessed over the central corneal area.

The significant increase in evaporation rate after the instillation of fluorescein was the result of disturbance in the tear film lipid layer by the fluorescein. A recent study by Greiner et al (2002) found that the instillation of fluorescein causes changes in the lipid layer, thus increasing the rate of evaporation. The greater the volume added, the greater the lipid layer alteration and the longer the recovery time. Instillation of 10μl of fluorescein had a 100% incidence of lipid layer alteration and a mean of recovery time 2.43±1.71 minutes. Instillation of 1μl of fluorescein had a 75% incidence of

lipid layer alteration and a mean recovery time of 1.24±1.04 minutes. The fact that only 0.7μl of fluorescein were added in the current study will have caused less lipid layer alteration and this may be the reason for observing only a small increase in the evaporation rate.

Increased evaporation might be expected in those individuals with a wider palpebral aperture and thus a greater exposed ocular surface area (Rolando and Refojo, 1983; Sotoyama et al., 1995). Changes in spontaneous eye-blink frequency have been noted when the palpebral aperture is deliberately changed by asking subjects to maintain a normal straight-ahead gaze or a superiorly or inferiorly-directed gaze (Stern et al., 1984; Karson, 1988; Tsubota and Nakamori, 1995; Nakamori et al., 1997). A significant increase in the blink rate was found as the exposed ocular surface area was increased, and vice versa. Increasing the exposed ocular surface spreads the tear film further, causing earlier break-up. The increased blink rate is due to either more rapid tear thinning producing greater evaporation and thus temperature change, or to more rapid full break-up. In either case the tear film changes are detected by the corneal and conjunctival nerves.

To compare the results of tear evaporation rate for normal subjects found in the current study with previous results published in the literature, it was important to convert the units used in the current study, gr/ m^2/ hr, to the units of $x10^{-7}g/cm^2/sec$, as most researchers reported values of evaporation rates in $x10^{-7}g/cm^2/sec$. This difference in units may be resolved if the value of evaporation rate found in this study, 61.01 gr/ m^2/ hr, is rendered to the same units ($x10^{-7}g/cm^2/sec$) by diving this value in gr/ m^2/ hr by a factor of 3.6. A value of 16.94 $x10^{-7}g/cm^2/sec$ was found, which is

equivalent to 61.01 gr/ m^2/ hr. This finding of evaporation rate is comparable to the values reported in the literature for normal subjects, but is much higher from the results of Goto et al (2003) who found a rate of 4.1 $x10^{-7}g/cm^2/sec$. This difference may be due to the different instrumentation used to assess tear evaporation rate, as they used microbalance technology to allow "continuous readings" of tear film evaporation.

This was the first time in the literature where the blink rate of a subject was correlated with the temperature change that occurs after a blink to test whether corneal temperature change during the inter-blink interval was the initiator for blinking. Previous studies have only reported the influence of environmental temperature change and humidity on blinking (Hata et al, 1994, 1995), demonstrating an increased blink rate in cooling conditions of room temperature and low humidity, as a result of increased tear evaporation.

There is increasing evidence that sensory stimuli arising from the exposed ocular surface area, and environmental factors that affect the ocular surface, are also determinants of blink rates (Tsubota, 1998). Higher blink rates have been recorded during conditions that favour evaporation (Nakamori et al., 1997; Tsubota and Nakamori, 1995).

In conclusion, the aim of the current study was to investigate the role of peripheral factors on the blink mechanism. Taking into account only the ocular surface area, and without affecting its properties, it was found that the stability of the tear film was the key determinant for the next blink. Following anaesthesia, the blink rate was

found to decrease substantially, supporting the hypothesis that the corneal nerves are able to detect early cooling changes of the tear film while it destabilises. Corneal sensitivity itself is another factor in normal blinking, whose role is better revealed in less sensitive subjects that cannot perceive early changes in the tear film and who subsequently blink less. The size and rate of temperature change that occurs following a blink, due to evaporation, was not a factor in blink rate. A faster or greater loss of temperature was not found to be an initiator for the next blink. Finally, no relationship was found between blink frequency and tear film evaporation rate, suggesting that early evaporative tear loss was not the trigger for blinking.

8. An Investigation of the Tear Film and Blinking in Contact Lens Wear Discomfort

8.1 Introduction-Purpose

When a contact lens is placed into the eye, the pre-corneal tear film is divided into two separate layers: the pre-lens tear film on the front surface of the contact lens and the post-lens tear films, which lies between the back surface of the lens and the corneal epithelium (Faber et al., 1991). These new tear layers must perform the normal functions of the pre-corneal tear film, as well as any new tear film functions arising from contact lens wear.

A stable pre-lens tear film is desirable for a number of reasons: it lubricates the surface of the lens, minimising the mechanical irritation of the tarsal conjunctiva and enhancing lens comfort; it creates an optically smooth surface to provide a sharp image quality; it maintains the bactericidal activity of tears to prevent surface contamination; it prevents lens dehydration, it facilitates lens rehydration and resists deposit formation (Andrasko and Schoessler, 1980; Efron et al., 1987; Fatt, 1990; Myers et al., 1991; Young and Efron, 1991; Jones, 1992; Little and Bruce, 1994). Irregularity of the pre-lens tear film may produce scattering of incident light, along with a reduction in the quality of vision (Timberlake et al., 1992). In addition, comfort may be reduced by pre-lens lipid layer instability, and associated lens surface drying (Caffery and Josephson, 1990; Forst, 1990; McMonnies, 1990).

The functions of the post-lens tear film are less clearly established than those of the pre-lens tear film. Nevertheless a few potential roles of the post-lens tear film have

been proposed. Post-lens tear film viscosity has been proposed as a determinant of the force required for lens movement (Bibby and Tomlinson, 1983). Coloured or patterned appearances of the post-lens tear film observed in specular reflection have been associated with minimal lens movement and implicated in the mechanism of hydrogel lens binding (Bruce and Brennan, 1988, 1992; Little and Bruce, 1994). However, the results of these studies cannot be considered conclusive, as only a small number of subjects participated. A few authors have also speculated that more efficient post-lens tear exchange may increase expulsion of debris and reduce metabolic epithelial insult (Golding et al., 1990; Stapleton, 1992).

Ocular discomfort in contact lens wear is a frequent patient complaint and the main cause of dissatisfaction and patient dropout from contact lens wear. Discomfort is often derived from ocular irritation and pain, dryness and lens deposits in silicone hydrogels lens wear (Fonn et al., 2000) . The aetiology of these symptoms has been attributed to the effect of contact lens wear on the tear film. This is due to the increased evaporation rates of the tear film when wearing contact lenses (Cedarstaff and Tomlinson, 1983). In addition, the tear break-up time on the corneal surface decreases significantly after wearing contact lenses (Faber et al., 1991; Young and Efron, 1991), and for prospective lens wearers the practitioner should make sure that the stability of the tear film is sufficient to allow successful contact lens wear. Another feature that is common during soft lenses wear, and especially of high water content lenses, is that they dehydrate. Some authors have suggested that lens dehydration, as a result of tear evaporation, is the cause of discomfort and dryness symptoms (Efron and Brennan, 1988; Lebow and Bridgewater, 1998; Young et al., 1995, 1997a, 1997b; Lemp et al., 1999), whereas other studies have shown no

correlation between the symptoms of dryness and lens dehydration (Pritchard and Fonn, 1995; Fonn et al., 1999a). It can therefore be concluded that the symptom may not have a solely tear film-related aetiology, but may be secondary to hyperaemia and/or mechanical stimulation of the conjunctiva as a result of friction from the lens surface or edge (Fonn et al., 2000).

When a contact lens is placed on the eye, blinking assumes additional functions. Normal blinking patterns remove debris from beneath the lens, re-oxygenate the tears beneath the lens, hydrate the lens, and clean the lens surface (Fatt and Hill, 1970; Collins et al., 1987; Efron and Carney, 1983).

The role of blinking during the wearing of rigid contact lenses has been well-documented. During rigid contact lens wear, the importance of blinking is enhanced through its contribution to the maintainance of normal corneal function through the tear exchange mechanism (Fatt and Hill, 1970). Even with the introduction of gas permeable rigid contact lens materials, adequate tear exchange, and hence blinking action, is still necessary (Hill, 1977). During soft contact lens wear, blinking and reformation of the tear film are important in maintaining contact lens hydration.

Rigid contact lens wear has been found to cause both an increased (Hill and Carney, 1984) and decreased blink rate (Brown et al., 1973) in neophyte subjects. Hill and Carney (1984) found that, even after comfortable wear was achieved, the blink rate was increased from 15.5 blinks/min to 23.2 blinks/min. They also found a change in the blinking pattern during contact lens wear, with all subjects exhibiting a more regular blinking pattern. In contrast, Brown et al (1973) found an altered blink action

and a decrease in the blink rate, from 15.8 blinks/min to 9.75 blinks/min, after the insertion of rigid lenses.

Blinking behaviour has been found to increase during soft lens wear. York et al (1971) reported an elevation in the blink rate in the early stages of contact lens wear, which they suggested was due to mechanical irritation of the ocular tissues. Brown et al (1973) found a small but not significant effect on blink rate after the insertion of soft lenses (Soflens^{TM}, Bausch & Lomb), which they proposed was due to the lens's superior comfort and diminished lid irritation. Carney and Hill (1984) reported an increase in blink rate from 12.1 blinks/min to 20.3 blinks/min. Similarly, Hill (1984) found an increase in the blink rate of seven new soft lens wearers from 12 blinks/min, before lens fitting to 20 blinks/min, once adapted to the lenses.

Incomplete blinking during contact lens wear, or an excessive proportion of incomplete blinks, can cause a variety of problems for both rigid and soft contact lens wearers. Inadequate tear exchange beneath the lens can lead to corneal oxygen deprivation, particularly in rigid lens wearers (Fatt and Hill, 1970). It can also lead to the accumulation of debris beneath the lens that may be responsible for sub-epithelial infiltration in extended lens wear (Zantos and Holden, 1978). It is associated with 3 and 9 o'clock corneal staining and increased lens surface deposits in rigid lens wearers. In soft lens wearers it may lead to lens dehydration, increased lens surface deposits, and inferior punctuate corneal staining (Kline and DeLuca, 1977).

This study will consider the role of the tear film in contact lens wear discomfort and how blinking behaviour alters in relation to the discomfort symptoms. Corneal

sensitivity will also be assessed to ascertain whether subjects with more sensitive corneas are more prone to discomfort. Thus, in an experienced group of contact lens wearers who both experience comfort and discomfort during contact lens wear, the relationship between blink-rate, tear film stability and corneal sensitivity will be examined.

8.2 Methods

Thirty experienced contact lens-wearing Caucasian subjects (8 males, 22 females; mean age, 20.45±1.98 years; range, 19-27) were recruited from the student population of Cardiff University. Subjects were excluded if they had any ocular or systemic pathology known to affect corneal sensitivity, e.g. ocular surgery, ocular diseases, diabetes, corneal dystrophy (Birndorf and Ginsberg, 1972; Schwartz, 1974; Lyne, 1977; Ishikawa et al., 1994; Ruben, 1994; Murphy et al., 1999a; Rosenberg et al., 2000). Also, pregnant women or women during the premenstruum, menstruation, or ovulating period, were excluded, as corneal sensitivity is depressed (Millodot, 1984, 1994; Martin and Safran, 1988). Ethical approval was obtained from the School of Optometry and Vision Sciences Research Ethics Committee. After explanation of the purpose of the study, subjects were asked to sign a consent form prior to participating and advised that they could withdraw from the experiment at any time.

Subjects were divided into two groups according to the subjective comfort levels of contact lens wear. *Comfort Group* (3 males, 12 females; mean age, 20.2±1.74 years) experiencing comfortable contact lens wear throughout the day; *Discomfort Group* (5 males, 10 females; mean age, 20.7±2.22 years) experiencing discomfort after five to seven hours of contact lens wear.

All the subjects wore soft, medium water content lenses (55-70%) on a daily basis. They were experienced wearers, having worn lenses for between 1.5 and 7 years (3.61±1.5 years).

Subjects were asked to attend the laboratory on two different days and each visit lasted for approximately forty-five minutes. All measurements were made after 12pm and onwards to avoid any possible diurnal bias in corneal sensitivity (Chapter 4), or tear film stability (Patel et al, 1988). At Visit 1, the subjects attended the laboratory without wearing the contact lenses on that day and at Visit 2 the subjects attended the laboratory wearing the contact lenses. The appointments for each subject were set at the same time for Visit 1 and 2, and the order of visits was randomised. For the group of subjects feeling discomfort, the appointments were set to coincide with onset of discomfort, which was on average after 5.7±0.88 hours wear. All ocular measurements were made on the *right eye only.*

At Visit 1 the blink rate of each subject was recorded using the digital video camera. Central corneal sensitivity was measured using the NCCA. Tear film stability was assessed first non-invasively using the Tearscope (NIBUT), and second invasively using the fluorescein tear film break-up time (TBUT).

At Visit 2 the blink rate was recorded while wearing contact lenses, and then the subject's comfort was assessed subjectively using the vertical analog comfort scale (Morgan and Efron, 2002; Morgan et al., 2003) (Fig 8.1). This scale uses sub-divisions of comfort and discomfort, whereby 0 represents pain and 100 represents excellent comfort. Then the measurement of the pre-lens tear film stability during

contact lens wear was was taken using the Tearscope. One minute after lens removal, the post-lens tear film stability was assessed using the Tearscope and then immediately after using the fluorescein break-up time. Corneal sensitivity was assessed last using the Non-Contact Corneal Aesthesiometer.

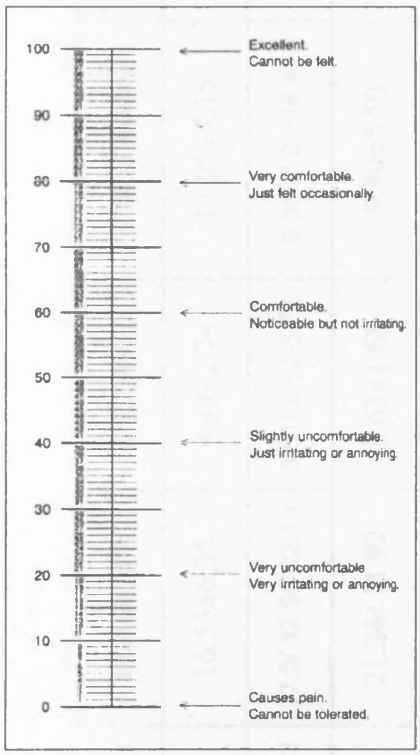


Fig 8.1: Vertical Analog Comfort scale

8.3 Results

The distributions for all measurements were assessed for normality (Shapiro-Wilk), using the SPSS11 (Lead Tools, Lead Technologies, Inc). The data was both normally and not normally distributed for both visits. Data were log transformed, and normality testing showed again most of the data to be not normally distributed, and so non-parametric statistical tests were used for statistical analysis, (Prism: GraphPad Software Inc, San Diego).

Table 8.1: Median/ Interquartile Range (IR) and Mean (± Standard Deviation) of all the measurement taken Visit 1 and Visit 2.

Visit 1

		CS (mbars)	Blinks/min	IBI (secs)	TBUT (secs)	NIBUT (secs)
Comfort	**Mean±SD**	0.80±0.46	16.55±6.35	4.47±2.74	11.95±7.16	13.37±7.64
	Median/ IR	0.95/ 0.32-1.3	17.2/ 12.95-22.13	3.51/ 2.82-5.72	9.30/ 7.27-16.47	9.83/ 8.43-18.5
Discomfort	**Mean±SD**	0.88±0.49	21.24±11.92	3.63±1.68	9.90±5.50	10.70±5.41
	Median/ IR	0.75/ 0.5-1.32	18.2/ 12.5-29.9	3.29/ 2.18-4.94	10.42/ 4.84-15.73	12.43/ 5.5-16.5

Visit 2

		CS after CL Removal (mbars)	Blinks/min	IBI (secs)	NIBUT During CL (secs)	NIBUT CL Removal (secs)	TBUT CL Removal (secs)	Comfort Response
Comfort	**Mean±SD**	0.82±0.46	20.49±7.28	3.44±7.28	3.28±1.22	6.71±1.38	6.10±1.38	81±13.26
	Median/ IR	0.75/ 0.471.27	20.00/ 15.7-27	2.99/ 2.27-5.12	2.66/ 2.29-4.73	7.06/ 5.3-7.93	6.33/ 4.82-7.15	80.00/ 72.5-95
Discomfort	**Mean±SD**	0.91±0.50	33.67±9.62	1.93±0.60	1.60±0.64	4.37±0.64	3.85±1.84	32.67±4.17
	Median/ IR	0.65/ 0.5-1.42	32.4/ 28.7-44.6	1.85/ 1.49-2.45	1.43/ 1.11-2.13	4.06/ 3.09-5.63	3.48/ 2.96-5.23	30.00/ 25-35

8.3.1 Visit 1

8.3.1.1 Blink Frequency

The blink frequency of the subjects was recorded and analysis of the blink rate (BR) and the inter-blink interval (IBI) was made. The *Comfort Group* exhibited a lower BR than the *Discomfort Group* but this difference was not statistically significant (Mann-Whitney test, p = 0.494) (Comfort: 17.21/ 12.95-22.30 blinks/min; Discomfort: 18.2/ 12.5-29.9 blinks/min) (Fig 8.2). A longer IBI with a greater variation was noted for the *Comfort Group* (Mann-Whitney test, p = 0.494) (Comfort: 3.51/ 2.82-5.72 secs; Discomfort: 3.29/ 2.18-4.94 secs) (Fig 8.3)

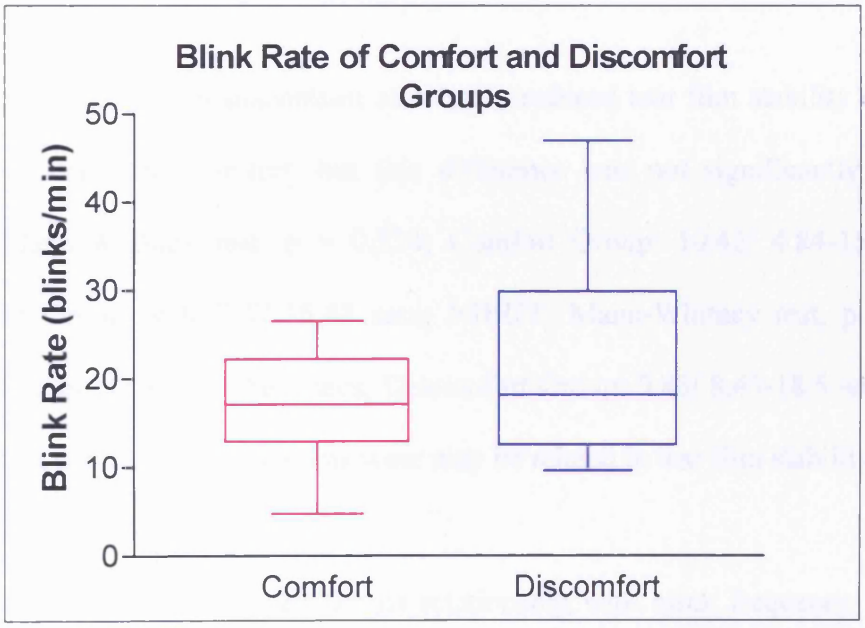


Fig 8.2: Median and interquartile range (IR) baseline blink rates for Comfort and Discomfort Groups.

