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Methods in eye research

A protective eye shield for prevention of media opacities during small animal ocular imaging

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1. Introduction

The use of non-invasive ocular imaging in small research animals is an increasingly important analytical tool. Many laboratories employ OCT and SLO in addition to routine conventional fundus photography to document the progression of ocular changes in research animals. A number of imaging systems are available commercially that allow investigators to assess ocular tissues *in vivo* in a variety of animal models, including rodents. While the instrumentation is readily obtained, high quality ocular images require that the animal procedures maintain the optical properties of the eye. In most situations, ocular images are obtained from anesthetized animals. Anesthesia abolishes the blink reflex, preventing the refreshment of the tear film required for the maintenance of corneal hydration. An eye continuously exposed to air quickly becomes dehydrated. A major challenge to the routine acquisition of high quality images involves maintaining hydration of the ocular surface. This issue is well known (Calderone et al., 1986; Hockwin and Koch, 1977; Ridder et al., 2002), but for the purpose of this methods paper, it is important to first illustrate the key features of the changes in the cornea and lens before presenting our approach to avoiding this problem.

Dehydration alters corneal topography, which begins soon after anesthesia induction and/or following topical mydriatic administration. Fig. 1 illustrates early corneal changes in a rat eye using macro photography (Fig. 1A–C), SLO (Fig. 1D) and OCT (Fig. 1E) imaging. A normal, unaffected cornea is shown by standard (Fig. 1A) and flash (Fig. 1B) macro photography. Corneal surface irregularities, revealed by flash macro photography (Fig. 1C), infrared SLO (Fig. 1D) and OCT (Fig. 1E), appear soon (<5 min) after anesthesia induction. As is evident in these illustrations, these changes include the appearance of corneal surface "pitting" (indicated by arrows in Fig. 1C–E). Once these surface changes occur, they are difficult to reverse and are typically not resolved following application of hydrating drops. Changes in the rat cornea are so pronounced that one can observe the phenomenon developing soon after anesthesia induction with the unaided eye. These irregularities alter the refractive properties of the cornea and degrade image quality by disrupting light transmission (Fig. 1F vs G).

The mechanisms leading to lens opacity are not well understood, although it has been shown that covering the rodent eye to prevent dehydration eliminates this problem (Ridder et al., 2002; Sparrow et al., 2013; Turner and Albassam, 2005). Lens opacities occur quickly, usually within 10 min in the absence of ocular surface protection (Hockwin and Koch, 1977; Nusinowitz et al., 2002; Ridder et al., 2002). Early changes are usually reversible and can fully absolve once the mouse has recovered from the effects of anesthesia (Bermudez et al., 2011).

We evaluated several approaches to maintain corneal hydration. Our initial attempts involved application of saline or artificial tears followed by the application of a small square of polyvinylidene

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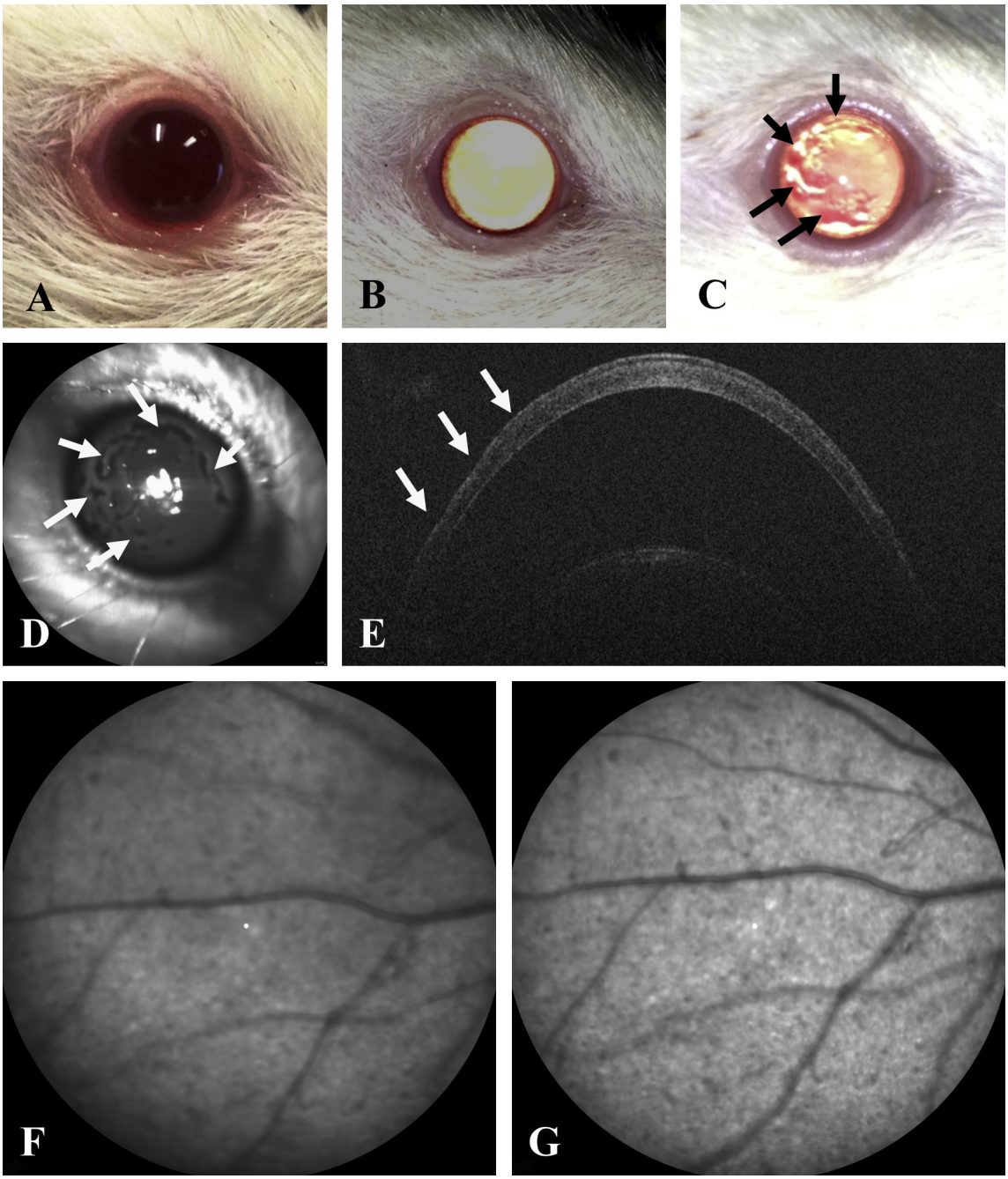

Fig. 1. Corneal changes imaged by digital macro photography (Fig. 1A–C, F, G), SLO (Fig. 1D) and OCT (Fig. 1E) in a Sprague–Dawley (SD) rat anesthetized using a combination of Ketamine (150 mg/kg) and Xylazine (12 mg/kg). Standard (no flash) macro photography of a recently anesthetized SD rat collected under ambient room lighting with normal cornea surface morphology (Fig. 1A). The two reflections present at 12 and 2 o'clock position are reflections from the fluorescent lamp fixtures located on the laboratory ceiling. Flash macro photography of the same rat before (Fig. 1B) and after (Fig. 1C) developing corneal perturbations (black arrows) several minutes after leaving the eye exposed to air (i.e. uncovered and unprotected against dehydration). The flash photography accentuates the corneal atrophy that occurs as a result of anesthesia induction and a lack of ocular protection against corneal tear film evaporation. This same phenomenon can be observed by infrared SLO imaging (Fig. 1D) which appears as "pitting" of the corneal surface (white arrows). OCT imaging (1000A-scans/B-scan) of an affected eye (Fig. 1E) shows epithelial and stromal thinning (white arrows) which contributes to a non-uniform surface for light refraction. Imaging instruments like SLO and OCT are highly dependent on corneal refraction for generating images of the posterior segment. OCT image dimensions are 1.5 mm (depth) × 6 mm (width). Images from the eye of a separate rat (pigmented SD Zucker) showing the influence of corneal perturbations on retinal SLO image quality (Fig. 1F–G). A cornea with perturbations causes a lack of image clarity (Fig. 1F). After performing a "Refresh-Reset" (see Detailed Methods section) procedure the retinal image quality dramatically improves (Fig. 1G) by rehydrating the corneal stroma and epithelial cells as well as smoothing out the tear film.