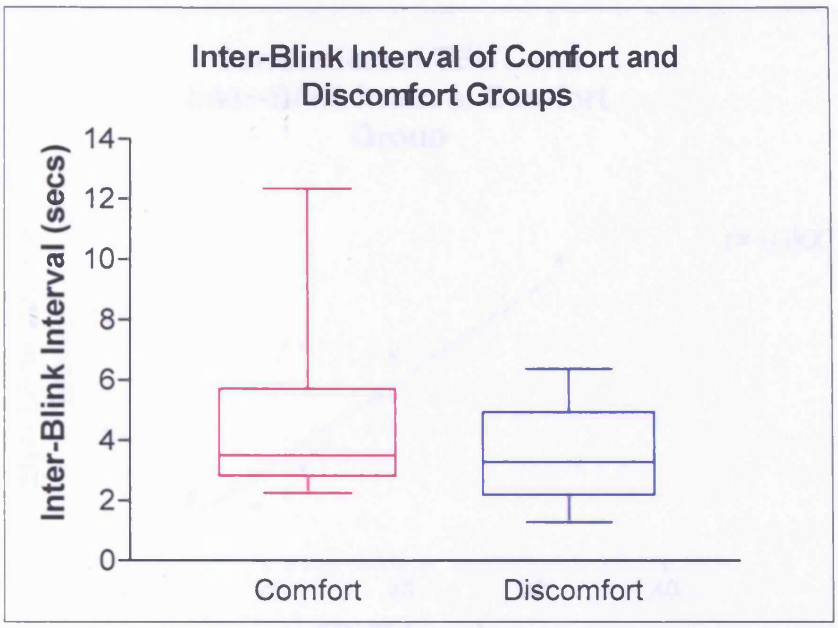


Fig 8.3: Median/ Interquartile Range (IR) inter-blink interval for the Comfort and Discomfort Groups.

The subjects with greater discomfort also had a reduced tear film stability compared to those experiencing comfort, but this difference was not significantly different (TBUT: Mann-Whitney test, p = 0.534; Comfort Group: 10.42/ 4.84-15.73 secs; Discomfort Group: 9.3/ 7.27-16.47 secs; NIBUT: Mann-Whitney test, p = 0.678; Comfort Group: 12.43/ 5.5-16.5 secs; Discomfort Group: 9.83/ 8.43-18.5 secs). This suggests that comfort in contact lens wear may be related to tear film stability.

Tear film stability was assessed for its relationship with blink frequency. For the *Comfort Group*, assessing tear film stability both invasively and non-invasively, strong correlations were seen, with IBI increasing as tear film stability increased (TBUT: Spearman, r = 0.800, p = 0.0003; Tearscope (NIBUT): Spearman r = 0.859, p<0.0001) (Figs 8.4, 8.5).

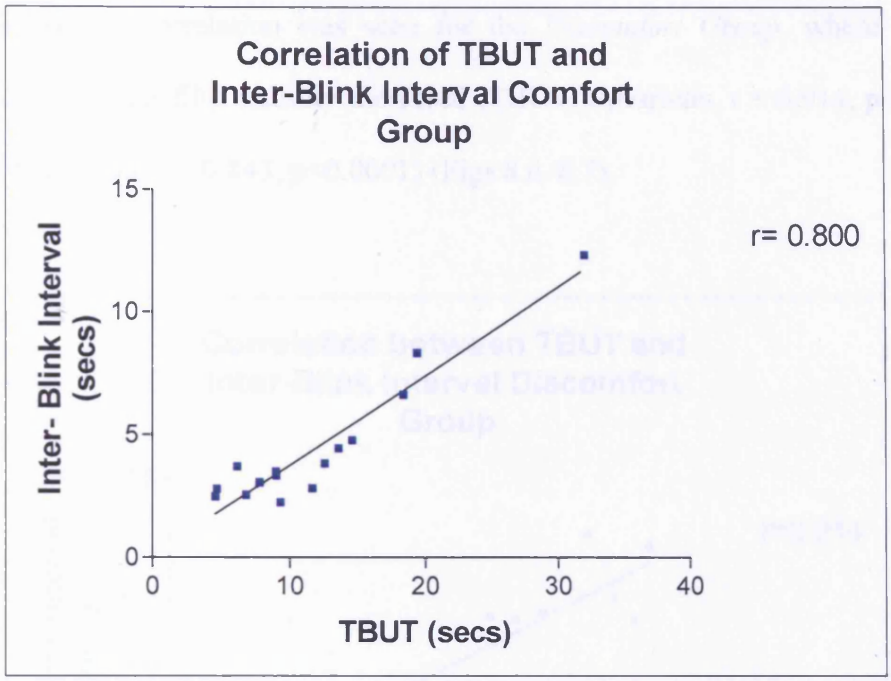


Fig 8.4: Correlation of tear film break-up time and inter-blink interval for the Comfort Group, measured with fluorescein.

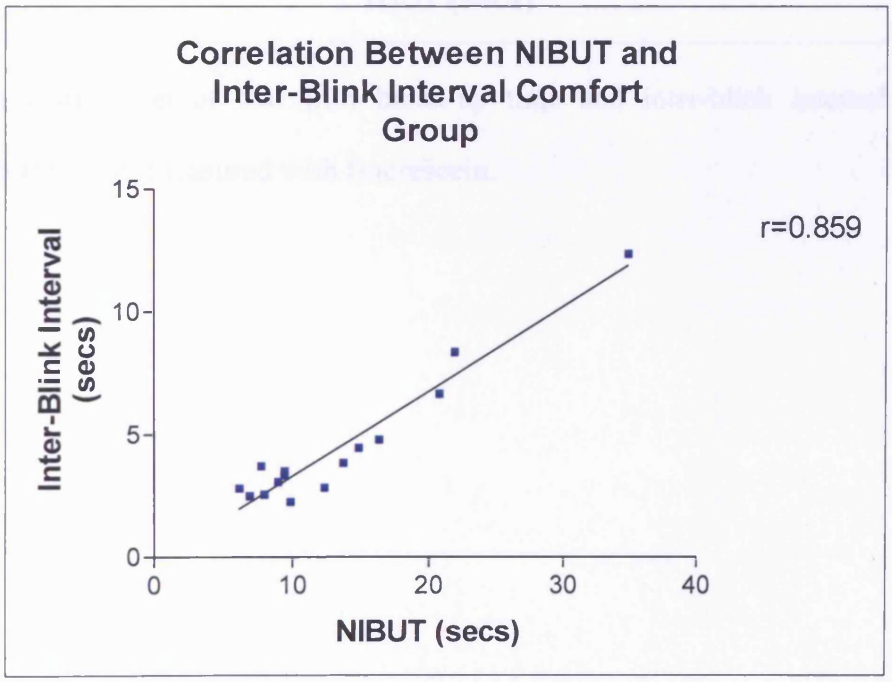


Fig 8.5: Correlation of non-invasive tear film break-up time and inter-blink interval for the Comfort Group, measured with Tearscope.

The same strong correlation was seen for the *Discomfort Group*, where the IBI increased as the tear film stability increased (TBUT: Spearman, r = 0.914, p<0.0001; NIBUT: Spearman, r = 0.843, p<0.0001) (Figs 8.6, 8.7).

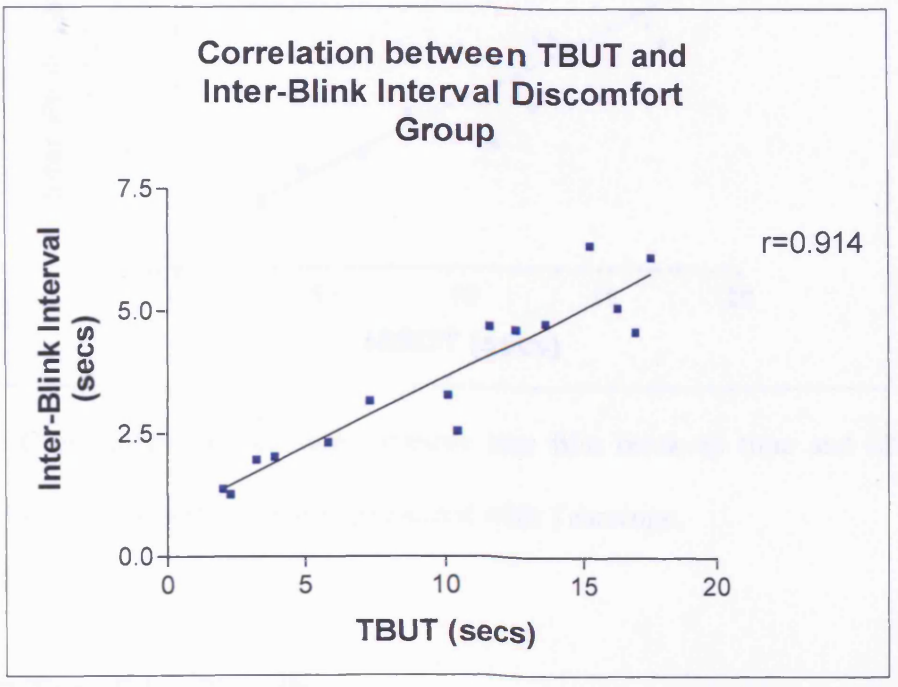


Fig 8.6: Correlation of tear film break-up time and inter-blink interval for the Discomfort Group, measured with fluorescein.

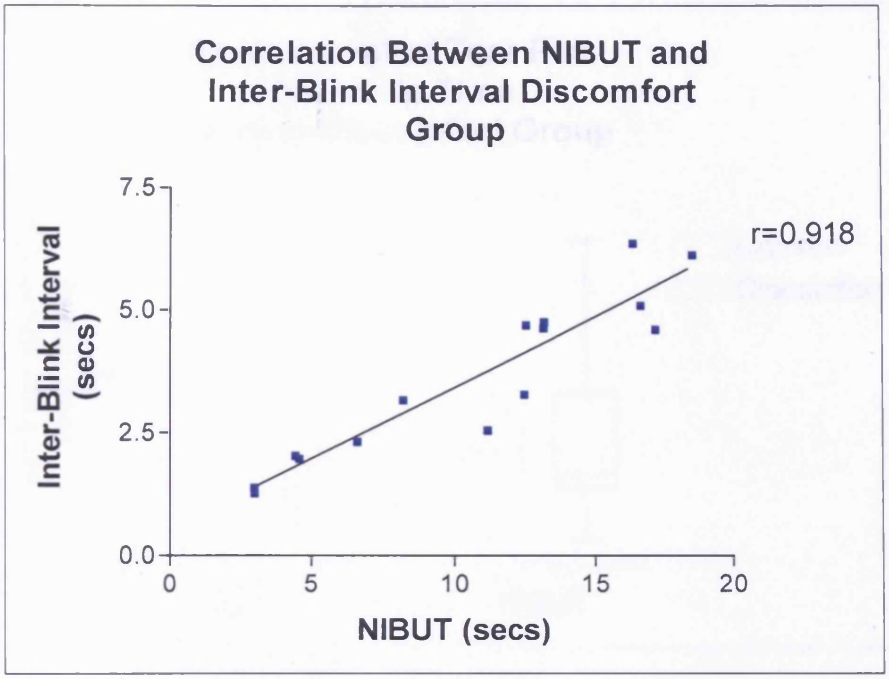


Fig 8.7: Correlation between non invasive tear film break-up time and inter-blink interval for the Discomfort Group, measured with Tearscope.

8.3.1.2 Tear Film Stability

Tear film stability measured with fluorescein and the Tearscope for both the *Comfort* and *Discomfort Groups*, gave comparable results (Comfort Group, Mann-Whitney test, $p = 0.454$; Discomfort Group, Mann-Whitney test, $p = 0.689$) (Fig 8.8).

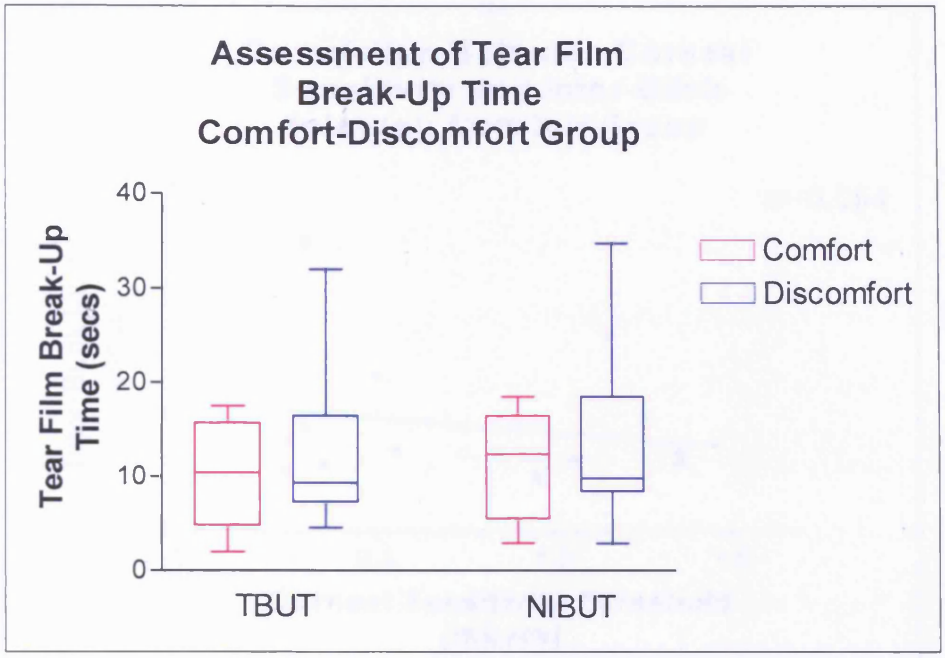


Fig 8.8: Assessment of tear film stability for the Comfort and Discomfort Group.

8.3.1.3 Corneal Sensitivity

The median/ interquartile range of corneal sensitivity for the *Comfort Group* was 0.95/ 0.32-1.3 mbars, and for the *Discomfort Group* was 0.75/ 0.5-1.32 mbars. No significant difference was found for corneal sensitivity between the *Comfort* and *Discomfort Groups* (Mann-Whitney test, p = 0.561). Interestingly, for the *Comfort Group* no correlation was found between corneal sensitivity and IBI (Spearman, r =-0.054, p = 0.849), but a significant correlation was found for the *Discomfort Group* (Spearman, r =0.665, p =0.007) (Figs 8.9, 8.10).

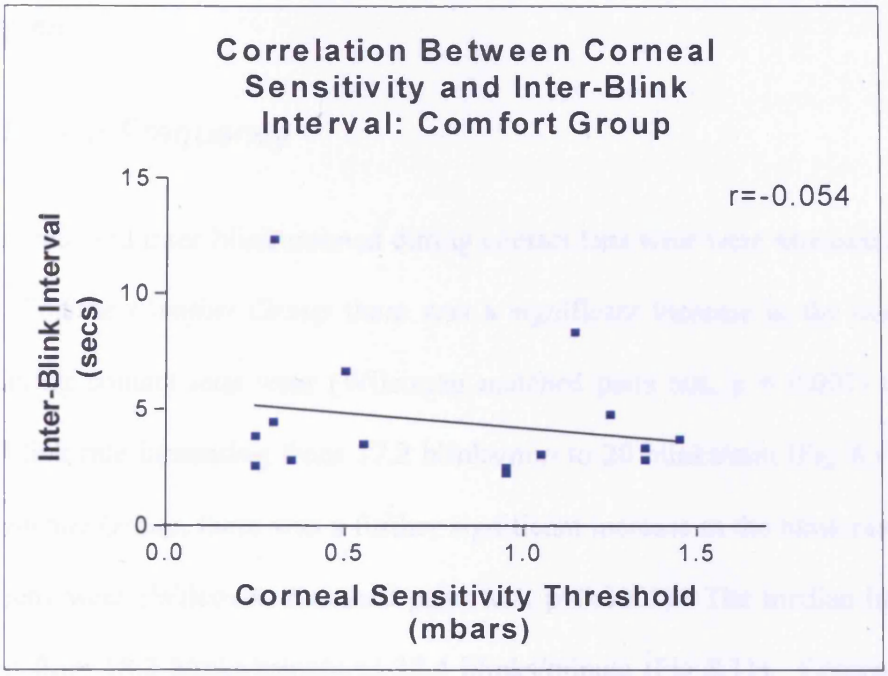


Fig 8.9: Correlation between corneal sensitivity and inter-blink interval: Comfort Group.

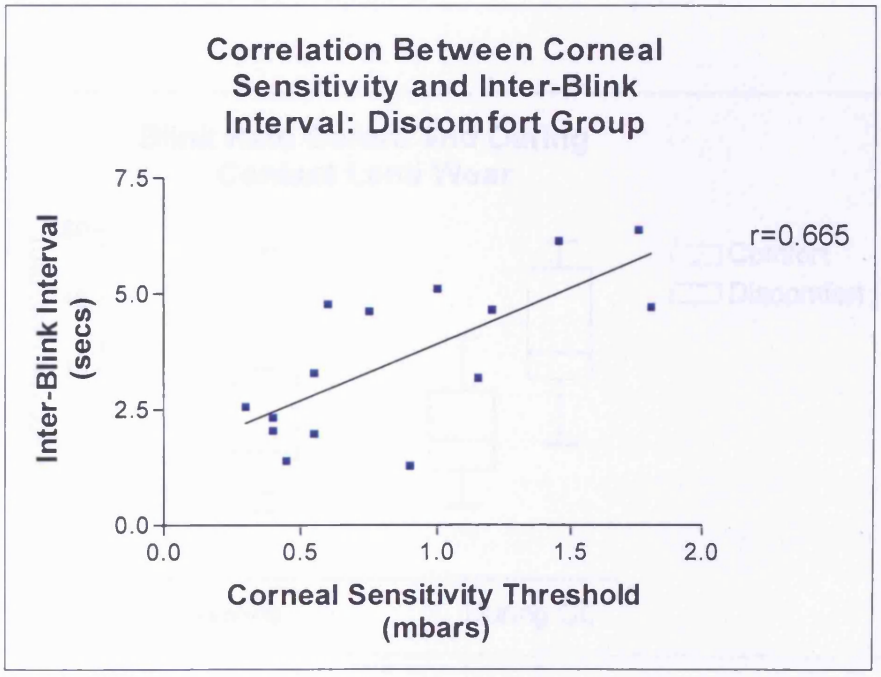


Fig 8.10: Correlation between corneal sensitivity and inter-blink interval: Discomfort Group.

8.3.2 Visit 2

8.3.2.1 Blink Frequency

The blink rate and inter-blink interval during contact lens wear were assessed for both groups. For the *Comfort Group* there was a significant increase in the number of blinks during contact lens wear (Wilcoxon matched pairs test, p = 0.007) with the median blink rate increasing from 17.2 blinks/min to 20 blinks/min (Fig 8.11). For *the Discomfort Group*, there was a further significant increase in the blink rate during contact lens wear (Wilcoxon matched pairs test, p<0.0001). The median blink rate increased from 18.2 blinks/minute to 32.4 blinks/minute (Fig 8.11). Comparing the blink rate for both groups while wearing contact lenses, a significant difference was found, indicating that the increase for the Discomfort Group was much greater than that in the Comfort Group (Mann-Whitney test, p = 0.001).

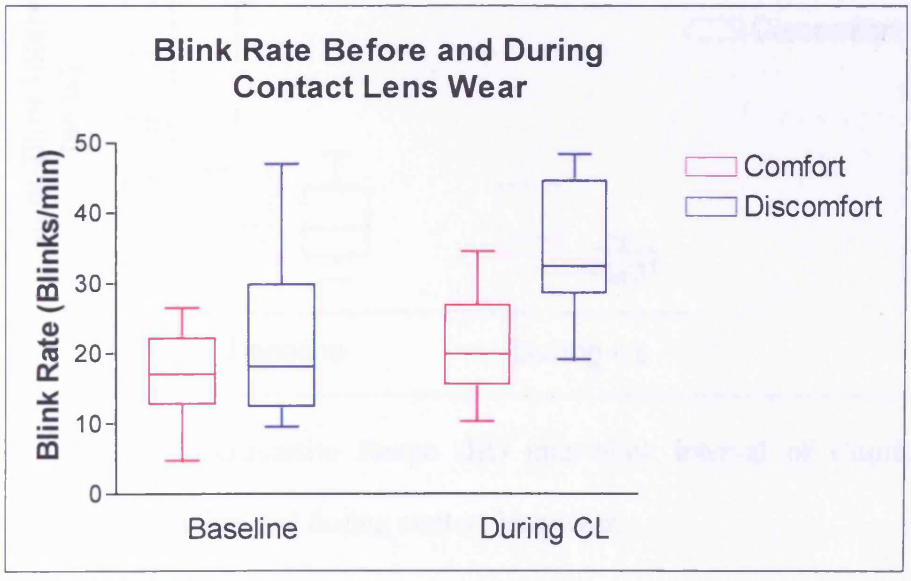


Fig 8.11: Median/ Interquartile Range blink rate of Comfort and Discomfort Group before and during contact lens wear.

In order to find out the blinking pattern exhibited before and during contact lens wear, the inter-blink interval was determined for both groups. For the *Comfort Group,* the median/ interquartile range (IR) IBI before contact lens wear was 3.51/ 2.82-5.72 secs and during contact lens wear was 2.99/ 2.27-5.12 secs, which was significantly different (Wilcoxon matched pairs test, $p = 0.035$). For the *Discomfort Group,* the median/ interquartile range (IR) before contact lens wear was 33.29/ 2.18-4.94 secs and during contact lens wear was 1.85/ 1.49-2.45 secs, being statistically different (Wilcoxon matched pairs test, $p<0.0001$). The IBIs of the *Discomfort Group* were much shorter than that of the *Comfort Group* and differed significantly during contact lens wear (Mann-Whitney test, $p = 0.0001$) (Fig 8.12).

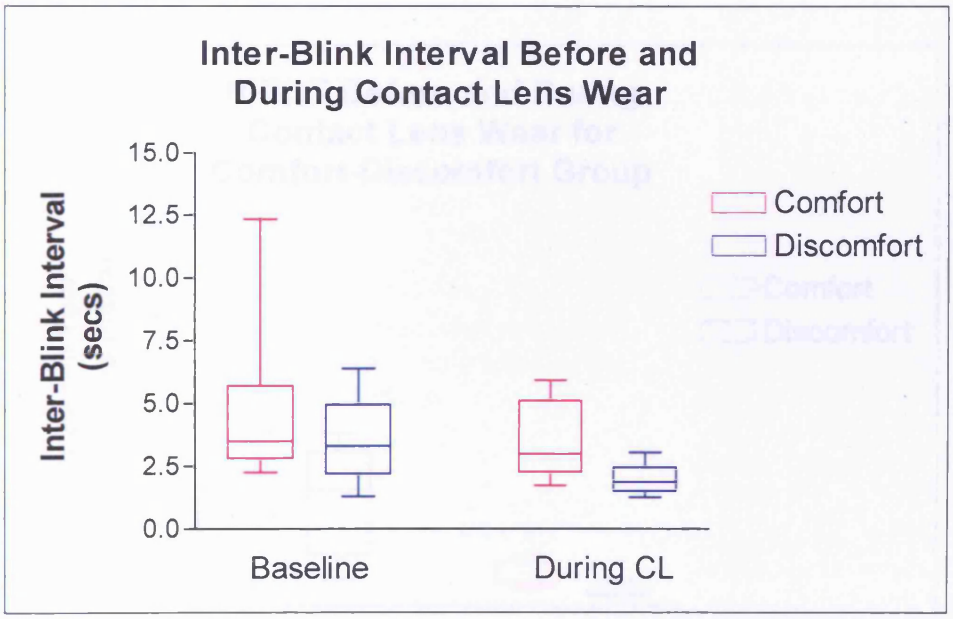


Fig 8.12: Median/ Interquartile Range (IR) inter-blink interval of Comfort and Discomfort Groups before and during contact lens wear.

8.3.2.2 Tear Film Stability

The pre-lens tear film stability was compared to the baseline data for both groups. For the *Comfort Group,* a significant difference was found between the pre-corneal and pre-lens tear film stability (Wilcoxon matched pairs test, $p<0.0001$), indicating the greater stability of the pre-corneal tear film. The median/ interquartile range (IR) NIBUT before and during contact lens wear was 9.83/ 8.43-18.5 secs and 2.66/ 2.29-4.73 secs, respectively. For the *Discomfort Group,* a similar significant difference was found (Wilcoxon matched pairs test, $p<0.0001$). The median/ interquartile range (IR) NIBUT before and during contact lens wear was 12.43/ 5.55-16.5 secs and 1.43/ 1.11-2.13 secs, respectively (Fig 8.13).

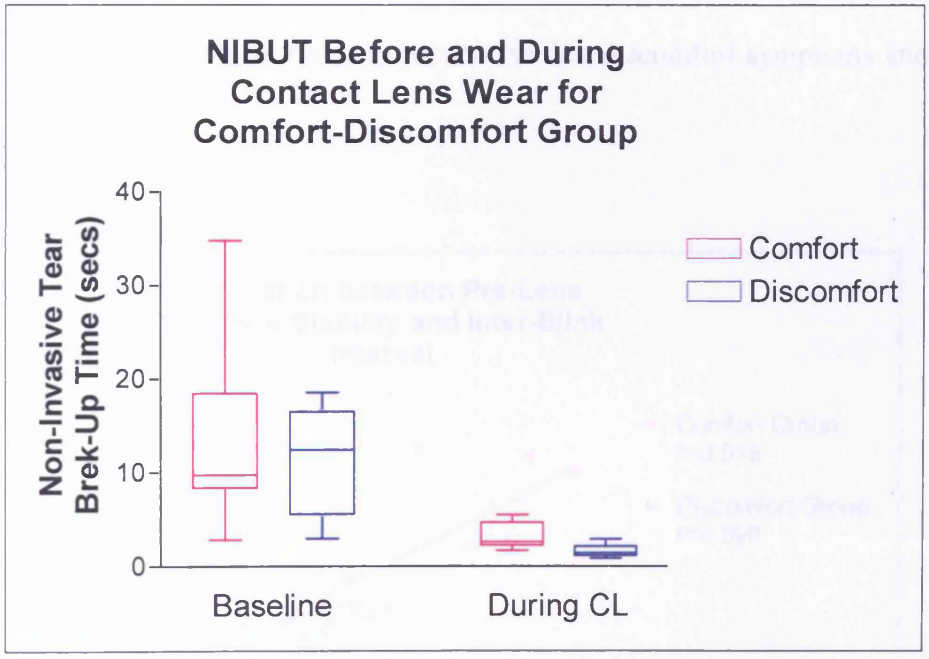


Fig 8.13: Median/ Interquartile Range (IR) NIBUT before and during contact lens wear, for both Comfort and Discomfort Groups.

The pre-lens tear film stability was less for the *Discomfort Group* compared to the *Comfort Group* (Mann-Whitney test, p = 0.002; Comfort: 2.66/ 2.29-4.73; Discomfort: 1.43/ 1.11-2.13).

For the *Comfort Group,* the results indicate a significant correlation between IBI and pre-lens tear film stability (Spearman, r = 0.519, p =0.003). This suggests either that the stability of the pre-lens tear film influences the blink rate during contact lens wear or that the underlying blink rate found without lens wear is unaffected by the altered ocular surface conditions (Fig 8.14). For the *Discomfort Group,* a similar significant relationship was found between the blink rate of the subjects and their pre-lens tear film stability (Spearman, r = 0.596, p = 0.019) (Fig 8.14). The weaker correlation implies that the blink rate is not entirely influenced by the stability of the pre-lens tear film, but that it increases in order to prevent further discomfort symptoms and blurry vision.

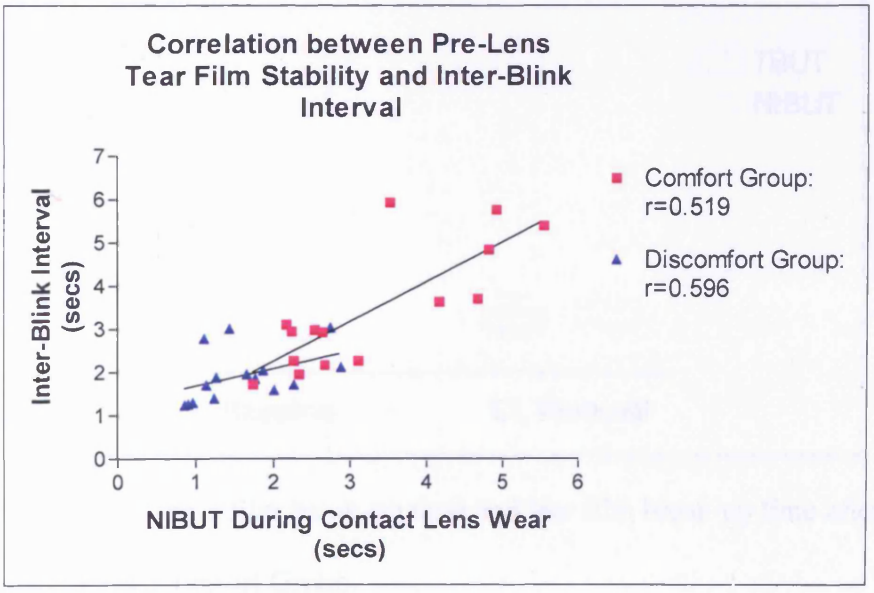


Fig 8.16: Correlation between pre-lens tear film stability (NIBUT) and inter-blink interval for both Comfort and Discomfort Group.

Post-wear pre-corneal tear film stability was assessed for both groups. For the *Comfort Group*, a significant reduction in the post-lens tear film stability was found compared to the pre-corneal tear film stability. The median/ interquartile range (IR) tear film stability (TBUT) before and after lens removal was 9.31/7.27-16.47 secs and 6.33/ 4.82-7.15 secs, respectively (TBUT, Wilcoxon matched pairs test, $p<0.0001$) (Fig 8.17). The same effect was found when tear film stability was assessed non-invasively using the Tearscope (NIBUT, Wilcoxon matched pairs test, $p<0.0001$), indicating that the baseline measurements were much greater than the pre-wear levels. The median/ interquartile range (IR) tear film stability (NIBUT) before and after lens removal was 9.83/ 8.43-18.5 secs and 7.06/ 5.3-7.93 secs, respectively (Fig 8.17).

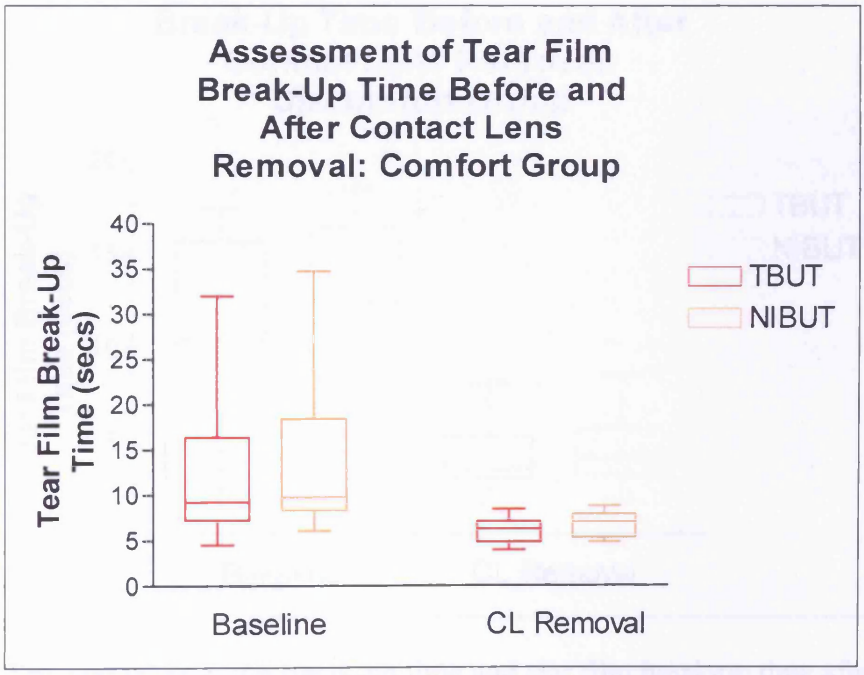


Fig 8.17: Pre-corneal tear film break-up time and tear film break-up time after contact lens removal for the Comfort Group.

Similar reductions in post-lens tear film stability were also found for the *Discomfort Group*. Assessing tear film stability with fluorescein, a significant drop in the tear film stability was found compared to the baseline data (TBUT, Wilcoxon matched pairs test, p = 0.001). The median/ interquartile range (IR) pre-corneal tear film stability (TBUT) was 10.42/ 4.81-15.73 secs and of the post-lens tear film stability was 3.48/ 2.96-5.23 secs (Fig 8.18). The same pattern was seen when post-lens tear film stability was assessed using the Tearscope (NIBUT, Wilcoxon matched pairs test, p = 0.001). The median/ interquartile range (IR) pre-corneal and post-lens tear film stability (NIBUT) was 12.43/ 5.55-16.5 secs and 4.06/ 3.09-5.6 secs, respectively (Fig 8.18).

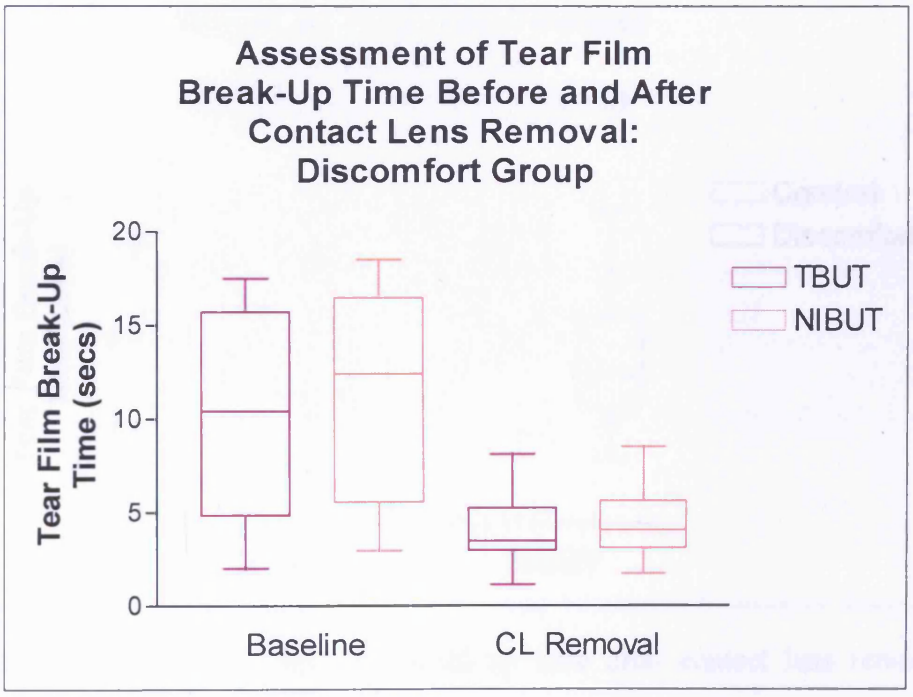


Fig 8.18: Pre-corneal tear film break-up time and tear film break-up time after contact lens removal for the Discomfort Group.

However, the subjects experiencing greater discomfort were found to have much lower post-lens tear film stability than those with comfort (TBUT: Mann-Whitney test, p = 0.001; NIBUT: Mann-Whitney test p = 0.001).

No significant change between the two methods of assessing post-lens tear film stability was found for the *Comfort Group* (Mann-Whitney test, p = 0.147) (Fig 8.19) and the *Discomfort Group* (Mann-Whitney test, p = 0.362) (Fig 8.19). Non-invasive assessment of post-lens tear film stability was always slightly higher than with fluorescein break-up time.

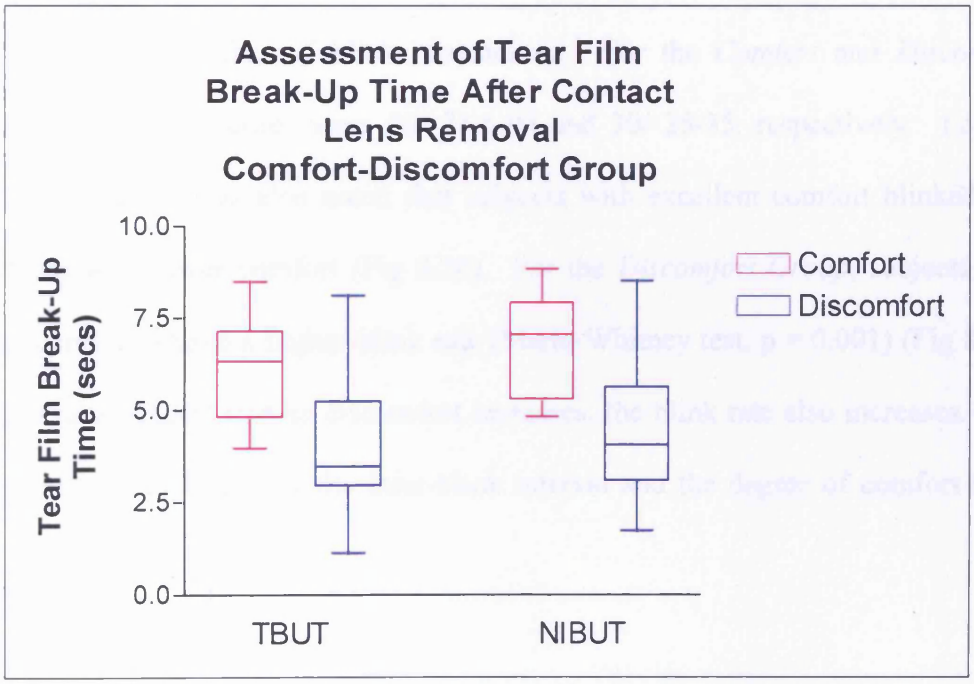


Fig 8.19: Comparison of tear film break-up time after contact lens removal for Comfort-Discomfort Group.

8.3.2.3 Corneal Sensitivity

For *Comfort* and *Discomfort Groups* no significant difference in corneal sensitivity was found between baseline and after lens removal (Comfort: Wilcoxon matched pairs test, $p = 0.791$; Discomfort: Wilcoxon matched pairs test, $p = 0.626$). Thus, despite a reduced oxygen supply to the cornea caused by contact lens wear, a further reduction in corneal sensitivity is not obtained after a few hours of lens wear.

8.3.2.4 Comfort Scores

The comfort scores were recorded for both groups and the relationship between comfort and the number of blinks considered. For the *Comfort* and *Discomfort Groups* the comfort scores were 80/ 71.5-95 and 30/ 25-35, respectively. For the *Comfort Group*, it was also noted that subjects with excellent comfort blinked less than those with lower comfort (Fig 8.20). For the *Discomfort Group*, subjects with lower scores also have a higher blink rate (Mann-Whitney test, $p = 0.001$) (Fig 8.21). These results suggest that as discomfort increases, the blink rate also increases. The same pattern was found for the inter-blink interval and the degree of comfort (Figs 8.22, 8.23).

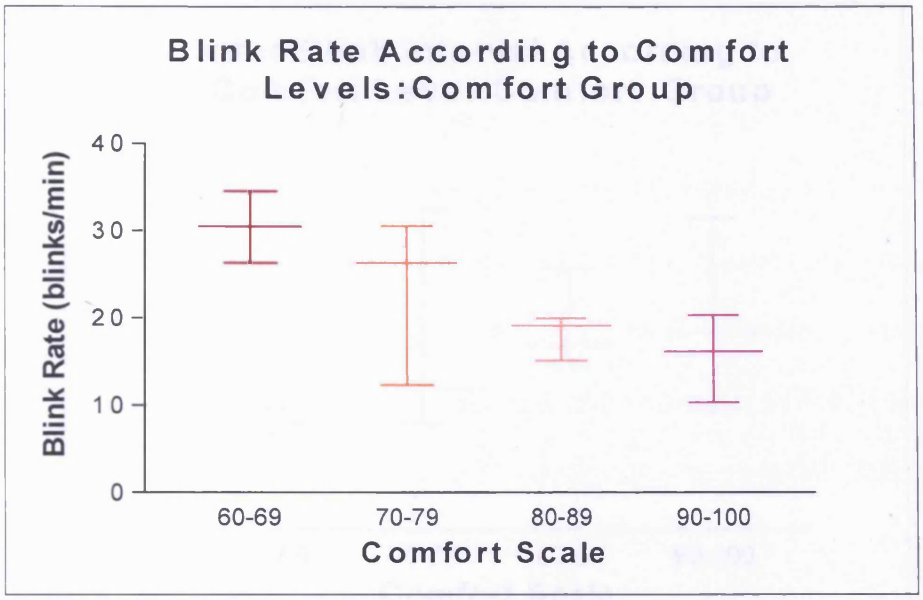


Fig 8.20: Median/ Minimun-Maximun blink rate and vertical analog comfort scale: Comfort Group.

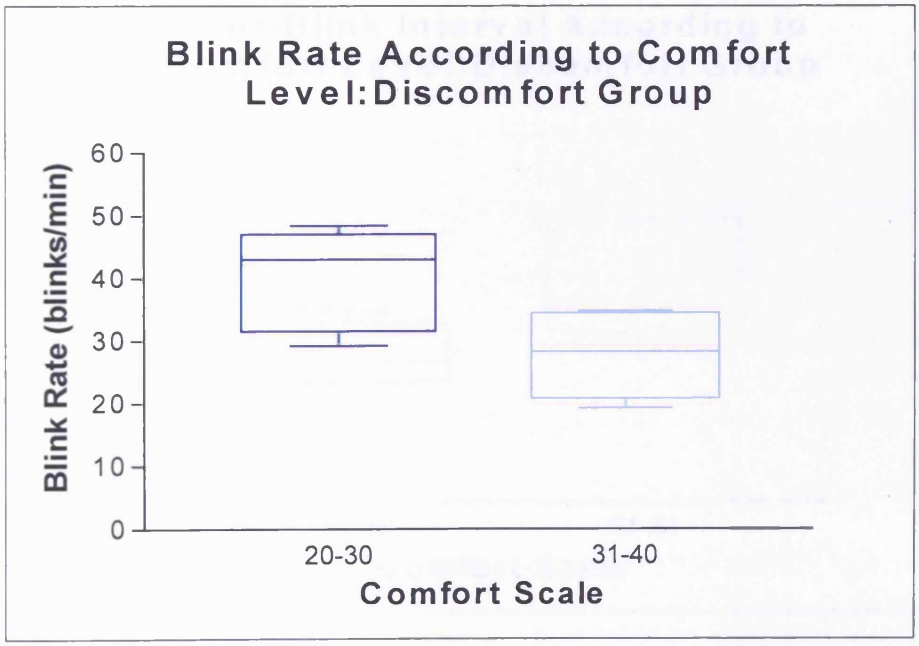


Fig 8.21: Blink rate and vertical analog comfort scale: Discomfort Group.

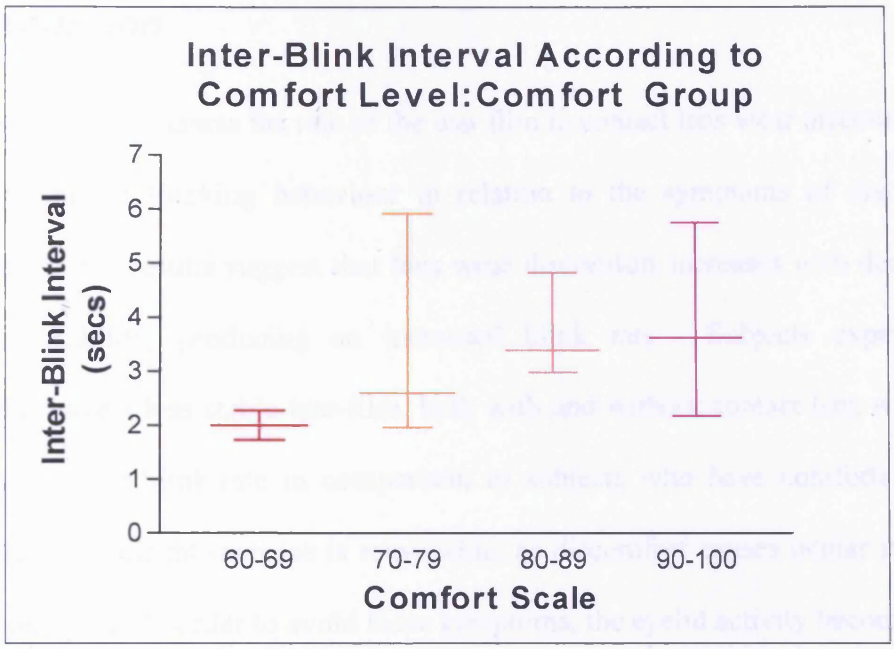


Fig 8.22: Inter-blink interval and vertical analog comfort scale: Comfort Group.

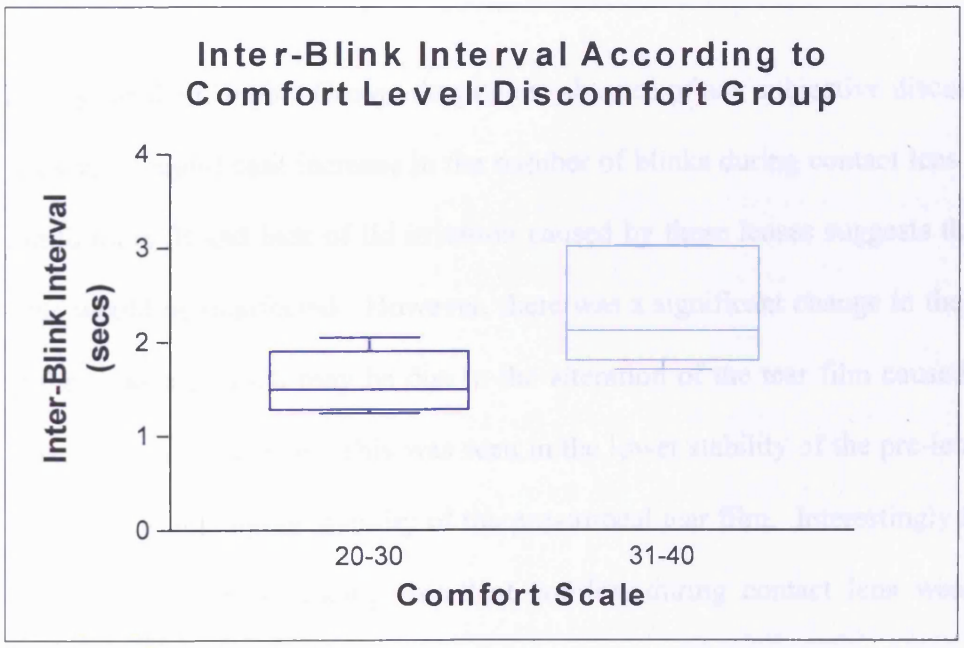


Fig 8.23: Blink rate and vertical analog comfort scale: Discomfort Group.

8.4 Discussion

This study aimed to assess the role of the tear film in contact lens wear discomfort and the alterations of blinking behaviour in relation to the symptoms of discomfort. Interestingly, the results suggest that lens wear discomfort increases with decreasing tear film stability, producing an increased blink rate. Subjects experiencing discomfort have a less stable tear-film, both with and without contact lens wear, and have an elevated blink-rate in comparison to subjects who have comfortable lens wear. This significant increase is reasonable, as discomfort causes ocular irritation, dryness and pain. In order to avoid these symptoms, the eyelid activity becomes more rapid to maintain contact lens hydration in order to minimise the mechanical irritation of the tarsal conjunctiva.

Specifically for the *Comfort Group*, despite the absence of any subjective discomfort, there was still a significant increase in the number of blinks during contact lens wear. The comfortable fit and lack of lid irritation caused by these lenses suggests that the blink rate would be unaffected. However, there was a significant change in the blink rate of the subjects, which may be due to the alteration of the tear film caused once the contact lens is in the eye. This was seen in the lower stability of the pre-lens tear film compared to the higher stability of the pre-corneal tear film. Interestingly it was found that subjects experiencing excellent comfort during contact lens wear had greater pre-corneal tear film stability than those experiencing less comfort, and blinked less during contact lens wear.

The same pattern was seen for the *Discomfort Group*, which exhibited a significant increase in the blink rate during contact lens wear. Subjective discomfort had a

greater effect on the number of blinks with the blink rate increasing as the level of discomfort increased. Subjects with increased discomfort had significantly decreased pre-corneal tear film stability, indicating that lens wear discomfort increases with decreasing tear film stability, producing an increased blink rate. While more frequent blinking takes place in the *Discomfort Group* in order to reform the tear film, it is reasonable to think that blinking more will also increase the discomfort due to the friction of the contact lens surface.

For the C*omfort Group* there was also a change in the regularity of the blinking action during contact lens wear. Before contact lens wear, most subjects showed irregularly occurring longer inter-blink intervals. Assessment of the inter-blink intervals during contact lens wear indicated that the blinking action became more regular and the long durations between blinks became less common. The increase in the blink rate is thus due to a reduction in the irregularity of the inter-blink interval, and not to a consistent shortening of the inter-blink interval itself. The blinking action remained normal with no significant change in the number of complete and incomplete blinks.

For the *Discomfort Group* there was a greater change in the regularity of the blinking action during contact lens wear. Before contact lens wear there was a considerable variability between subjects in their frequency distributions. During lens wear the nature of this pattern changed, with all subjects exhibiting a more regular and frequent blinking pattern. The increased blink rate was achieved by a reduction in the range of inter-blink intervals exhibited, with the irregularly between blinks effectively being eliminated. The occurrence of complete or incomplete blinks did not alter during contact lens wear.

layer over the hydrogel contact lens (Young and Efron, 1991; Craig, 2002). As a result, the stability of the tear film is affected in contact lens wear (Guillon, 1986; Guillon and Guillon, 1988, 1993; Young and Efron, 1991). For a stable tear film to be formed on the ocular surface, the contact lens would be required to be entirely biocompatible with the tear fluid and surrounding tissues, allowing a continuous film, complete with lipid layer, to be formed over the surface. The relatively hydrophobic nature of contact lens materials, especially rigid lens materials, does not allow the formation of such a film (Guillon and Guillon, 1993; Craig, 2002).

Pre-lens tear film stability values found in this study were lower than previously reported. Patel (1987) reported pre-lens tear thinning times of 6.1±0.7 secs for experienced contact lens wearers using Igel 67% contact lenses. Bourassa and Benjamin (1989) measured break-up times of 6.3±0.08 secs for rigid gas permeable lenses. Golding et al (1990) reported values of 6-7 secs for symptomatic experienced soft HEMA lens wearers. Young and Efron (1991) measured break-up times of 7.3±0.7 secs for a variety of hydrophilic lenses. Faber et al (1991) reported values of 6.1±1.1 secs for two types of low and high water content material (Optima 38%, Igel, 67%). Higher break-up times of 15.2 secs (7.6-25 secs) have been reported for Igel "Prima" and Bausch & Lomb "Series 70" contact lenses using the Tearscope (Guillon and Guillon, 1988).

The lower values of pre-lens tear film stability found in this study may be due to lower baseline pre-corneal tear film stability, different water content of contact lenses, different instrumentation used, or that the lenses used in this study were previously worn. Most of the previous studies used unworn lenses that presumably had a

smoother anterior surface without scratches and depositions, thus increasing the stability of the pre-lens tear film. Young and Efron (1991) found a trend for high water contact lenses to support a more stable tear film, and this is consistent with the greater surface hydrophilicity of high water content materials. However, high water materials collect more surface deposition, which may result in a reversal of this trend. The mean water content used in the study was 61.3%, which is lower in comparison to most previously reported results. Perhaps this lower percentage, in conjunction with lens deposits, is the key factor for the lower values of pre-lens tear film stability found in this study.

Tear film stability was lower immediately after lens removal compared to pre-wear levels for both the *Comfort* and *Discomfort Group*, with a substantial decrease for the latter group. A number of mechanisms can be proposed to explain this drop. On insertion of the lens, the lipid layer is trapped under the lens and may contaminate the mucous layer, rendering it hydrophobic and poorly wettable (Holly, 1986). Further disruption of the mucous layer is likely to continue from the mechanical presence of the contact lens. This occlusive effect of the lens would exacerbate the effect by preventing resurfacing of the layer with blinking. A number of other studies have suggested that there is a minimal aqueous layer beneath a hydrophilic lens (Polse, 1979; Fatt and DiMartino, 1985; Bruce and Brennan, 1988; Faber et al., 1991). This may arise from deficiency of the lipid layer of the pre-lens tear film compared to the normal pre-corneal lipid layer, leading to an increased evaporation, lens dehydration, and subsequent evaporation of the post-lens aqueous layer (Fatt, 1989). This reduction of post-lens tear film aqueous, combined with a disturbance of the pre-corneal mucous layer, provides a possible explanation for the observed loss of tear

film stability found in our study after a few hours contact lens wear. The further reduction of the post-lens tear film stability found in the *Discomfort Group* probably arises from the reduction of post-lens tear film aqueous. The measurement of break-up time after lens removal can provide useful information for estimating the extent to which the tear film under a soft contact lens is disturbed (Kline and DeLuca, 1975; Hamano, 1981; Faber et al., 1991).

This study was the first to consider the effect of hydrogel contact lenses on blinking behaviour in a group of experienced lens wearers and to take into consideration the ocular comfort response of the subjects. Previous studies considered only the effect of soft lenses on blink rate, in naïve subjects (Brown et al., 1973; Carney and Hill, 1984; Pointer, 1988). All of these studies recorded an increased blink rate during the first weeks of soft contact lens wear, apart from Brown et al (1973) who found that the blink rate was essentially unaffected by the initial wear of soft lenses.

In conclusion, this study has shown that lens wear discomfort increases with decreasing tear-film stability, producing an increased blink-rate. There is a significant increase in the blink rate in soft lens wear, and the degree of change is dependent on the comfort level of the subject. Discomfort causes an elevated blink rate in order to provide a sharp image. Assessment of tear film stability before contact lens wear was strongly correlated to the blink rate of the subjects, repeating the previous finding that changes in tear film stability, occurring during the inter-blink intervals, are detected by the corneal nerves. Tear film stability during contact lens wear was also correlated with the blink rate of the subjects, and the blink rate was found to be quick enough to avoid full tear film break-up time. This was sufficient to maintain complete wetting

of the lens surface throughout the inter-blink interval, in order to lubricate the surface of the lens and enhance lens comfort. The findings of this study are useful to practitioners, since discomfort during contact lens wear is closely related to the tear film stability. Measurements of tear film stability should always be considered before contact lens fitting, since it was found to be responsible for the initial and ongoing comfort.

9. Final Discussion

Why is blinking so interesting? Everybody blinks regularly everyday, and we do so for a number of reasons, including for: protection of the eye from external noxious threats - physical or imagined, mechanical or chemical; shielding of the eye from bright sunshine; assisting the tear film in removing foreign bodies; helping form the tear film and allowing the tear film to complete its functions, and it can even be used for communication by winking!

However, these actions are affected by various conditions. There is evidence that various mental activities including reading, visual task activities, memory use or emotions modify blink rate (Stern et al., 1984; Karson, 1988). The performance of simple behavioural tasks such as listening, talking or arithmetical exercises significantly increases the blink rate (Karson et al., 1981; Tanaka and Yamaoka, 1993). The use of video display terminals that require strong visual attention has been reported to decrease blink rate (Patel et al.,1991; Tsubota and Nakamori, 1993; Acosta et al., 1999). These findings suggest that blink frequency depends on the type and difficulty of the task, as well as on the degree of attention (Stern, 1994). Significantly altered blink frequencies are observed in several neuro-psychiatric disorders that are known to affect dopaminergic neuro-transmission (Taylor et al., 1999; Kimber and Thompson; 2000) and blink activity can be influenced by experimental manipulation of dopaminergic circuits in the basal ganglia. . Blinking is also influenced by sensory stimuli arising from the exposed ocular surface, and consequently by the environmental factors that favour tear evaporation (Nakamori et al., 1987). It can

therefore be seen that the factors involved in the control of spontaneous blinking are likely to involve central and peripheral triggers.

The blinking action is also interesting because it is a regular action. Excluding blinking that occurs in response to external threats, sunshine or under voluntary control, involuntary blinking takes place during our waking hours without us actually being aware of it occurring. The next question that arises is, why do we blink without thinking? Is there an unconscious metronome in our brain that triggers a blink every x seconds, or do we blink in response to stimuli?

The reports of cortical control of blinking suggest a "blink-trigger" centre in the brain whose action is modified by other areas of the brain. Similar autonomic control is used for other physical activities such as heart rate or breathing. There are certain advantages of such a regular unconscious mechanism in maintaining the function that the action serves. For example, in blinking the important function of tear film volume renewal and remove of foreign bodies would be maintained, even without any ocular surface stimulus.

However, just as other brain areas can influence this "blink-trigger" centre ocular surface conditions, can also be involved in modifying the blink frequency. This thesis assessed the role of the tear film and corneal nerves in the normal blink mechanism, based on the hypothesis that localised cooling in the tear film, produced by tear thinning prior to tear break up, is detected by the corneal nerves, thereby triggering a blink.

From the series of observations that were conducted, it was found that the blink frequency was strongly correlated with tear film break-up time: the more stable the tear film, the less frequent the blinks. Therefore, it seems conceivable that temperature change and thinning of the tear film, during eye opening, act as stimuli for the corneal sensory nerves and trigger a blink before full break-up occurs. More sensitive eyes are better able to detect these changes than less sensitive eyes, and trigger more blinks. On the other hand, less sensitive corneas are not able to detect the early changes, thus producing a lower blink frequency.

The next question then is, what stimuli are the corneal nerves detecting? Since blink rate is initiated before full break-up, and cooling of the ocular surface occurs during the inter-blink interval, the hypothesis was made that the size or rate of temperature change during the inter-blink interval could be the trigger for normal blinking. However, no such relationship was found, but this may be because the measurements of temperature change were taken only at the centre of the cornea and not across the ocular surface. The overall temperature change produced by tear thinning during the inter-blink interval would be a better measurement for correlation with the blink frequency. Since tear film thinning and break-up occurs randomly across the ocular surface, it is highly unlikely that tear thinning will occur at the same location as temperature measurement. In any case, if we consider the corneal nerve architecture, the large overlapping receptive fields promote summation of temperature change across the surface, rather than localisation at a particular corneal location. By measuring total ocular surface temperature change a better understanding of the temperature change stimuli to the corneal nerves would be obtained. In addition, if the thermal camera was modified to assess tear film thinning and break-up

simultaneously with thermography (perhaps by using fluorescein or incorporating a Mengher grid) the actual effect of tear thinning on tear film temperature at specific corneal locations could be determined.

Since temperature change is moderated, to some extent, by tear film evaporation, and tear film thinning may produce increased evaporation, measuring tear evaporation rate was considered as an alternative way of assessing the blink trigger. However, evaporative tear loss during the inter-blink interval was not found to be correlated with the blink frequency. This was not unexpected since the Servo-Med Evaporimeter measures across the full ocular surface and is unable to assess local variation in evaporation rate. Similarly, the temperature change was not related to evaporation rate due to these disparities in measurement techniques.

We can therefore conclude that blinking is affected by changes in the tear film that are detected by corneal nerves, provoking a blink. However, the mechanism is not clear, other than a change in the ocular surface temperature, but the amount of change in temperature required was not determined in these studies.

Considering these interactions between corneal sensitivity, tear film break-up time and blink rate, a circular interacting model can therefore be proposed (Fig 9.1).

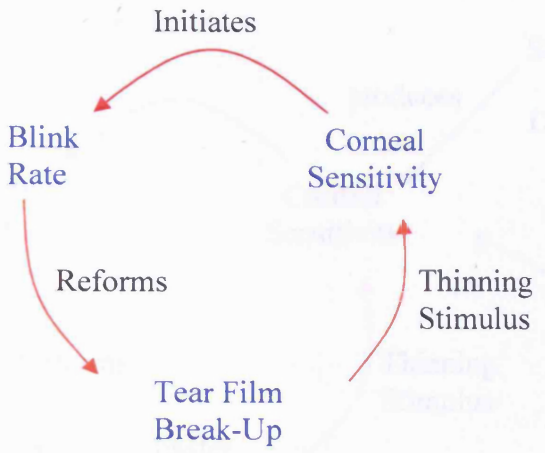


Fig 9.1: Circular interacting model between corneal sensitivity, tear film break-up time and blink rate.

When this model is functioning correctly, ocular surface health is maintained. We can also deduce that:

1) High corneal sensitivity detects early changes in the tear film producing a higher blink rate.
2) More frequent blinking improves tear film stability by increasing tear film aqueous and lipid production.
3) Good tear film stability promotes corneal epithelial health preserving corneal nerve function.

However, if any of the three parameters of the model are altered, dry eye may be produced (Fig 9.2).

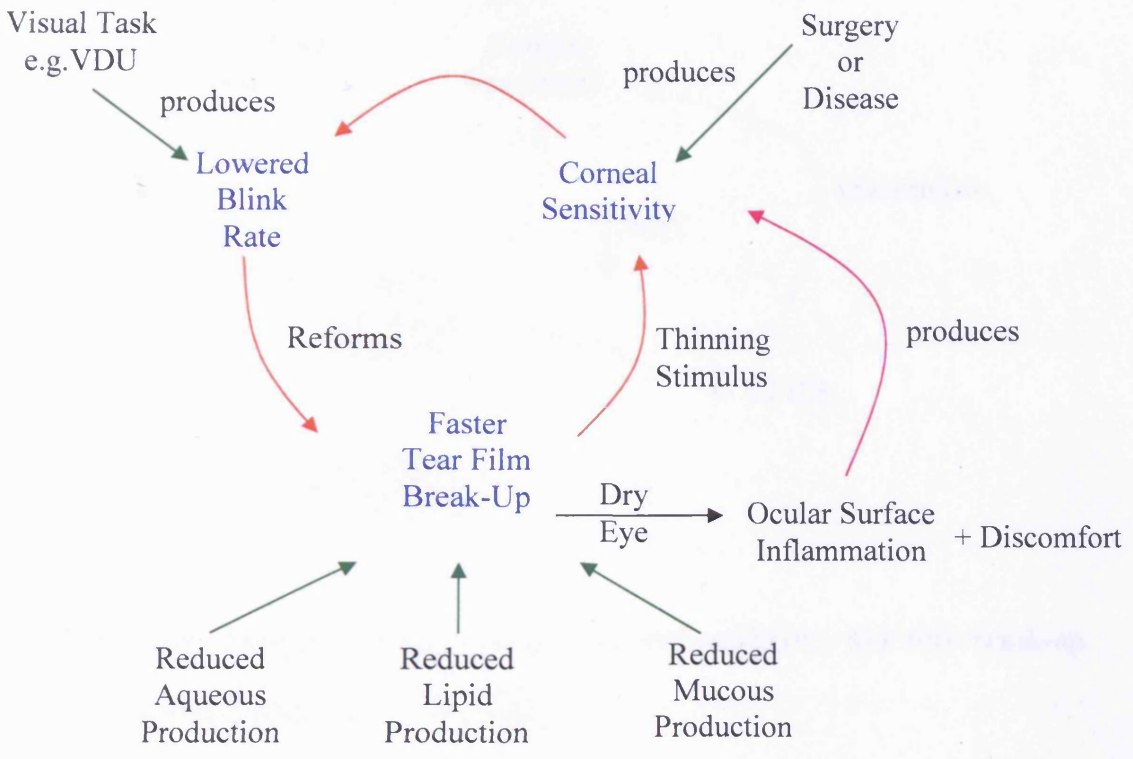


Fig 9.2: Altered circular interacting model between corneal sensitivity, tear film break-up time and blink rate.

From this altered model it can be seen that various factors can influence the normal function of each parameter. Although each parameter has some capacity to accomodate to these influences, there is a limit to the adjustments that can be made. The ultimate consequence is the production of the signs and symptoms of dry eye.

An example of a break down in this model was observed in the contact lens discomfort study reported in Chapter 8. For all contact lens wearers, tear film stability was reduced, promoting an increased blink rate in response to the more frequent detection of tear film changes by the corneal nerves.

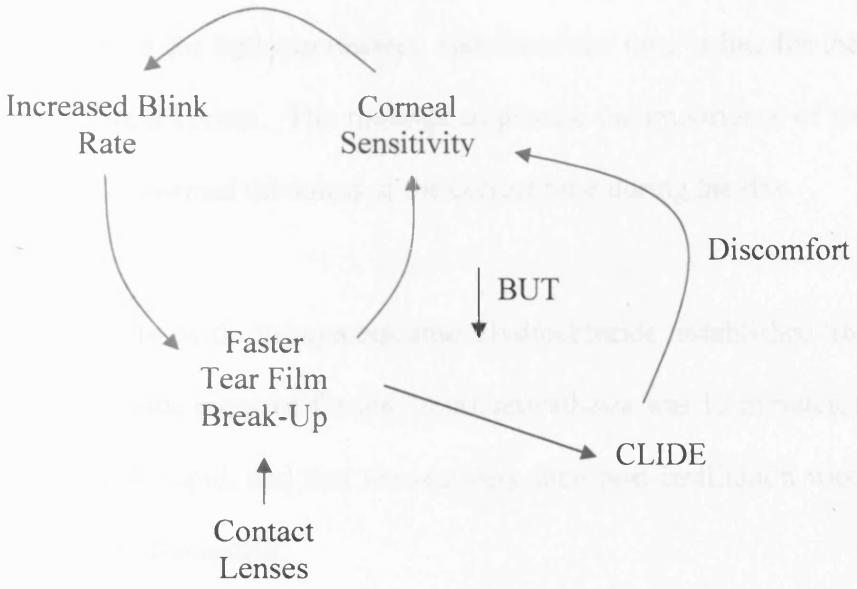


Fig 9.3: Circular interacting model between corneal sensitivity, tear film break-up time and blink rate during contact lens wear.

The patients with discomfort had a lower tear film stability than the patients without discomfort, indicating the development of Contact Lens Induced Dry Eye (CLIDE). This condition is not as severe as pathological dry eye, although the signs and symptoms are often similar. Resolution of the condition is easily made by ceasing contact lens wear. However, the rapid tear film break-up with contact lens wear leads to exposure of the corneal surface, stimulating the nerves and leading to discomfort. This was the situation observed in our patients.

Apart from the studies considering the role of ocular surface conditions in the blink mechanism, significant results were found in the three preliminary studies.

The study on diurnal variation in corneal sensitivity and thickness established the diurnal change pattern for both parameters, and found the time points for the highest sensitivity and thinnest cornea. The findings emphasise the importance of measuring corneal sensitivity or corneal thickness at the correct time during the day.

The anaesthetic study with Proxymetacaine Hydrochloride established the useful information that the time required for maximum anaesthesia was 15 minutes, although onset was much more rapid, and that the recovery time post-instillation was greately prolonged to at least 60 minutes.

This study also suggested a weak anaesthetic effect in the tested eye from contralateral anaesthesia. Such an unusual finding has not been detected previously for an anaesthetic, although sympathetic swelling in the fellow cornea of an eye with a low Dk soft lens has been noted (Fonn et al., 1999). Further work on this contralateral effect is proposed in order to determine the possible mechanism of action. By increasing the anaesthetic dose or duration of anaesthesia it could be proved whether systemic absorption is causing the effect.

The iris colour study found that as iris pigmentation increases, corneal sensitivity decreases. The findings were in accordance with previous results that assessed mechanical corneal sensitivity. The fact that the same variation in corneal sensitivity was found for both the mechanical and cold sensors of the corneal epithelium is a good indicator that the different nerve receptor types are affected by the same factors. The reason for this variation has been attributed to the correlation that might exist between the melanin in the iris and the amount of neuro-melanin in areas of the

central nervous system. It would therefore been interesting for future work to consider how the neuro-melanin may affect the architecture and fine nerve endings of the corneal nerves.

In conclusion, this series of studies on corneal sensitivity, tear film and blinking has been able to assess their interaction and help in the understanding of a possible model explaining their relationship. Further work is now necessary to test the model's usefulness for clinical practice.

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Appendix: 1. RAW DATA

An Investigation of the Diurnal Variation of Corneal Sensitivity and Thickness

Non Contact Corneal Aesthesiometer Measurements (NCCA) (mbars)

Subject	08.00	09.00	10.00	11.00	12.00	14.00	16.00	18.00	20.00	22.00
1	0.35	0.25	0.2	0.2	0.15	0.15	0.15	0.1	0.1	0.05
2	0.75	0.7	0.6	0.65	0.55	0.65	0.6	0.4	0.4	0.4
3	0.55	0.4	0.35	0.25	0.2	0.2	0.2	0.2	0.2	0.15
4	0.4	0.35	0.4	0.35	0.2	0.45	0.35	0.35	0.35	0.2
5	0.35	0.25	0.25	0.2	0.15	0.15	0.15	0.15	0.15	0.15
6	0.4	0.3	0.25	0.25	0.2	0.15	0.15	0.15	0.15	0.15
7	0.4	0.35	0.3	0.3	0.25	0.25	0.25	0.25	0.25	0.25
8	0.35	0.25	0.2	0.2	0.15	0.15	0.15	0.15	0.15	0.15
9	0.35	0.3	0.3	0.25	0.25	0.2	0.2	0.2	0.15	0.15
10	0.4	0.35	0.3	0.3	0.25	0.2	0.2	0.3	0.25	0.2
11	0.55	0.4	0.35	0.35	0.25	0.2	0.2	0.2	0.2	0.2
12	0.6	0.55	0.5	0.45	0.4	0.4	0.4	0.4	0.4	0.35
13	0.25	0.2	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
14	0.7	0.65	0.55	0.5	0.45	0.45	0.45	0.4	0.4	0.4
15	0.55	0.55	0.45	0.4	0.35	0.35	0.35	0.35	0.35	0.35
16	0.25	0.25	0.2	0.2	0.15	0.15	0.15	0.15	0.15	0.15
17	0.35	0.3	0.3	0.25	0.2	0.2	0.2	0.2	0.2	0.2
18	0.8	0.75	0.65	0.65	0.6	0.6	0.55	0.6	0.6	0.6
19	0.6	0.6	0.5	0.45	0.45	0.45	0.45	0.45	0.5	0.45
20	0.7	0.6	0.6	0.55	0.5	0.5	0.5	0.5	0.55	0.5
Mean	0.483	0.418	0.370	0.345	0.293	0.300	0.290	0.283	0.283	0.260
SD	0.17	0.17	0.15	0.15	0.15	0.17	0.15	0.14	0.15	0.15

Cochet-Bonnet Aesthesiometer Measurements (gr/mm^2)

Subject	08.00	09.00	10.00	11.00	12.00	14.00	16.00	18.00	20.00	22.00
1	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
2	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
6	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
7	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
8	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
9	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
10	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
11	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
12	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
13	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
14	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
15	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
16	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
17	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
18	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
19	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
20	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Mean	0.415	0.410	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
SD	0.036635	0.030779	4.83E-09	4.83E-09	4.83E-09	4.83E-09	4.83E-09	4.83E-09	4.83E-09	4.83E-09

Haag-Streit Pachometer Measurements (mm)

Subject	08.00	09.00	10.00	11.00	12.00	14.00	16.00	18.00	20.00	22.00
1	0.59	0.58	0.59	0.58	0.56	0.58	0.6	0.57	0.56	0.54
2	0.6	0.58	0.54	0.56	0.55	0.53	0.49	0.49	0.47	0.47
3	0.6	0.59	0.57	0.54	0.52	0.56	0.58	0.52	0.54	0.53
4	0.56	0.54	0.52	0.51	0.5	0.5	0.52	0.52	0.52	0.5
5	0.54	0.54	0.54	0.52	0.52	0.5	0.54	0.58	0.56	0.56
6	0.54	0.52	0.54	0.53	0.51	0.51	0.52	0.57	0.56	0.54
7	0.5	0.46	0.48	0.46	0.44	0.48	0.46	0.44	0.46	0.46
8	0.56	0.54	0.5	0.5	0.5	0.46	0.49	0.48	0.5	0.48
9	0.57	0.55	0.54	0.54	0.54	0.54	0.54	0.53	0.53	0.53
10	0.56	0.54	0.53	0.54	0.52	0.52	0.54	0.54	0.53	0.52
11	0.52	0.49	0.48	0.47	0.48	0.48	0.47	0.48	0.5	0.48
12	0.44	0.42	0.42	0.43	0.42	0.42	0.44	0.42	0.45	0.43
13	0.5	0.47	0.44	0.43	0.44	0.46	0.45	0.44	0.44	0.45
14	0.54	0.52	0.48	0.54	0.5	0.5	0.54	0.5	0.5	0.51
15	0.51	0.48	0.46	0.48	0.44	0.46	0.48	0.48	0.5	0.5
16	0.58	0.56	0.54	0.5	0.46	0.46	0.46	0.46	0.44	0.44
17	0.5	0.48	0.48	0.46	0.5	0.48	0.46	0.44	0.5	0.5
18	0.48	0.48	0.46	0.46	0.45	0.46	0.45	0.49	0.48	0.48
19	0.52	0.51	0.48	0.48	0.51	0.48	0.48	0.52	0.54	0.56
20	0.44	0.42	0.42	0.4	0.42	0.41	0.41	0.42	0.4	0.43
Mean	0.53	0.51	0.50	0.50	0.49	0.49	0.50	0.49	0.50	0.50
SD	0.05	0.05	0.05	0.05	0.04	0.04	0.05	0.05	0.05	0.04

Patient Training with the NCCA (test/re-test of NCCA)

Day 1

Subject	08.00	09.00	10.00	11.00	12.00	14.00	16.00	18.00	20.00	22.00
1	0.35	0.25	0.2	0.2	0.15	0.15	0.15	0.1	0.1	0.05
2	0.75	0.7	0.6	0.65	0.55	0.65	0.6	0.4	0.4	0.4
3	0.55	0.4	0.35	0.25	0.2	0.2	0.2	0.2	0.2	0.15
4	0.35	0.25	0.25	0.2	0.15	0.15	0.15	0.15	0.15	0.15
5	0.4	0.3	0.25	0.25	0.2	0.15	0.15	0.15	0.15	0.15
6	0.4	0.35	0.3	0.3	0.25	0.25	0.25	0.25	0.25	0.25
7	0.35	0.25	0.2	0.2	0.15	0.15	0.15	0.15	0.15	0.15
Mean	0.45	0.36	0.31	0.29	0.24	0.18	0.17	0.10	0.10	0.11
SD	0.15	0.16	0.14	0.16	0.14	0.18	0.17	0.10	0.10	0.11

Day 2

Subject	08.00	09.00	10.00	11.00	12.00	14.00	16.00	18.00	20.00	22.00
1	0.25	0.2	0.15	0.15	0.15	0.15	0.15	0.15	0.1	0.15
2	0.50	0.5	0.45	0.45	0.4	0.35	0.4	0.3	0.25	0.3
3	0.5	0.4	0.3	0.2	0.2	0.2	0.15	0.15	0.15	0.1
4	0.3	0.3	0.25	0.25	0.2	0.15	0.15	0.15	0.15	0.15
5	0.25	0.2	0.2	0.15	0.1	0.15	0.15	0.15	0.15	0.1
6	0.35	0.3	0.3	0.25	0.25	0.2	0.2	0.25	0.25	0.2
7	0.25	0.2	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Mean	0.34	0.30	0.26	0.23	0.21	0.19	0.19	0.19	0.17	0.16
SD	0.11	0.12	0.11	0.11	0.10	0.07	0.09	0.06	0.06	0.07

An Investigation of the Anaesthetic Effect of 0.5% Proxymetacaine Hydrochloride (Proparacaine) on Corneal Sensation

Instillation of Proxymetacaine-Proxymetacaine

Corneal Sensitivity Threshold (mbars)

Subject	Baseline	2 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	Iris Colour
1	0.70	1.00	1.15	1.90	2.25	2.15	1.95	0.85	0.80	Brown
2	1.20	2.80	4.30	4.70	5.00	5.10	4.85	2.70	1.70	Blue
3	0.90	1.50	2.05	2.35	2.45	1.85	1.70	1.70	1.60	Brown
4	1.70	2.15	2.20	2.35	2.45	1.85	1.75	1.70	1.70	Blue
5	1.30	1.85	2.05	2.80	2.95	1.70	1.60	1.55	1.40	Brown
6	0.85	3.15	3.25	3.45	3.80	3.70	3.20	2.30	2.05	Blue
7	0.90	1.65	2.00	2.60	2.85	4.30	2.30	1.95	1.95	Brown
8	0.70	2.90	3.00	2.40	2.20	1.80	1.50	1.40	1.05	Brown
9	1.45	1.75	2.90	3.35	3.95	3.85	3.75	3.00	2.00	Brown
10	0.95	2.45	3.90	4.00	4.40	2.70	1.90	1.70	1.55	Blue
11	1.15	2.50	4.00	4.90	5.20	3.60	2.75	1.30	1.20	Brown
12	0.85	0.90	1.85	1.85	2.10	1.65	1.40	1.00	0.90	Blue
13	1.30	2.95	3.60	4.30	4.55	3.30	2.30	1.90	1.75	Blue
14	0.85	1.30	2.35	4.50	4.80	3.65	3.10	2.10	1.65	Brown
15	0.10	0.30	0.50	0.95	0.70	0.50	0.40	0.20	0.15	Brown
16	0.85	1.05	1.25	1.65	2.05	1.80	1.35	1.00	0.95	Blue
17	0.60	1.10	1.90	2.95	3.30	2.70	1.85	1.45	1.05	Blue
Mean	0.96	1.84	2.49	3.00	3.24	2.72	2.21	1.64	1.38	
SD	0.37	0.85	1.08	1.17	1.27	1.20	1.05	0.69	0.51	

Instillation of Proxymetacaine-Saline

Subject	Baseline	2 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	Iris Colour
1	0.65	1.80	2.50	2.70	3.05	2.70	1.65	1.30	1.20	Brown
2	1.10	3.75	3.95	4.75	5.10	5.45	4.00	2.35	1.80	Blue
3	1.00	1.20	1.30	3.15	3.20	2.70	2.00	1.40	1.25	Brown
4	1.65	2.15	2.75	2.90	3.20	2.50	2.20	1.80	1.80	Blue
5	0.90	1.05	2.70	3.05	3.70	3.45	2.60	1.55	1.45	Brown
6	0.90	1.15	2.85	3.15	3.30	2.70	1.80	1.40	1.20	Blue
7	0.75	1.65	2.05	2.35	2.65	1.60	1.20	1.05	1.00	Brown
8	0.65	1.40	1.90	2.05	2.20	1.45	0.75	0.55	0.50	Brown
9	1.40	1.75	3.00	3.10	3.50	2.45	1.70	1.60	1.25	Brown
10	0.80	2.20	2.70	2.70	3.60	2.55	2.15	1.65	1.25	Blue
11	0.80	3.15	3.40	4.45	6.05	5.70	3.85	1.85	1.10	Brown
12	0.80	1.15	2.15	2.45	2.80	2.45	1.90	1.80	1.70	Blue
13	1.35	4.25	5.90	6.85	6.95	6.10	5.10	4.80	4.30	Blue
14	0.90	2.10	3.10	3.35	3.55	2.95	2.70	1.90	1.40	Brown
15	0.10	0.75	0.85	0.95	1.05	0.30	0.45	0.10	0.10	Brown
16	0.90	1.75	1.95	2.30	2.45	1.85	1.50	1.40	1.10	Blue
17	0.65	1.30	2.20	2.55	3.05	2.45	1.90	1.20	0.90	Blue
Mean	0.90	1.91	2.66	3.11	3.49	2.90	2.20	1.63	1.37	
SD	0.35	0.97	1.12	1.29	1.41	1.53	1.18	0.97	0.87	

Instillation of Saline-Saline

Subject	Baseline	2 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	Iris Colour
1	0.6	0.65	0.55	0.6	0.55	0.55	0.5	0.55	0.6	Brown
2	1.05	1.1	1.05	1.1	1.05	1	1	1.05	1.05	Blue
3	1	1.05	1	0.95	0.95	0.9	0.95	1	0.8	Brown
4	1.5	1.55	1.55	1.5	1.45	1.55	1.6	1.5	1.5	Blue
5	0.9	0.95	0.9	0.85	0.9	0.9	0.95	0.9	0.9	Brown
6	0.95	1.05	0.95	0.9	0.9	1	0.95	0.95	0.9	Blue
7	0.95	1.15	1.05	1	1	0.95	1	0.95	0.9	Brown
8	0.85	0.95	0.9	0.85	0.85	0.8	0.85	0.9	0.85	Brown
9	1.35	1.45	1.35	1.3	1.35	1.35	1.4	1.3	1.3	Brown
10	0.95	0.9	0.95	0.95	0.9	1	1	1	1	Blue
11	0.75	0.8	0.75	0.75	0.75	0.75	0.75	0.75	0.75	Brown
12	1.05	1.15	1.05	1.1	1.1	1.05	1.1	1.05	1	Blue
13	1.35	1.55	1.35	1.3	1.35	1.35	1.4	1.3	1.3	Blue
14	1.1	1.05	1.1	1.1	1.05	1.1	1.1	1.1	1.15	Brown
15	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	Brown
16	0.75	0.75	0.75	0.75	0.75	0.8	0.75	0.75	0.7	Blue
17	0.65	0.75	0.65	0.65	0.6	0.55	0.65	0.65	0.6	Blue
Mean	0.93	1.00	0.94	0.93	0.92	0.92	0.94	0.93	0.91	
SD	0.32	0.34	0.33	0.32	0.33	0.34	0.35	0.32	0.32	

Instillation of Saline-Proxymetacaine

Subject	Baseline	2 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	Iris Colour
1	0.60	0.75	0.80	0.75	0.75	0.75	0.60	0.65	0.70	Brown
2	1.10	1.75	2.20	1.95	1.85	1.75	1.60	1.55	1.50	Blue
3	1.00	1.35	1.55	1.45	1.30	1.30	1.25	1.35	1.25	Brown
4	1.60	1.75	1.75	1.90	1.80	1.75	1.65	1.65	1.60	Blue
5	0.90	1.35	1.45	1.20	1.20	1.05	1.05	1.00	0.90	Brown
6	0.95	1.10	1.15	1.10	1.10	1.10	0.95	0.95	0.95	Blue
7	0.90	1.30	1.35	1.25	1.10	1.10	1.00	1.00	0.90	Brown
8	0.75	0.80	0.95	1.20	0.95	0.95	0.85	0.60	0.60	Brown
9	1.20	1.45	1.55	1.75	1.95	2.20	1.70	2.00	1.95	Brown
10	0.95	0.90	1.75	1.60	1.30	1.00	1.10	1.20	1.35	Blue
11	0.85	1.50	1.90	1.70	1.40	1.20	1.10	1.05	0.90	Brown
12	0.85	0.90	1.15	1.40	1.60	1.75	1.55	1.40	1.35	Blue
13	1.20	1.40	1.55	1.50	1.45	1.35	1.35	1.30	1.25	Blue
14	1.45	1.75	1.70	1.75	1.65	1.70	1.60	1.55	1.50	Brown
15	0.10	0.20	0.15	0.10	0.10	0.10	0.10	0.10	0.10	Brown
16	0.80	0.75	0.75	0.70	0.70	0.70	0.60	0.60	0.70	Blue
17	0.65	0.85	0.75	0.65	0.65	0.70	0.75	0.70	0.65	Blue
Mean	0.93	1.17	1.32	1.29	1.23	1.20	1.11	1.10	1.07	
SD	0.34	0.43	0.52	0.51	0.49	0.52	0.45	0.48	0.46	

An Investigation of the Effect of Iris Colour and Ethnic Origin on Corneal and Skin Sensitivity and on Tear Film Stability and Blink Rate

Caucasians

CS: Corneal Sensitivity Threshold *SS*: Skin Sensitivity Threshold, *TBUT*: Tear Break-Up Time Blinks/min: Blink Rate

Grade 1

Subject	CS (mbars)	SS (mbars)	TBUT (sec)	Blinks/min
1	0.7	0.6	29.47	5.6
2	0.45	0.6	6.79	3.4
3	0.75	0.45	15.42	7
4	0.7	0.65	11.55	16.4
5	0.55	0.5	6.01	25
6	0.7	0.65	4.02	11.6
7	0.55	0.65	5.46	21
8	0.5	1.3	11.3	16.8
9	0.65	0.5	5.87	51.6
10	0.65	0.65	8.95	11.2
11	0.85	0.7	7.03	8
12	0.8	0.65	9.3	7.2
13	0.75	0.25	7.29	30.2
14	0.7	0.65	7.99	26.8
15	0.9	0.6	9.35	7.4
16	0.8	0.85	25.4	3.8
17	0.85	0.85	6.84	7.8
18	0.35	0.3	29.97	6.8
19	0.5	0.45	3.33	24.2

20	0.4	0.85	7.36	24.6
Mean	0.66	0.64	10.94	15.82
SD	0.16	0.22	7.99	12.01

Grade 2

Subject	CS (mbars)	SS (mbars)	TBUT (sec)	Blinks/min
1	0.75	0.55	9.3	19
2	0.65	0.6	6	24.4
3	0.95	0.85	8.56	11.4
4	0.85	0.55	3.63	23.6
5	0.9	0.5	12.87	14.2
6	1	0.85	9.17	11.6
7	0.55	0.65	5.56	11.4
8	0.9	0.9	9.14	14.8
9	0.85	0.75	13.11	31.6
10	0.7	0.75	12.38	13.6
11	0.85	0.45	3.63	34.6
12	0.55	0.55	8	11
13	0.85	0.4	4.99	28.4
14	0.55	0.75	5.03	21.4
15	0.1	0.6	10.89	13.4
16	0.6	0.45	5.93	20
17	0.9	0.4	13.69	7.6
18	0.35	0.5	6.98	30.6
19	0.6	0.55	8.73	13.6
20	0.65	0.45	13.63	8.8
Mean	0.71	0.60	8.56	18.25
SD	0.22	0.16	3.34	8.17

Grade 3

Subject	CS (mbars)	SS (mbars)	TBUT (sec)	Blinks/min
1	0.9	0.85	8.42	12.2
2	0.9	0.55	8.98	21.8
3	0.8	1.15	10.11	7.8
4	1.3	1.15	6.27	7.2
5	0.95	0.55	13.22	8
6	0.9	0.85	8.4	10.4
7	1.4	1.5	8.46	13.2
8	0.6	0.7	10.89	18.6
9	0.6	0.65	12.49	24.2
10	0.55	0.75	24.12	4.4
11	0.65	0.35	19.91	6.4
12	0.6	0.7	2.96	23.2
13	0.65	0.3	21.2	7.2
14	0.65	0.45	7.28	15
15	0.95	0.45	5.04	22
16	1.75	0.7	6.47	12.2
17	0.6	1	14.87	7.6
18	0.75	0.85	21.22	12.4
19	0.65	0.35	15.75	8
20	1	0.4	8.36	23.6
Mean	0.85	0.73	11.90	12.73
SD	0.31	0.31	6.00	6.62

Grade 4

Subject	CS (mbars)	SS (mbars)	TBUT (sec)	Blinks/min
1	0.75	0.4	5.37	16
2	1.25	0.95	15.9	9
3	0.9	0.3	5.1	8
4	0.95	0.8	8.42	21.6
5	1.4	0.6	12.47	12.8
6	1.35	0.8	5.65	18
7	1.2	0.85	5.84	31.2
8	1.45	2.15	4.98	37.6
9	1.05	1.2	6.39	15.2
10	1.05	0.8	22.92	9.2
11	1.1	0.6	15.37	4
12	0.85	0.35	10.53	10.8
13	1.45	0.55	15.3	11
14	0.95	0.6	15.21	5.4
15	1.05	0.55	15.58	11
16	0.4	0.45	4.39	26.4
17	0.95	1	17.51	3
18	0.35	0.7	3.02	24
19	0.7	0.7	11.3	17.2
20	1.05	0.7	7.35	16.4
Mean	1.01	0.75	10.43	15.39
SD	0.31	0.40	5.56	9.10

Grade 5

Subject	CS (mbars)	SS (mbars)	TBUT (sec)	Blinks/min
1	1.05	0.6	12.38	9.4
2	1.3	0.45	15.6	5.2
3	1.7	0.75	45.9	2
4	1	0.8	7.64	19.4
5	1.4	0.45	19.27	7
6	0.85	0.6	10.65	29
7	1.1	0.6	15.37	4
8	1.05	0.8	22.92	9.2
9	1.65	1.8	20.45	3
10	0.9	0.35	19.64	4.6
11	0.55	0.80	7.04	8.00
12	1.15	0.85	14.73	6.6
13	1.1	1.05	17.28	6.3
14	1.45	1.8	14.85	8.33
15	0.8	0.65	16.35	10
16	1.35	0.4	19.45	5.6
17	0.65	0.55	6.56	12.6
18	0.5	0.8	5.36	18.6
19	1.5	0.85	16.6	4.6
20	1.15	0.6	15.4	8
Mean	1.11	0.78	16.17	9.07
SD	0.34	0.39	8.58	6.52

Assians

Grade 4

Subject	CS (mbars)	SS (mbars)	TBUT (sec)	Blinks/min
1	0.9	0.65	16.7	10.8
2	0.85	0.35	14.07	4.8
3	0.85	0.65	6.64	41.8
4	0.8	0.45	25.89	10.6
5	0.8	0.9	4.36	26.8
6	0.85	1.3	14.6	15.6
7	1.8	0.6	8.73	21.2
8	0.9	1.15	16.57	6
9	1.3	0.6	5.48	21
10	0.45	1.2	3.36	14.4
11	0.65	1.05	5.17	14
12	0.85	1.1	8.73	10.2
13	0.85	0.55	4.57	13.6
14	0.65	1.7	7.63	15.2
15	0.75	1.05	7.49	18
16	0.6	0.5	16.6	8.6
17	0.75	1.35	4.24	30.4
18	0.9	0.9	15.18	15.18
19	0.7	0.9	22.65	3.4
20	0.7	0.7	19.84	11.2
Mean	0.85	0.88	11.43	15.64
SD	0.28	0.35	6.78	9.20

Grade 5

Subject	CS (mars)	SS (mbars)	TBUT (sec)	Blinks/min
1	0.8	0.9	17.22	10.6
2	0.85	0.75	16.38	11.6
3	0.7	0.55	15.94	15.6
4	1	0.55	10.33	9
5	1.7	0.55	9.13	31.6
6	0.6	0.45	5.96	19
7	0.6	0.55	12.02	4.6
8	0.75	0.8	26.5	4.4
9	1	0.65	29.54	14.6
10	0.8	0.45	9.42	42.2
11	0.95	0.4	16.44	8.6
12	0.85	0.85	26.2	11
13	1.2	1.65	7.02	30.6
14	1	1.1	5.22	26
15	0.85	1.85	16.7	6
16	0.8	0.5	10.75	14
17	1.3	0.85	9.64	14
18	1.15	0.6	13.41	9.4
19	0.4	0.4	5.11	29.2
20	1.4	0.75	18.81	8.4
Mean	0.94	0.76	14.09	16.02
SD	0.30	0.39	7.12	10.46

Chinese

Grade 4

Subject	CS (mbars)	SS (mbars)	TBUT (sec)	Blinks/min
1	0.7	2.35	4.06	22
2	1.2	0.6	10.95	16.6
3	1.55	1.25	11.95	1.8
4	0.9	0.9	5.8	24
5	1	0.85	21.34	4.6
6	0.6	0.4	7.3	39.6
7	0.55	0.55	7.93	31.8
8	0.35	0.65	3.33	40
9	0.75	0.45	14.69	16.2
10	0.8	0.6	13.46	8
11	0.75	0.5	9.65	10
12	0.5	1.05	6.44	7
13	0.7	0.75	9.38	10
14	0.85	0.5	6.67	5.2
15	0.3	0.35	4.09	10
16	0.55	0.45	4.39	33.3
17	0.7	0.65	21.59	6.4
18	0.3	0.6	5.39	9.33
19	0.6	0.65	10.15	11.3
20	0.5	0.75	6.45	15
Mean	0.71	0.74	9.25	16.11
SD	0.30	0.44	5.25	11.79

Grade 5

Subject	CS (mbars)	SS (mbars)	TBUT (sec)	Blinks/min
1	1.2	1.05	4.83	21.6
2	0.95	0.4	3.68	38.8
3	1.7	0.6	12.88	6.4
4	0.55	0.45	11.16	10.2
5	2.3	0.6	17.98	5.3
6	1.6	0.45	13.49	13.8
7	1.5	1	15.33	10.2
8	1	0.9	5.43	29.6
9	1.35	0.45	3.52	26
10	1.35	0.65	6.34	22
11	0.95	0.65	14.41	9.2
12	0.55	0.5	20.67	2.8
13	0.75	0.9	12.3	5.8
14	0.5	0.75	8.04	11
15	1.55	0.6	10.2	4
16	0.25	0.25	3.91	36
17	0.55	1.4	12.77	22
18	0.65	0.45	7.53	4
19	0.3	0.6	19.52	18.8
20	0.75	0.6	4.31	3
Mean	1.02	0.66	10.42	15.03
SD	0.54	0.27	5.48	11.20

Africans

Grade 5

Subject	CS (mbars)	SS (mbars)	TBUT (sec)	Blinks/min
1	1.75	1.5	17.95	4
2	1.20	1.3	29.84	5
3	1.65	2.4	23.78	6.4
4	1	0.25	10.93	9.6
5	1.4	1.05	10.34	7
6	0.95	0.4	10.64	17.2
7	1.05	1.45	7.35	18.2
8	1.05	0.4	4.02	28
9	1.15	1.9	9.97	14.2
10	1	0.95	5.25	33.3
11	1.6	0.6	23.6	8
12	0.85	0.6	4.34	19.2
13	0.95	1.85	7.32	28
14	0.95	0.9	19.72	8
15	1.2	1.3	26.43	4.4
16	1.4	0.6	20.83	4
17	1.6	2.1	18.77	8
18	1.4	2.15	15.85	4
19	2.05	1.35	16.03	5.6
20	1.05	0.55	14.29	8.33
Mean	1.23	1.21	14.10	12.58
SD	0.28	0.67	8.08	9.36

An Investigation of the Ocular Surface Sensory Trigger for Blinking

Visit 1

Subject	CS (mbars)	Evap Baseline ($g/m^2/h$)	Evap after Fluor ($g/m^2/h$)	Baseline Temp (°C)	Temp after Fluor (°C)	Besline Temp Diff at 8 secs (°C)	Temp Diff at 8 sec after Fluor (°C)
1	0.3	62.59	44.30	36.16	35.92	0.88	0.44
2	0.55	80.11	85.92	35.81	35.78	0.43	0.55
3	1.4	74.62	88.94	35.54	35.63	0.92	0.70
4	0.4	89.16	76.62	37.44	36.47	0.49	0.69
5	0.6	86.16	75.74	34.45	34.67	0.38	0.48
6	0.35	44.54	53.47	34.42	34.66	0.54	0.93
7	1.15	64.80	84.92	37.62	37.01	1.02	0.66
8	0.55	100.34	101.05	35.22	35.27	0.51	0.31
9	0.25	158.39	156.10	36.15	36.38	0.63	0.49
10	0.3	41.85	65.70	34.83	35.79	0.74	0.53
11	0.5	41.97	63.62	36.16	36.86	0.47	0.41
12	0.6	69.21	64.79	34.77	34.54	0.48	0.50
13	1.1	36.98	59.80	35.84	36.42	0.68	0.43
14	0.45	147.70	106.60	34.50	34.54	0.91	0.94
15	0.45	57.70	84.42	35.64	35.66	0.89	0.71
16	0.3	84.16	88.59	34.67	34.91	2.15	0.72
17	0.9	44.62	89.71	35.08	35.21	1.02	0.66
18	0.95	69.72	95.95	34.93	34.97	1.09	0.70
19	0.45	112.82	144.84	35.06	35.16	1.27	0.71
20	0.6	56.81	87.88	34.96	35.06	1.21	0.75
Mean	0.61	76.21	85.95	35.46	35.55	0.84	0.61
SD	0.32	33.37	27.34	0.91	0.77	0.41	0.17

Subject	Baseline Temp Change (1/2 life) at 8 secs (secs)	Temp Change (1/2 life) at 8 secs after Fluor (secs)	TBUT (sec)	Blinks/min	IBI (secs)	Complete Blinks	Incomplete Blinks	Room Temp (°C)	Room Humidity (%)
1	0.75	1.61	38	3.6	16.74	18	0	23.8	37
2	1.58	1.62	8.2	22	2.71	110	1	24.3	26
3	0.61	0.80	15.3	10.8	5.57	54	0	24.2	30
4	1.06	0.66	15.04	15.6	3.86	78	0	23.8	33
5	1.26	0.81	18.21	6.6	9.10	29	4	25.5	28
6	1.38	1.86	8.24	18.8	3.18	94	0	26.5	27
7	1.59	0.92	19.36	9	6.69	45	0	25	33
8	0.41	1.29	9.77	17.2	3.49	85	1	24.5	35
9	0.36	0.43	12.74	15.8	3.81	78	1	22.5	32
10	1.72	1.99	25.21	6	9.93	30	0	22.6	39
11	0.94	0.93	16.48	13.8	4.37	69	0	24.4	34
12	1.01	0.30	8.3	28.4	2.11	134	8	26.6	26
13	0.57	0.43	19.12	5.2	11.33	19	7	24.3	34
14	1.42	1.59	9.21	18.4	3.27	92	0	25.7	27
15	0.59	0.47	12.23	20.8	2.88	104	0	25	30
16	0.96	0.83	8.26	28.6	2.12	142	1	26.2	27
17	0.91	0.72	22.3	5.6	10.30	28	0	23	35
18	0.89	0.81	21.33	5.6	10.72	28	0	23.7	36
19	0.95	0.88	16.97	10.2	5.82	50	1	24.2	30
20	0.86	0.74	14.97	19	3.15	94	1	23.4	31
Mean	0.99	0.98	15.96	14.05	6.06	69.05	1.25	24.46	31.45
SD	0.40	0.50	7.31	7.64	3.99	37.98	2.34	1.20	3.84

Visit 2

Subject	CS (mbars)	Baseline Evap ($g/m^2/h$)	Evap 2min Anaes ($g/m^2/h$)	Evap 20 min Anaes (g/m2/h)	Initial Temp Basel (°C)	Temp 2 min Anaes (°C)	Temp 20 min Anaes (°C)	Baseline Temp Cooling at 8 secs (°C)	Temp Cooling at 8 sec 2 min Anaes (°C)
1	0.25	13.02	47.01	62.35	36.23	36.77	35.24	0.698	0.726
2	0.55	29.19	101.49	73.14	35.99	37.78	36.27	0.679	0.866
3	1.3	67.76	84.79	93.48	36.75	36.88	35.81	0.928	1.055
4	0.4	46.34	57.70	97.68	36.69	37.06	36.92	0.558	0.578
5	0.55	30.05	77.19	87.25	35.72	36.39	36.99	0.316	0.459
6	0.45	48.72	63.61	68.54	37.00	36.93	36.56	0.503	0.674
7	1.05	83.26	111.75	109.21	35.84	35.80	37.15	1.574	1.659
8	0.45	141.18	76.19	73.84	35.50	35.80	35.39	1.541	1.659
9	0.25	73.56	53.72	62.11	34.26	34.76	34.85	0.416	1.289
10	0.25	78.99	62.26	70.99	35.45	35.77	34.20	0.649	0.680
11	0.45	46.74	62.16	62.32	36.16	34.66	35.74	0.466	0.616
12	0.65	38.44	56.24	66.93	34.88	35.19	34.50	0.931	0.706
13	1.1	65.02	62.89	69.51	33.80	33.81	35.47	0.596	0.269
14	0.4	84.51	104.90	128.58	36.31	36.31	33.97	0.643	1.050
15	0.5	36.99	38.84	50.88	36.86	37.12	36.32	0.565	0.568
16	0.3	77.24	97.10	108.22	37.12	35.64	36.74	0.730	0.654
17	0.85	38.41	54.23	63.97	35.79	35.61	35.65	0.693	0.649
18	0.85	55.84	75.65	98.49	36.19	35.97	35.63	0.712	0.725
19	0.45	89.79	113.61	170.00	36.49	36.10	35.96	0.674	0.665
20	0.6	75.07	78.82	90.26	36.46	36.13	36.01	0.669	0.729
Mean	0.58	61.01	74.01	85.39	35.97	36.02	35.81	0.73	0.81
SD	0.30	28.81	22.07	28.24	0.87	0.95	0.91	0.32	0.36

Subject	Temp Cooling at 8 secs 20 min Anaes (°C)	Baseline Temp Change (1/2 life) (secs)	Temp Rate Change (1/2 life) 2 mins Anaes (secs)	Temp Change(1/2 life) 20 mins Anaes (secs)	Blinks/min 15 min Anaes	IBI (secs)	Complete Blinks	Incomplete Blinks	Room Temp	Room Humidity
1	0.52	0.71	1.32	1.32	3	20.23	15	0	24.4	30
2	0.81	0.39	0.45	0.45	8.2	7.34	39	2	25.5	29
3	1.50	0.50	0.42	0.42	6.2	9.58	31	0	26	28
4	0.56	0.72	1.05	1.05	5.4	11.04	27	0	23.1	33
5	0.52	1.60	1.81	1.81	2.2	28.72	11	0	22.8	34
6	0.85	1.47	1.46	1.46	7.6	8.08	38	0	25.6	32
7	1.07	1.22	0.69	0.69	8.6	6.99	43	0	23.8	33
8	0.79	1.13	0.69	0.69	9.8	6.05	49	0	24.5	35
9	1.92	0.75	0.62	0.62	12.4	4.99	62	0	23.2	30
10	0.93	1.72	1.36	1.36	2.4	25.40	10	2	21.7	41
11	0.43	0.47	2.04	2.04	4.33	14.11	13	0	24.4	32
12	0.88	0.33	0.73	0.73	6.6	9.13	12	21	25.3	29
13	0.54	0.41	1.09	1.09	4.4	13.30	17	5	24.7	44
14	1.00	2.12	1.50	1.50	8	7.48	40	0	25.7	27
15	0.66	1.00	0.96	0.96	16.6	3.55	83	0	24.8	30
16	0.80	0.51	1.26	1.26	10.2	5.87	48	3	25.6	33
17	0.78	0.87	1.11	1.11	3.8	20.28	14	0	22	35
18	0.82	0.98	1.18	1.18	4.6	12.80	23	0	25.1	33
19	0.78	1.10	1.20	1.20	7.8	7.64	39	0	25.4	29
20	0.81	0.89	1.14	1.14	5.6	10.76	19	9	23.9	30
Mean	0.85	0.94	1.10	0.88	6.89	11.67	31.65	2.10	24.38	32.35
SD	0.34	0.49	0.43	0.28	3.56	6.93	19.42	5.00	1.26	4.18

An Investigation of the Role of Tear Film and Blinking in Contact Lens Wear Discomfort

Comfort Group – Visit 1

Subject	CS (mbars)	Blinks/min	IBI (secs)	Complete Blinks	Incomplete Blinks	TBUT (secs)	NIBUT (secs)	Type of Lenses	% Water	Months of Wear
1	0.35	21.4	2.81	105	2	4.67	6.13	Soflens B&L	66	32
2	0.3	4.8	12.36	24	0	32	34.8	Freshcare Advance	55	32
3	1.15	7.2	8.36	43	3	19.4	21.86	Easy Vision	69	60
4	1.05	19.6	3.07	98	0	7.77	8.93	Cooper Vision Torics	55	25
5	0.25	23.2	2.56	116	0	6.78	7.93	Cooper Vision Proclear	62	28
6	1.35	20.6	2.83	103	0	11.64	12.3	Survue J&J	58	58
7	0.3	13.5	4.46	54	0	13.56	14.78	Easy Vision Specsavers	62	60
8	0.55	17.2	3.51	86	0	8.97	9.42	Cooper Vision Torics	55	36
9	0.25	15.8	3.84	79	0	12.54	13.68	Survue J&J	58	54
10	1.35	18	3.34	89	1	8.99	9.38	Soflens Toric B&L	66	22
11	0.95	26.6	2.26	133	0	9.3	9.83	Acuvue J&J	58	58
12	1.25	12.4	4.79	61	1	14.57	16.27	Standard Lens	55	24
13	0.5	9	6.66	43	2	18.36	20.72	Soflens B&L	59	28
14	1.45	16.2	3.72	79	2	6.15	7.71	Sauflon UV	55	40
15	0.95	24.2	2.49	119	2	4.56	6.87	Cooper Vision Proclear	62	34
Mean	0.80	16.65	4.47	82.13	0.87	11.95	13.37		59.67	39.40
SD	0.46	6.35	2.74	31.70	1.06	7.16	7.64		4.64	14.42

Comfort Group – Visit 2

Subject	Blinks/min during CL	IBI during CL (secs)	Complete Blinks	Incomplete Blinks	NIBUT during CL (secs)	NIBUT after CL Removal (secs)	BUT after CL Removal (secs)	CS after CL Removal (mbars)	Comfort Response
1	20	2.99	99	1	2.53	5.13	4.59	0.35	100
2	11	5.4	55	0	5.53	7.06	6.63	0.25	80
3	10.4	5.76	52	0	4.9	5.8	5.47	1.4	90
4	20.4	2.93	102	0	2.63	8.26	7.45	0.9	75
5	26.4	2.27	132	0	2.26	5.28	4.97	0.75	70
6	34.6	1.73	171	0	1.73	7.56	6.79	1.15	60
7	15.2	5.93	76	0	3.5	8.63	7.98	0.45	85
8	19.2	3.11	93	1	2.16	5.03	4.67	0.5	85
9	12.4	4.84	62	0	4.8	7.13	6.33	0.35	75
10	20.4	2.95	101	1	2.23	6.86	6.22	0.7	95
11	26.4	2.27	132	0	3.1	7.36	6.85	0.95	60
12	16.2	3.7	81	1	4.66	7.6	6.59	1.7	95
13	16.6	3.63	81	2	4.15	8.83	8.49	0.5	100
14	30.6	1.96	153	0	2.33	4.83	4.48	1.6	70
15	27.6	2.17	138	0	2.66	5.33	3.98	0.75	75
Mean	20.49	3.44	101.87	0.40	3.28	6.71	6.10	0.82	81.00
SD	7.28	1.41	36.27	0.63	1.22	1.38	1.36	0.46	13.26

Discomfort Group – Visit 1

Subject	CS (mbars)	Blinks/min	IBI (secs)	Complete Blinks	Incomplete Blinks	TBUT (secs)	NIBUT (secs)	Type of Lenses	% Water	Months of Wear	Comfort Response
1	1.15	18.8	3.18	91	3	7.32	8.16	Dollond&Aichison	55	50	100
2	1.2	13	4.65	65	0	12.49	13.1	Acuvue 2 J&J	58	72	80
3	1.45	9.8	6.14	47	2	17.5	18.53	Focus Dailies	69	35	90
4	0.6	12.6	4.78	63	0	13.54	13.13	EasyVision Specsavers	69	66	75
5	0.9	47	1.28	235	0	2.28	2.97	Acuvue 2 J&J	58	84	70
6	0.4	25.6	2.33	128	0	5.81	6.56	Focus Dailies	69	58	60
7	1.8	12.4	4.71	62	0	11.56	12.48	Cooper Vision Proclear	62	32	85
8	0.4	29.4	2.04	146	1	3.87	4.4	Cooper Vision Proclear	62	20	85
9	1.75	9.6	6.38	43	5	15.22	16.36	Cooper Vision Proclear	62	62	75
10	0.3	23.6	2.56	118	0	10.42	11.13	Soflens B&L	66	56	95
11	0.55	18.2	3.29	91	0	10.08	12.43	EasyVision	69	18	60
12	0.45	43.4	1.39	217	0	2.01	2.96	Focus Monthlies	55	21	95
13	1	11.8	5.11	57	2	16.24	16.63	Survue J&J	58	64	100
14	0.55	30.4	1.98	151	1	3.21	4.54	EasyVision Specsavers	62	45	70
15	0.75	13	4.62	65	0	16.92	17.16	Soflens B&L	66	29	75
Mean	0.88	21.24	3.63	105.27	0.93	9.90	10.70		62.67	47.47	81.00
SD	0.49	11.92	1.68	60.15	1.49	5.50	5.41		5.12	20.74	13.26

Discomfort Group – Visit 2

Subject	Blinks/min during CL	IBI during CL (secs)	Complete Blinks	Incomplete Blinks	NIBUT during CL (secs)	NIBUT after CL Removal (secs)	BUT after CL Removal (secs)	CS after CL Removal (mbars)	Comfort Response
1	29.2	2.05	146	0	1.86	3.03	2.63	1.2	30
2	19.2	3.01	94	2	1.43	5.23	3.7	0.65	35
3	34	1.7	168	2	1.13	4.6	3.65	1.4	35
4	30.6	1.95	153	0	1.65	4.36	3.29	0.45	30
5	48	1.26	236	4	0.9	3.79	2.28	1.2	30
6	37.6	1.59	188	0	2	3.16	3.5	0.5	30
7	32.4	1.85	161	1	1.76	4.06	4.38	1.9	30
8	48.4	1.24	241	1	0.85	2.56	3.48	0.5	30
9	21.6	2.78	108	0	1.1	3.56	3.42	1.55	35
10	43	1.4	215	0	1.23	4.1	3.31	0.4	25
11	34.8	1.72	174	0	2.26	6.03	6.09	0.45	40
12	46.2	1.3	231	0	0.96	1.73	1.13	0.55	30
13	19.8	3.03	97	2	2.73	8.13	6.78	1.45	35
14	32	1.88	160	0	1.26	2.66	2.07	0.55	35
15	28.2	2.12	141	0	2.87	8.5	8.1	0.85	40
Mean	33.67	1.93	167.53	0.80	1.60	4.37	3.85	0.91	32.67
SD	9.62	0.60	47.98	1.21	0.64	1.93	1.84	0.50	4.17

Appendix: 2. Related Publications

1505-M.OF./Review

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The effect of contact lens wear on corneal sensation

A. M. NTOLA, P. J. MURPHY

From the Department of Optometry and Vision Sciences Cardiff University, Wales, UK

The corneal nerves play an important role in the protection and maintenance of corneal health, and the corneal epithelium has the highest density of free nerve endings in the body. Contact lenses are increasingly used to correct refractive error or for cosmetic purposes. It is therefore important to study the relationship between these factors. Studies have revealed that contact lens wear can produce a reduction in corneal sensitivity, with the extent of sensation loss related to the type of contact lens, the material it is made from, and the frequency and duration of wear. In summary, as the time of wear increases, both in the short-term (days) and long-term (-months), the greater the loss of sensation. Recovery to normal levels, with the cessation of lens wear, is also prolonged with extended durations of contact lens wear. Newer lens materials that have improved oxygen permeability have less of an effect. The two principle mechanisms by which the corneal nerves are affected are the mechanical action of the lens and interference with the metabolic function of the cornea, as a result of the reduced oxygen supply. The impaired metabolic function produces an increase in acidosis and a change in corneal pH as a result of hypercapnia. Both of these can alter nerve function, and so reduce corneal sensitivity.

Key words: **Contact lenses - Cornea, physiology - Cornea, metabolism.**

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Address reprint requests to: A. M. Ntola - Cardiff University, Department of Optometry and Vision Sciences, Redwood Building, King Edward VII Avenue, Cardiff CF10 3 NB, Wales, UK.

Contact lenses have become one of the principal methods for correcting refractive error over the last 30 years. The development of the soft contact lens by Wichterle *et al.*[1] created a safe and comfortable method for patients to change from spectacle wear. New developments in lens materials led to the introduction of rigid gas permeable (RGP) lenses and, more recently, to silicone-hydrogel lenses. Indeed, the silicone hydrogel lens holds the promise of the first successful continuous wear lens.[2]

The clinical care of contact lens wear has revealed a wide number of unwanted adverse reactions, *e.g.* corneal oedema, neovascularisation, papillary conjunctivitis, dry eye, marginal ulcers.[3] However, each reaction has encouraged the development of new lens designs and materials to avoid/overcome the problem.

One of the more unusual side effects of contact lens wear is a reduction in corneal sensation. For PMMA (poly-methylmethacrylate) and RGP lens wear, the practitioner is actively encouraging a reduction in sensitivity, but this improved comfort also brings the risk of undetected foreign bodies or pathology. It is this paradoxical situation that we will review in this paper.

Corneal nerve supply

The cornea has the highest density of free nerve endings in the body, and these produce an exquisite level of sensitivity to noxious stimulation.[4] They play a vital role in the detection and prevention of damage to the cornea and anterior ocular surface. The cornea performs several important roles in the eye - transmission of light to the retina, refraction of the light as part of focussing the image, maintaining the intraocular pressure and protection of the internal eye. The tears, corneal nerves and eyelids all perform roles in the defence of these functions. The nerves also play a role in the maintenance and health of the corneal epithelium. Research on rabbits has shown that a total lack of corneal nerve supply will result in impaired wound healing, decreased corneal metabolism and reduced epithelial cell adhesion.[5] The manner in which this influence is exerted is unknown, but may be due to axonally transported substances, such as proteins.[6]

The corneal nerves are derived from the Nasociliary nerve, a branch of the Ophthalmic nerve, a division of the Vth Cranial Nerve (Trigeminal). The nerves supplying the cornea pass along the long ciliary nerve branch of the nasociliary nerve. They penetrate the posterior of the eye and pass between the sclera and choroid, coursing anteriorly to supply the cornea, iris and the sensory fibres of the ciliary body, trabecular meshwork and sclera.

Upon reaching the cornea, 70-80 nerve axons (in man) enter the corneal stroma in a radial fashion from various sites around the corneal limbal circumference. The nerves enter in the middle third of the stroma and run towards the centre of the cornea, giving rise to branches that innervate the anterior and mid stroma. As the axons pass towards the epithelium, they ramify and divide to form a poorly characterised nerve plexus beneath Bowman's Layer in the superficial stroma.[4, 7-9] The nerves then penetrate Bowman's Layer at an estimated 400 sites to enter the basal epithelial layer.[10] As they do so, the nerve bundles lose their remaining Schwann cell coverings. These nerves then combine with nerves that enter the basal epithelium from the limbus to form the basal epithelial plexus.[11]

The nerve fibres continue to divide and ramify anteriorly within the corneal epithelium to distribute free nerve endings across the whole of the cornea anterior surface. The nerve fibres innervating the cornea are of several different types, each responding to a different set of stimuli. These different nerve types are also arranged within the corneal epithelium according to their type - myelinated Aδ fibres that respond to mechanical stimuli run parallel to the corneal surface within the basal cell layer, unmyelinated C fibres that respond to thermal and mechanical stimuli turn upwards from the epithelial plexus towards the surface.[12] These two nerve types are the principal moderators of the corneal nerve response to the current corneal aesthesiometers used to assess corneal sensitivity.

Corneal sensation measurement

The two principal methods used to assess corneal nerve function in this review are the Cochet-Bonnet Aesthesiometer and the Non-Contact Corneal Aesthesiometer (NCCA). The Cochet-Bonnet instrument uses a thin nylon thread that is pressed against the corneal surface.[13] This produces a mechanical deformation in the anterior corneal surface that stimulates the Aδ fibres. A variation in the intensity of the stimulus is achieved by varying the length of the nylon thread, which in turn alters the force that must be applied to produce a bend in the thread. By this indirect method the stimulus intensity can be determined.[14] In contrast, the NCCA uses a controlled pulse of air, of a predetermined intensity and duration, to produce a localised cooling of the pre-corneal tear film.[15] This cooling stimulus is transferred to the corneal epithelium and detected by the C fibres.[16, 17] For both instruments, the patient is asked to respond verbally as to whether they felt the stimulus or not - for the Cochet-Bonnet Aesthesiometer the subject feels a touch on the eye, and for the NCCA the subject feels a gentle cooling of the eye.

Pattern of corneal sensitivity loss and recovery

The extent of corneal sensitivity loss and recovery with contact lens wear depends on the contact lens type, the oxygen permeability of the material, the number of hours of daily lens wear, the number of years of wear, and the length of any recovery period. For this review, we shall consider the pattern of sensitivity loss and recovery. of hard and soft contact lenses, over both short-term and long-term wear.

Short term effects

Hard/PMMA contact lenses.—As mentioned earlier, Hard/PMMA lenses actually require a reduction in corneal sensitivity to improve their comfort and allow long-term wear. This is most clearly seen when a lens is inserted in the eye of a naïve wearer. An immediate lacrimal response occurs, which gradually reduces with neural adaptation. A further more significant reduction in corneal sensitivity occurs over a full days wear.

A large number of studies have investigated this effect.[13, 18-33] The most interesting series of studies were completed by Millodot. He found a reduction in corneal sensitivity of about 110% over a 12-hour wear period. His subjects had worn their lenses for at least 3 months and he assessed their corneal sensation prior to insertion, after 4 hours, 8 hours and 12 hours of continuous uninterrupted wear. He found that sensitivity diminishes progressively with the length of wear to a maximum after the 12-hour wear period. He also found a high correlation between central and peripheral corneal sensitivity, although the loss was less in the centre than in the periphery.[34] This effect presumably relates to the increased mechanical effect of the edge of the lens. It is reasonable to assume that if the lenses are worn for more hours without removal, the loss will continue to some unknown maximum level.

The recovery of sensitivity after short-term PMMA contact lens wear is rapid. When lenses are removed after 8 hours of wear, a statistically significant recovery occurs within 1 hour, although complete recovery takes longer and is related to the number of hours of contact lens wear.[29, 34]

Soft contact lenses.—Soft contact lenses do not require any reduction in corneal sensitivity to improve their ease of wear because of the flexibility of the material. At the same time, investigations have shown that soft contact lenses still produce a progressive decline of corneal sensitivity, but to a much lesser degree than hard contact lenses.

A number of studies have considered this effect,[27, 30, 37-39] although the most interesting studies were completed by Millodot and Velasco *et al.* Millodot measured the corneal sensitivity in 15 subjects before and after 4 hours, 8 hours and 12 hours of uninterrupted HEMA soft lens wear. He observed a small, but significant, decrease in corneal sensitivity after 8 hours of wear, and this loss increased with continuing wear.

Velasco *et al.* found a significantly greater decrease in corneal sensitivity with 38% water content hydrogel lenses than with 55% water content lenses. This reflects the influence of lens water content on the oxygen concentration at the corneal surface - more hydrated lenses produce a higher corneal surface oxygen tension. Similar investigations found that high water content soft lenses produce practically no change in corneal sensitivity over a 12-hour period, although different lenses and fits cause slightly different results.[35, 42, 43]

The recovery of sensitivity after soft contact lens wear is usually more rapid than that found with hard contact lenses and depends on the nature and duration of wear. Recovery usually occurs within one hour of lens removal.[40, 41]

Long term effects

Hard/PMMA contact lenses.—Most reports of sensitivity reduction have paid attention to short term wear of contact lenses. However, a number of investigations have considered the effects of longer periods of wear.[13, 19, 25-27, 30, 33, 44, 45]

The most interesting studies were completed by Millodot who assessed the effect of long-term PMMA lens wear. He found a marked decline in sensitivity after the first

few years of wear. In one study, the subjects were divided between an experimental group, who had worn hard contact lenses for 1-22 years, and a control group who had never worn lenses. Subjects who had worn lenses for only 1-2 years had no significant difference in corneal sensitivity when compared to those subjects in the control daily group. This indicates that the recovery which occurs after removal of the lens is sufficient to return sensitivity to normal levels. However, the effect of prolonged wear is easily seen after 5-7 years. Subjects in the experimental group have a significant decrease in corneal sensitivity in comparison with the control group. If we describe corneal sensitivity in terms of its inverse, the corneal touch threshold (CTT), then 5-7 years wear produces a 100% increase in CTT and after 17-22 years wear a 200% increase in CTT.

A similar study has been completed by Sanaty and Tenel who found the same pattern of sensitivity loss. They also found a greater loss of sensitivity in the periphery, which presumably again relates to the increased mechanical edge effect of the lens.

The recovery of corneal sensitivity after long-term wear can take many months and depends on the length of time the subject has worn PMMA lenses. For example, with a subject who has worn lenses for 10 years, recovery to normal levels can occur within 1 month, but for a subject who has worn lenses for 15 years, recovery takes 4 months. The main point is that the longer the initial wear, the longer it takes to recover.[29, 46]

Soft contact lenses.—A number of studies have considered the effect of long-term daily wear of soft contact lenses on corneal sensitivity. Two studies considered the effect of high water content extended wear lenses. Larke and Hirji followed patients who were wearing Sauflon 85 lenses and Millodot examined people who were wearing X-Ten lenses. In both studies, corneal sensitivity reduced progressively over the weeks of wear, with approximately a 50% increase in CTT by the end of 3 months with the X-Ten lenses. From these results it is evident that, even with lenses of high oxygen permeability, some loss of corneal sensitivity occurs.

A more recent study by Murphy *et al.* assessed the long-term effects of daily-wear soft contact lenses and rigid gas permeable (RGP) contact lenses on corneal sensitivity using the non-invasive air-pulse stimulus (-NCCA). Interestingly, while both lens reduced corneal sensitivity from normal levels, no significant difference was found between the results of each contact lens types: soft and RGP. A similar pattern of significance was found when the results for the peripheral test locations were compared, suggesting that there is no topographical variation in the effect of the two lens types. RGP lenses generally have a higher oxygen permeability than soft lenses and so should produce less of an effect on corneal sensitivity as a result of an impaired metabolic function. However, RGP lenses also produce a mechanical adaptation effect in the corneal nerves and this adds to the effect from the reduced metabolic function. In contrast, soft lenses do not have a mechanical action. When the metabolic and mechanical effects are combined for each lens type, they appear to produce a similar total effect on corneal sensitivity.

The second significant finding was that the duration of lens wear for both soft and RGP lenses doesn't affect the extent of sensitivity loss. It appears that with adaptation to the metabolic change and mechanical action of lens wear, a new balance between the metabolic requirements of the corneal nerves and their oxygen supply produces an altered corneal touch threshold.

The last important finding was the lack of topographical variation across the cornea in corneal sensitivity change. For negative power lenses, we might expect to find greater sensitivity loss in the periphery due to the increased lens thickness. However, the periphery should also receive more oxygen dissolved in the tears, via tear exchange under the lens, and so these effects may cancel each other out. In contrast, RGP lenses do not cover the corneal periphery, but have an increased mechanical action from the edges of the lens during blinking that may cause the corneal sensitivity loss in the periphery.

There have been no published studies that have considered the recovery of sensitivity af-

ter long-term soft lens wear. Nevertheless, we can expect a similar pattern of recovery to occur as with long-term PMMA lens wear. The only main speculation might be the length of time required to recover to normal levels. However, since soft lenses generally produce less of an effect on corneal sensitivity, a more rapid recovery should occur.

Possible mechanisms of corneal sensitivity loss

There are two main answers to the question of what causes the sensitivity loss with contact lens wear - metabolic impairment of the cornea or mechanical pressure on the cornea.

Polse passed 100% nitrogen gas over a subject's eye via a modified swimming goggle. After 2 hours wear, corneal sensitivity was unaltered, although corneal swelling was present. In a second experiment, he fitted subjects with PMMA lenses and this produced a loss of sensitivity, but no swelling. Polse concluded that it was not the oedema which induced the changes in corneal sensitivity, but rather the effect from mechanical stimulation.

Although there is some anectodal evidence that lenses which produce less mechanical stimulation give rise to a smaller decrease in corneal sensitivity, the mechanical action of a lens on the corneal nerves cannot be the only mechanism for corneal sensitivity loss. This is evident in a number of ways. Firstly, soft lenses still produce a reduction in corneal sensitivity. Secondly, when the eyes are closed overnight, corneal sensitivity declines as a result of a lower oxygen pressure at the corneal surface and not as a result of mechanical stimulation.[48] Thirdly, when the cornea is exposed to a reduced partial pressure of atmospheric oxygen, a reduction in sensitivity occurs.[49] In an experiment by Millodot and O'Leary (1980), the cornea was exposed to two different gas mixtures containing 2.1% oxygen and 3.15% oxygen, (normal atmospheric oxygen contains 10% oxygen). They found a strong relationship between the time of exposure to a reduced pressure of atmospheric oxygen and a reduced corneal sensitivity. They also found a time delay between the start of the experiment and the reduction in corneal sensitivity. With the 2.1 and 3.15% oxygen pressures, it took 3 and 4 hours respectively to produce a measurable change sensitivity. In the study by Polse described earlier, no change in corneal sensitivity occurred with a 100% nitrogen atmosphere, but the measurement was taken after only 2 hours and a longer period may be needed before any change can be detected.

The comparative impact between the mechanical action and the corneal oxygen supply can be demonstrated by considering the differing effects of PMMA and RGP lens wear on corneal sensitivity. In one experiment, subjects were fitted with a PMMA lens in one eye and a RGP (CAB) lens in the other. After 3 months of wear, a reduction in sensitivity was measured in the PMMA wearing eye, while practically no change occured in the RGP wearing eye.[36] Another experiment compared the effect of three RGP lenses, each with a different oxygen permeability, and found a relationship between the epithelial oxygen availability and changes in corneal sensitivity.[50] Bergenske and Polse also found that patients who are refitted with RGP lenses after having worn PMMA lenses often regain lens awareness.

From this series of experiments we can conclude that corneal sensitivity reduction is mediated by a change in the oxygen supply to the cornea and not simply by any mechanical stimulation. However, the mechanism by which the corneal nerves are affected by a reduced oxygen pressure is not clear. There is some evidence that acetylcholine is involved in corneal sensitivity. The corneal epithelium has the highest concentration of acetylcholine in the body. Tanelian *et al.* showed that acetylcholine instilled into the eye increases the action potential in the long ciliary nerves of the rabbit cornea. Pesin and Candia proposed that acetylcholine in the corneal epithelium plays a role in the regulation of sodium positive and chloride negative transport, both of which are necessary in the production of nerve impulses. The synthesis of choline acetyltransferase, the enzyme that

synthesises acetylcholine, is interfered with when the oxygen supply is reduced.[54, 55] Since such a situation occurs in contact lens wear, this may be one pathway for a reduced corneal nerve function.

Lastly, the reduction in corneal sensation may be due to corneal acidosis during contact lens wear. The pH of the body is carefully regulated to 7.4 and even a change of 0.05 can produce severe complications. Metabolic acidosis, and specifically lactic acidosis, can lead to depression of neural activity ranging from weakness and lethargy through to coma, depression of vital functions and ultimately death. Respiratory acidosis, due to hypercapnia (the accumulation of carbon dioxide), can lead to depression of neural function as well. The stromal pH is usually maintained at 7.54,[56] which is higher than that of the body, but closed eye wear of a PMMA lens can lead to a decrease of pH to 7.1.[57] Such a change would cause severe depression of neural function elsewhere in the body. Since both lactate accumulation and carbon dioxide are evident during contact lens wear, their increased concentration may be responsible for corneal hypoesthesia.

Conclusions

This review of the different studies has revealed the gradual effect of improved lens design on corneal sensitivity changes with contact lens wear. Early contact lenses, particularly PMMA but also soft lenses, had a greater impact on corneal physiology than more recent designs. These improvements have largely resulted from an improved oxygen supply to the anterior cornea. However, this continuing improvement in contact lens design may produce an interesting complication for silicone-hydrogel contact lens wearers. The high oxygen permeability of these lenses has encouraged their use in extended wear. However, the improved oxygen supply may also ensure that a higher level of corneal sensitivity is maintained, thereby reducing corneal comfort with the lens. Such a situation has not been reported anecdotally and there have been no published studies reporting on corneal sensation with silicone-hydrogel lenses, but this area still merits attention. Other areas of contact lens wear that require investigation are the influence of new generation, high oxygen permeable RGP lenses, and the recovery of corneal sensitivity after ceasing long-term daily soft lens wear.

This review has demonstrated the usefulness of assessing corneal sensitivity as a measure of corneal health with contact lens wear. Unfortunately, using the corneal sensitivity measurement as a predictor for contact lens wear success is not as useful. There are too many other variables, such as patient motivation and ambient environmental conditions, which can also have a significant impact.

Riassunto

Lenti a contatto e sensibilità corneale

Le fibre nervose corneali svolgono un ruolo importante nei meccanismi di protezione e di omeostasi della cornea e nell'epitelio corneale si osserva il maggior numero di fibre nervose libere dell'intero organismo. Le lenti a contatto vengono utilizzate sempre più spesso per correggere difetti di rifrazione o ai fini cosmetici. Di conseguenza, è importante studiare i rapporti fra uso delle lenti a contatto e innervazione corneale. Le indagini finora condotte hanno rilevato che l'impiego di lenti a contatto è in grado di causare una riduzione della sensibilità corneale di entità correlata al tipo e al materiale di costruzione delle lenti nonché alla frequenza e durata del loro impiego. Nel complesso, aumentando il periodo di tempo in cui le lenti vengono usate, sia in termini di giorni che di mesi, aumenta la perdita della sensibilità. Protraendo l'uso delle lenti, inoltre, si eleva il tempo necessario per riacquistare la sensibilità corneale originaria una volta sospeso l'uso delle lenti stesse. I materiali di costruzione di recente introduzione, caratterizzati da una maggiore permeabilità all'ossigeno, esercitano minori effetti sulla cornea. I sue meccanismi patogenetici principali alla base dell'alterazione della sensibilità corneale sono costituiti dall'irritazione meccanica da parte delle lenti e dell'interferenza con le funzioni metaboliche della cornea, secondarie al ridotto apporto di ossigeno. La ridotta funzione metabolica porta a un aumento dell'acidosi e a un'alterazione del pH corneale in conseguenza dell'ipercapnia. Entrambi questi fattori possono alterare la funzione delle fibre nervose e quindi ridurre la sensibilità corneale.

Parole chiave: Lenti a contatto - Cornea, fisiologia - Cornea, metabolismo.

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more objective criteria of adequate fixation during evoked potential recording.

Colour specificity of the motion after-effect

E. Laviers and D. McKeefry

Department of Optometry, University of Bradford, Richmond Road, Bradford BD7 1DP, UK

Purpose: It has been demonstrated that a motion after-effect (MAE) can be both induced and nulled using isoluminant chromatic stimuli (Cavanagh and Favreau, 1985; Derrington and Badcock, 1985; Mullen and Baker, 1985). It has also been suggested that, at low velocities at least, the motion-processing pathway is sensitive to the chromaticity of motion stimuli (Hawken *et al.*, 1994, Gegenfurtner and Hawken, 1995, 1996; Burr *et al.*, 1998; McKeefry, 2001). Therefore, it would follow that the MAE would display some degree of colour selectivity. To investigate the colour selectivity of the MAE, we used a 2AFC motion-nulling paradigm to measure the strength of the MAE induced using isoluminant adapting stimuli modulated along the cardinal chromatic axes in MBDKL colour space and isoluminant test stimuli modulated along a number of different chromatic axes. We hypothesised that the MAE would be greatest when the adapting and test stimulus were modulated along the same axis, and weakest when test and adapting stimuli were modulated along orthogonal axes.

Methods: Subjects adapted to a sinusoidal grating drifting to the left at 2 deg s^{-1} and subsequently viewed a test grating of variable velocity and direction (i.e. left or rightward motion) then made a choice as to whether the test grating drifted towards the left or the right. The test velocity at which the MAE was nulled was recorded as the MAE strength. All stimuli were presented at equal multiples of contrast detection threshold.

Results: The MAE was strongest when the test stimulus was modulated along the same chromatic axis as the adapting stimulus and MAE strength showed the general trend of decreasing with increasing deviation of the test axis from that of the adapting stimulus.

Conclusion: The results confirm our hypothesis that the MAE is colour selective, for these conditions.

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Repeatability of the Complete Ophthalmic Analysis System (COAS®) on determining higher order aberrations on model eyes

A. Cerviño, S. L. Hosking, S. A. Naroo and M. C. M. Dunne

Neurosciences Research Institute, Aston University, Birmingham B4 7ET, UK

Purpose: Evaluating the repeatability of the Complete Ophthalmic Analysis System (COAS®, WaveFront Sciences, Albuquerque, NM, USA) on the determination of higher order aberrations of model eyes.

Method: Twelve model eyes were examined with the COAS. Two sessions of 10 measurements each, as well as another session of 10 more measurements without refocusing, were performed. Values obtained were analysed to assess repeatability of the instrument, variations on the intrasession and intersession repeatability, as well as those induced by human manipulation during the focusing procedure.

Results: Root mean square (RMS) of the confidence interval (CI) obtained for the whole sample was reduced to 1.10% of the mean value. High correlation was obtained between the repeatability obtained from two measurements and that obtained from ten consecutive measurements ($r = 0.950$; $p < 0.001$; 95% CI for $r = 0.7755$ to 0.9898) with the values not being significantly different (mean difference: 0.0258, S.D.: 0.1005, 95% CI: −0.0514 to 0.1031, $p = 0.4628$; paired t-test), suggesting a high performance of the instrument. When refocusing, up to four measurements are needed to get a correlation above 0.500 ($r = 0.5715$, $p = 0.1080$; 95% CI for $r = -0.1493$ to 0.8957) and for the values not to be significantly different (mean difference: −0.0175, S.D.: 0.0499, 95% CI: −0.0558 to 0.0209, $p = 0.3246$; paired t-test), suggesting that at least four measurements are needed to get a reliable value. Comparison of values obtained in two sessions (refocusing for each measurement) shows variable correlation for the different eyes, suggesting a non-constant human factor in the manipulation of the instrument.

Conclusions: The COAS wavefront sensor shows good performance with high repeatability. However, the comparability of results diminishes significantly due to human manipulation of the instrument, and there is variability between sessions. This suggests a need for improving the reference for focusing, so that the whole image capture procedure is less clinician-dependent.

Diurnal variation of corneal sensitivity and thickness

A. M. Ntola and P. J. Murphy

School of Optometry and Vision Sciences, Cardiff University, Cardiff CF10 3NB, UK

Aim: To assess the diurnal pattern of change in corneal sensitivity (CS) and corneal thickness (CT).

Methods: Twenty Caucasian subjects were recruited (males = 7, females = 13, age = 23.7 ± 3.18 years). Subjects with any ocular condition known to affect CS were excluded. Ethical approval was obtained and subjects were asked to sign a consent form prior to participating. Central CS was assessed using the non-contact corneal aesthesiometer (NCCA) and Cochet-Bonnet aesthesiometer (C-BA). The NCCA stimulates the cold C fibres, while the C-BA stimulates the Aδ mechano-sensors. CT was measured using the Haag-Streit optical pachometer. All measurements were taken on the left eye, which was patched overnight. The patch was removed 5 min before measurements began, to create a standard point for all subjects. Measurements were taken every hour from 8 AM to 12 PM, and then every 2 h from 2 PM to 10 PM. The order of measurements was randomised at each time period. To assess patient training with the NCCA, seven subjects were re-measured on a second day.

Results: A significant diurnal change in CS was found with the NCCA, with sensitivity being lower in the morning and higher in the evening (ANOVA, $p = 0.0285$). No significant change was found using the C-BA (ANOVA, $p = 0.0545$). With CT, a significant change was found from 8 AM to 12 PM (ANOVA, $p = 0.0401$), but no significant change was found from 2 PM to 10 PM (ANOVA, $p = 0.9693$). A significant correlation between the first and second measurements, for the re-test subgroup of NCCA was found ($r^2 = 0.9637$).

Conclusions: The NCCA was able to detect the diurnal variation in sensitivity of the C-fibres. The C-BA was unable to detect any variation, because of its truncated stimulus range. CT decreased through the day, having an inverse relationship with CS ($r^2 = 0.6910$). Measurement of corneal sensitivity with the NCCA has only a small learning component.

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ARVO Abstract 2004

Program#/Poster#:	4823/B147
Abstract Title:	The Anaesthetic Effect of 0.5 % Proxymetacaine Hydrochloride (Proparacaine) on Corneal Sensation
Presentation Start/End Time:	Thursday, Apr 29, 2004, 10:30 AM -12:30 PM
Location:	Hall BC
Reviewing Code:	315 No Reviewing Code applies – CO
Author Block:	*A.Ntola, P.J. Murphy.* School of Optometry & Vision Sciences, Cardiff University, Cardiff, United Kingdom.
Keywords:	552 innervation: sensation,474 cornea: clinical science,623 pharmacology,552 innervation: sensation,474 cornea: clinical science,623 pharmacology,552 innervation: sensation,474 cornea: clinical science,623 pharmacology

Purpose: To assess the duration, depth and recovery time of anaesthesia produced by the topical instillation of 0.5% proxymetacaine hydrochloride using the Non-Contact Corneal Aesthesiometer (NCCA).

Methods: Seventeen Caucasian subjects were recruited (m=2, f=15, age=26±3.6). Subjects with any ocular condition known to affect corneal sensitivity (CS) were excluded. Ethical approval was obtained and subjects were asked to sign a consent form prior to participating. CS was assessed using the NCCA which stimulates the cold sensitive C fibres. All measurements were made on the right eye only. 20µl of 0.5% proxymetacaine hydrochloride or 20µl of saline was then instilled in either the right or left eye. Central CS was measured under four conditions: (P-P) proxymetacaine in both eyes, (P-S) proxymetacaine in the right eye and saline in the left eye, (S-S) saline in both eyes and, (S-P) saline in the right eye and proxymetacaine in the left eye. CS was measured before instillation and at 2, 5, 10, 15, 20, 30, 45, and 60 minutes post instillation. Subjects were further classified according to iris colour into two groups: brown or blue.

Results: (1) For conditions P-P and P-S onset of anaesthesia was observed at 2 min post-instillation (P-P, t-test $p=0.001$; P-S, t-test $p=0.002$), with a maximum depth of anaesthesia at 15 min post-instillation (P-P, t-test $p<0.001$; P-S, t-test $p<0.001$), after which sensitivity began to recover. CS did not return to baseline levels at 60 min post-instillation (P-P, t-test $p=0.0003$; P-S, t-test $p=0.0133$). No significant difference in depth of anaesthesia was noted between p-p and p-s. (2) No change in CS was found in the right eye over the period of the trial, for conditions s-s and s-p. (3) No significant difference was found between the brown and blue iris colour sub-groups, at 2, 15, and 60 min (P-P, P-S, t-test $p>0.05$). **Conclusions:** (1) The anaesthetic effect of 0.5% proxymetacaine hydrochloride is more prolonged than the 20 minutes previously thought. (2) The maximum anaesthetic effect does not occur until 15 minutes after instillation. (3) The results suggest that 0.5% proxymetacaine affects Aδ and C nerve fibres in different ways. (4) The iris colour of the subject has no effect on the anaesthetic action of 0.5% proxymetacaine hydrochloride.

Commercial Relationship:	**A. Ntola**, None; **P.J. Murphy**, None.

ARVO Abstract 2005

Program#/Poster#:	4397/B755
Abstract Title:	**The Effect of Iris Pigmentation and Ethnic Origin on Corneal and Skin Sensitivity, and on Tear Film Break-Up Time and Blink Rate**
Presentation Start/End Time:	Wednesday, May 04, 2005, 11:15 AM - 1:00 PM
Location:	Hall B/C
Reviewing Code:	317 No Reviewing Code applies - CO
Author Block:	*A.Ntola, P.J. Murphy.* School of Optometry & Vision Sciences, Cardiff University, Cardiff, United Kingdom.
Keywords:	552 innervation: sensation, 474 cornea: clinical science, 481 cornea: tears/tear film/dry eye

Purpose: To assess the effect of iris pigmentation and ethnic origin on corneal sensitivity (CS), skin sensitivity (SS), tear film break-up (BUT) and blink rate.
Methods: Subjects (n=172) were recruited for the study and allocated to four ethnic groups: 1) 86 Caucasian (m=28, f=58, age=23.5±3.7), 2) 40 Asian (m=5, f=35, age=21.9±3.5), 3) 30 Chinese (m=10, f=20, age=24.3±5.6), and 4) 16 Black African (m=6, f=10, age=24.2±3.9). Ethical approval was obtained and subjects were asked to sign a consent form prior to participating. Subjects were also classified according to the Iris Color Classification System. All measurements were made on the right eye only and after 12 noon to avoid any diurnal bias. Central CS was measured using the Non-Contact Corneal Aesthesiometer (NCCA), which stimulates the cold sensitive C fibres. SS was assessed at the upper closed eyelid using the NCCA. Tear film break-up time was assessed following the instillation of 0.7µl of fluorescein. The blink rate was recorded for 5 minutes without the subject's knowledge.
Results: 1) Caucasians had a progressive decrease in sensitivity with increasing iris pigmentation (R^2=0.97). 2) No difference in CS was found between ethnic groups (ANOVA, $p>0.05$) except for Asian/African groups, with Africans being less sensitive than Asians (t-test, $p=0.02$). 3) A significant difference in SS was found only between the Chinese and Africans groups (t-test, $p<0.05$). 5) No significant correlation was found for CS and SS between each ethnic group. 6) A weak but significant correlation was found between blink rate and BUT for all ethnic groups, with blink rate decreasing as BUT increases (R^2=0.28).
Conclusions: 1) Iris pigmentation influences CS; as iris pigmentation increases, CS decreases. 2) No significant difference was found in CS, SS, BUT and blink rate between the ethnic groups. 3) BUT and blink rate are weakly correlated; as blink rate increases, BUT decreases.

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